HIGH PRESSURE CO_2 INACTIVATION IN FOOD: A MULTI-REACTOR APPARATUS FOR INACTIVATION KINETIC DETERMINATION

Tiziana Parton*, Nicola Elvassore, Alberto Bertucco
Dipartimento di Principi e Impianti di Ingegneria Chimica, Università di Padova
via Marzolo, 9 - I-35131 Padova PD ITALY
Sergio De Sanctis, Sonia Dal Corso, Luana Grimolizzi
Direzione Marketing e Innovazione, SAPIO Produzione Idrogeno Ossigeno S.r.l.
via S. Pellico, 48 - I-20052 Monza MI ITALY
tiziana.parton@unipd.it +39 049 827 5461

High pressure CO_2 treatment represents an alternative to the traditional methods for preservation of foods against pathogenic and spoilage microorganisms, and enzymatic activity. This happens with regard to the natural and nutritive properties of food because of mild conditions of treatment.
Preliminary studies are required on CO_2-substrate interactions, particularly for the determination of the inactivation kinetics of microorganisms and possible physical-chemical changes occurring in foodstuffs during the treatment.
In this work, a new batch apparatus is presented for investigating food inactivation by high pressure CO_2. The multi-reactor system allows to save time and materials, and a better collection of experimental data in terms of reliability and homogeneity. Moreover, the knowledge of properly documented inactivation kinetics is enlarged, very important for mechanism understanding and high pressure CO_2 technology development in food pasteurization.
Preliminary results are presented about pasteurization of tomato sauce and grape must.

INTRODUCTION

Lately the nourishment importance in people health and wellbeing was very much valorized. The market request of products maintaining as much as possible its natural characteristics is then more and more consistent. On the other hand, foodstuffs have to be preserved in order to allow their consumption delayed in time: at present, the mostly employed inactivation technology at industrial level is the thermal treatment. This is an efficient process, but often involving undesired consequences on the food quality, as the denaturation of some important alimentary components (proteins and vitamins), the change of part of organoleptic properties, alterations on the product appearance (colour and texture) and, at times, on the taste. Thus the studies were directed towards the improvement of the current thermal process and, primarily, towards alternative preservation techniques.
The exploitation of high hydrostatic pressure and the use of high pressure carbon dioxide are between the main experimental processes of pasteurization and sterilization known as “cold processes”. For the first application very good results were obtained in some cases [1], but they are strongly dependent on the food characteristic, so that the process becomes

inapplicable [2, 3]; moreover, the excessive plant costs further restrict the possibility of development of this technology on large scale (the utilized pressures reach even 10000 bar).
The use of CO_2 at high pressure, conversely, seems to be an approach more feasible, because it allows to operate in conditions of relatively low pressure and temperature (close to room temperature), and it seems not to induce alterations incompatible with alimentary functions of the substrates; nevertheless it is able to have a pasteurizing effect on foodstuffs [4, 5]. In this way, treated food can maintain intact its natural nutritional characteristics after the preservation treatment.
In this work a new batch apparatus for studies on food pasteurization by high pressure CO_2 is presented; in particular, it was equipped for inactivation kinetic determination with improvement of reliability of the results by means of a series of reactors of little volume connected in parallel.
Results are presented for alimentary substrates, grape must and tomato sauce, both at natural condition and inoculated with microorganisms isolated from their natural flora.

I - BATCH APPARATUS

Figure 1 shows the schematic of the batch plant for pasteurization by high pressure CO_2.

