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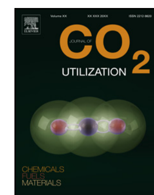

Continuous inactivation of alkaline phosphatase and *Escherichia coli* in milk using compressed carbon dioxide as inactivating agent

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ABSTRACT

This work investigated a continuous processing of milk for inactivation of alkaline phosphatase and *Escherichia coli* ATCC 25922 using supercritical carbon dioxide as inactivating agent. For this purpose, the influence of CO_2 to milk mass ratio in the range of 0.05–0.45 wt%, temperature from 30 to 70 °C, pressure from 80 to 180 bar and apparent residence time from 10 to 30 min on the enzyme and microbial inactivation were investigated by means of a central composite design for three independent variable. Enzyme inactivation of 94.5% was reached when the process was operated at CO_2 to milk mass ratio of 0.05, 70 °C, 80 bar and apparent residence time of 30 min. At this condition, the rate of microbial inactivation was 0.09 min^{-1}. Results presented in this work contribute for the development of innovative process for food processing and preservation. The continuous operation consists in an advance in the use of supercritical carbon dioxide for food treatment.

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1. Introduction

Alkaline phosphatase (ALP, orthophosphoric monoester phosphohydrolase EC 3.1.3.1) is an endogenous enzyme in milk which is slightly more resistant to heat than most pathogenic bacteria and is used as good indicators for the severity or effectiveness of heat treatment of milk and milk products [1]. For example, the absence of alkaline phosphatase activity in milk is commonly used as a standard test for the determination of proper pasteurization of milk. During the pasteurization, the ALP activity should decrease about 500-fold. A higher ALP activity level may indicate serious deficiencies in the pasteurization process [2].

Consumer demand for fresh-like food products with minimal degradation of nutritional and organoleptic properties has stimulated new treatments or combined processes in food industry [3]. Recently, attempts have been made to apply non-thermal treatments in milk processes, including pulsed electric field [1], ultrasound [4], ohmic heating [5], high hydrostatic pressure [6], supercritical carbon dioxide [6], among others. The main difference between high hydrostatic pressure and supercritical carbon dioxide processes is that much lower processing pressure is employed with supercritical carbon dioxide (SC-CO_2), usually of two orders of magnitude. In fact, when talking about SC-CO_2, processing pressure is in the range of 100–200 bar, while for high (hydrostatic) pressure, a value as high as 10,000 bar is achieved. One disadvantage of such technology is the high capital expenditure (CAPEX) compared to supercritical carbon dioxide. Besides, improved mass transfer due to higher diffusivity is present when using supercritical carbon dioxide, and hence processing time might be shorter with consequent preservation level of essential food elements, which means minimal degradation of nutritional and organoleptic properties.

Among the technologies for food preservation using high-pressure, supercritical carbon dioxide (SC-CO_2) processing is being used to inactivate enzymes and pathogens micro organisms, resulting in a minimal degradation of thermo-labile nutrients of foods, preserving sensory and nutritional characteristics, also increasing shelf-life [7]. The use of SC-CO_2 to inactivate micro organisms is well reported in the literature either in the free form or directly on food [8–13]. Specifically about alkaline phosphatase inactivation, Fadiloglu et al. [3] reported that the treatment of buffer exposed to CO_2 under atmospheric pressure decreased the enzyme activity, but the inactivation was more influenced by the temperature than CO_2.

Most of the studies reported above [8–13] are focused on the SC-CO_2 treatment maintaining the food in contact with

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Table 1
Chemical characterization of whole milk before and after SC-CO_2 treatment.

Chemical analysis	Before SC-CO_2 treatment	After SC-CO_2 treatment
pH	6.9	6.9
Protein (wt%, wet basis)	0.83	0.83
Fat (wt%, wet basis)	3.95	3.94
Acidity (°D)	15	16
Lactose (wt%, wet basis)	4.71	4.70
Calcio (mg/100 g)	44.56	44.40
Iron (mg/100 g)	0.07	0.07
Potassim (mg/100 g)	64.18	64.16
Magnesium (mg/100 g)	6.05	6.02

pressurized CO_2 for a certain period of time in a batch process, whereas the literature concerning the use of continuous processes for SC-CO_2 treatment are rather scarce, although continuous processes are highly desirable in industry. In this sense, the main objective of the present study was to evaluate the continuous process for inactivation of alkaline phosphatase and *Escherichia coli* in milk using supercritical carbon dioxide. For this purpose, a central composite design was conceived so as to assess the influence of CO_2 to milk mass ratio (0.05–0.45), temperature (30–70 °C) and pressure (80–180 bar) on the processing inactivation. At the optimized condition, it was investigated the influence of residence time on total count of *E. coli.*

2. Experimental

2.1. Sample

The samples of milk (in nature whole milk) were obtained in a local farm and maintained under refrigeration until the supercritical fluid treatments. Table 1 presents the chemical characterization of milk. Carbon dioxide with a purity of 99.5% (liquid phase) was purchased from White Martins S.A. (Caxias do Sul, Brazil).

2.2. Apparatus and experimental procedure

The experimental high-pressure continuous system used in this work, schematically presented in Fig. 1, is similar to that used previously by Bertoldi et al. [14]. The treatments of milk were carried out in duplicate using a microtube reactor with capacity of approximately 37.0 mL made of stainless steel tubing (316L HIP 1/4" internal diameter of ~0.5 cm). The crude milk was placed in a closed Erlenmeyer, which was submitted to a gentle nitrogen flow to remove residual air, and mixed by means of a mechanical stirring device and then were fed into the inactivation system by a high-pressure liquid pump (Acuflow). Carbon dioxide was added to the system at a pre-established flow rate using a syringe pump (Isco, model 500D). The microtube reactor was placed in a furnace with controlled temperature and monitored by two thermocouples directly connected at the inlet and outlet of the inactivation system. With this arrangement, the system temperature was controlled with a precision better than 5 K. The system pressure was controlled by a control loop composed by a pressure transducer (Smar, model A5), a PID controller (Novus, model N1100) and an electropneumatic valve (BaumannTM, model 51000) (Fig. 1).

Samples were collected periodically in a glass vial placed at the reactor outlet of the inactivation system after reaching the steady state condition, i.e., after a reactor space-time had been elapsed at least three times. Preliminary experiments were performed for some experimental conditions to check whether the system reached the steady-state, taking samples for at least three additional apparent residence times, and in all cases excellent system stabilization was verified, thus assuring the reliability of the experimental measurements. In this work the apparent residence time was computed dividing the volume of the system (mL) by the flow rate of substrates (mL min^{-1}) set in the liquid pump, a true, engineering parameter, which was defined as apparent residence time. Based on duplicate experiments, the