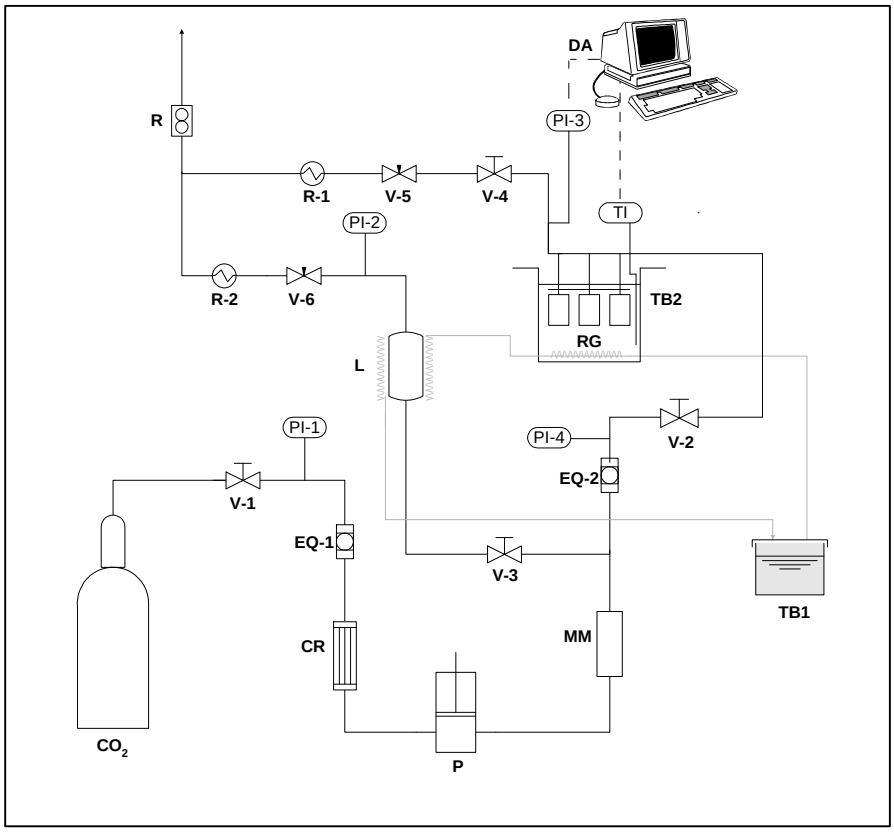
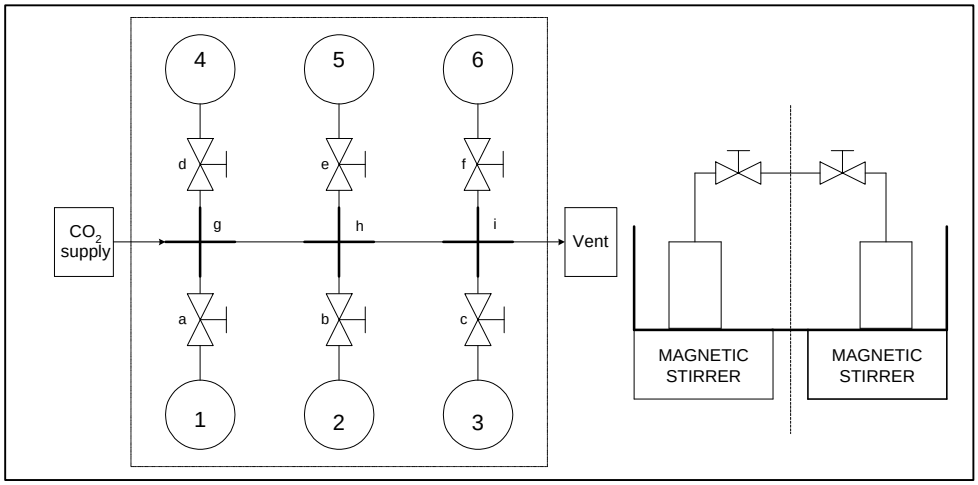


Figure 1. Schematic of batch plant for kinetic determinations in food inactivation by high pressure CO_2. V-1, V-2, V-3, V-4: on-off valves; V-5, V-6: millimetric regulation valves; EQ-1, EQ-2: filters; CR: CO_2 cooling system; P: piston pump; MM: manometric modulus; TB1, TB2: thermostatic baths; L: CO_2 reservoir; R1, R2: heating coils; R: gas counter; RG: reactors group; DA: data acquisition.

All piping in contact with high pressure CO_2, so as the valves, filters and CO_2 lung, are made in AISI 316 stainless steel. The CO_2 reservoir is a cylindrical vessel for CO_2 storage at high pressure, provided with two free screwed flanges and neoprene O-Ring gaskets. It is essential in order to avoid pressure fluctuations and, primarily, for very rapid attainment of the operative pressure into reactors. So, the typical "death-time" in pressurization step, a systematic error current in many kinetic determinations, can be avoided. The time required for pressurization of reactors system presented in this work is always less than 10 seconds.



(a) (b)

Figure 2. Final configuration of six reactors group in the high pressure CO_2 plant: (a) high prospect, where 1, 2 ,3, 4, 5, 6: reactors, a, b, c, d, e, f: on-off valves, g, h, i: cross pipe-fittings; (b) front view.

The new equipment consists in six identical reactors connected in parallel, so that each experimental run provides simultaneously six kinetic points. Reactors were arranged as showed in Figure 2. Each reactor is connected with an on-off valve that, after pressurization of the whole system, is closed at the moment of depressurization of individual reactor, when the kinetic time set for that position is passed. Other reactors can remain under operative pressure for the desired time. The six reactors are immerged in a thermostatic water bath so that the temperature is uniform everywhere. Six magnetic stirrers were inserted under the bath, each in correspondence of a reactor, where magnetic stirrer bar was preventively inserted. Turbulence created by magnetic stirrers enables an homogeneous distribution of CO_2 into the treated substrate and, in particular, makes the mass transfer between the two phases faster, so that the determined kinetics can be independent of this phenomenon and is only related to the physical-chemical effects occurring between CO_2 and microorganism cells. Thus, the new plant configuration allows collection of experimental data which are very interesting and useful in order to better know the microbial inactivation mechanism induced by high pressure CO_2.

Each model reactor consists in a cylinder (50 mm diameter) with closed bottom, united to the body reactor, and screwed cap. The total height of the reactor is 80 mm, and it was designed to resist at 339 bar at most, at a temperature of 80°C. Into the reactor there is a little cavity

that can hold up to a volume of 2 mL of sample. The seal is ensured by neoprene O-Ring inserted in its specific seat, located in the reactor cap.
The sample was submitted to different treatment times, at the same temperature and pressure, and the microbial inactivation attained was measured afterwards by means of microbiological methods (plates count). Each reactor of the group provides a kinetic point relative to certain treatment time, so that the whole kinetic curve can be obtained carrying out a single experimental run.
Moreover, this new plant configuration involves advantages in results reliability also, because each kinetic point of the determined curve is relative to identical experimental conditions of pressure, temperature and contaminated substrate. Finally, the control sample is the same for all treated samples in the six reactors, ensuring homogeneity in results and saving of work time and laboratory material consumption.

II - MATERIALS AND METHODS

First of all, we carried out a microbiological research for identification of microorganisms that are present in the alimentary substrates considered. This study included three steps: seeding, isolation and identification of microorganisms. Within the individualized cell branches, microorganisms were chosen and cultivated for subsequent inoculation in foods.
In different experimental runs a bacterium (*Bacillus subtilis*) as vegetative form and a yeast (*Pichia awry 1272*) were inoculated in grape must in quantity of 10^6 cfu/mL (cfu, *colony-forming units*). Tomato sauce and grape must were also treated as natural foods, with their natural microbial flora.
For plates count, untreated foods were serially diluted 1:10, until 10^{-3} if natural, until 10^{-7} if inoculated, whereas treated foods were serially diluted 1:10 until 10^{-2}. All samples were seeded in adequate solid broths for growth, as reported in Table 1.

Table 1. Solid broths used for plates count.

Food	Solid Broth
Natural grape must	Sabouraud (SAB), Brain Heart Infusion Agar (BHIA)
Inoculated grape must: *Pichia awry1272*	Sabouraud (SAB)
Inoculated grape must: *Bacillus subtilis*	Brain Heart Infusion Agar (BHIA)
Natural tomato sauce	Tomato Juice Agar (TJA), Brain Heart Infusion Agar (BHIA)

After temperature equilibration, the group of six reactor, charged each with 1.5 mL of sample, was pressurized with CO_2. At prearranged time, one by one the reactors were closed acting on its correspondent valve, depressurized and brought under sterile cabinet for subsequent microbiological analysis.
The plates seeded with samples were incubated in a thermostat at 32°C for 72 hours, and then the colonies count was executed. Inactivation was expressed as $Log N_0/N$, where N_0 is the number of microorganisms initially contained in sample at time *0*, and N is the number of microorganisms counted after treatment at time *t*.

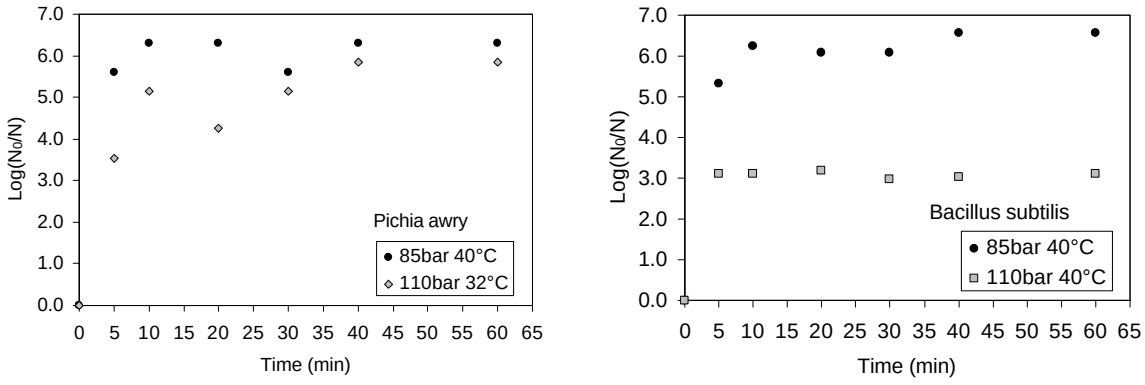
III - RESULTS AND CONCLUSIONS

Sample dilutions were always seeded in twice in Petri dishes, so results are the mean of two separate counts.
Table 2 reports the results obtained for natural must subjected to high pressure CO_2 at 80 bar and 30°C, for rising intervals of time. Treatment was more efficient for moulds and yeasts rather than for bacteria. In BHIA the surviving of microorganisms growing in this solid broth (typically bacteria) was 25% about. This number includes spores, but they are present in small quantity (15% about).

Table 2. Results of experimental run in natural grape must at 80 bar and 30°C.

Time (min)	***SAB***		***BHIA***	
	N	***Log(N_0/N)***	***N***	***Log(N_0/N)***
0	7200	0	2100	0
5	250	1.46	798	0.42
10	63	2.06	533	0.60
20	43	2.23	585	0.56
30	25	2.46	280	0.88
40	15	2.68	485	0.64
50	25	2.46	355	0.77

Figure 3(a) and 3(b) shows some results of grape must inoculated with wild *Pichia awry 1272*, and with vegetative wild form of *Bacillus subtilis* respectively.



(a) (b)

Figure 3. Inactivation kinetics of wild *Pichia awry 1272* (a) and vegetative wild *Bacillus subtilis* (b) inoculated in grape must.

It is possible to note that microbial charge reduction obtained after 10 minutes is very close to that obtained after 60 minutes. Both for yeasts and bacteria the best operative conditions for inactivation are 85 bar and 40°C.

In Figure 3(b) it can be seen that an increase of pressure does not produce directly necessarily an increase in microbial inactivation; this is a clear indication that the inactivation mechanism is quite complicate.
Table 3 shows results for natural tomato sauce treated at 110 bar and 40°C.

Table 3. Results of experimental run in natural tomato sauce at 110 bar and 40°C.

Time (min)	***TJA***		***BHIA***	
	N	***Log(N_0/N)***	***N***	***Log(N_0/N)***
0	31000	0	25000	0
10	1110	1.45	8640	0.46
20	1140	1.43	6965	0.56
30	2250	1.14	7980	0.50
45	985	1.50	8330	0.48
60	110	2.45	7365	0.53
75	1055	1.47	7130	0.54

Natural tomato sauce displays a certain resistance to the high pressure CO_2 pasteurization treatment and further investigations need, in particular on mass transfer phenomena occurring between dense gas and substrate.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs Loretta Cesaro [6] for cooperation in experimental runs and for useful discussions.

REFERENCES :

[1] SMELT, J.P.P.M., Trends in Food Science and Technology, Vol. 9, **1998**, p. 152

[2] PORRETTA, S., BIRZI, A., GHIZZONI, C., VICINI E., Food Chemistry, Vol. 52, **1995**, p. 35

[3] BUTZ, P., EDENHARDER, R., FERNANDEZ GARCIA, A., FISTER, H., MERKEL, C., TAUSCHER, B., Food Research International, Vol. 35, **2002**, p. 295

[4] DILLOW, A.K., DEHGHANI, F., HRKACH, J.S., FOSTER, N.R., LANGER, R., Proc. Natl. Acad. Sci. USA, Vol. 96, **1999**, p. 10344

[5] WEI, C.I., BALABAN, M.O., FERNANDO, S.Y., PEPLOW, A.J., Journal of Food Protection, Vol. 54 (3), **1991**, p. 189

[6] CESARO, L., Master Thesis, March **2004**, University of Padova, Italy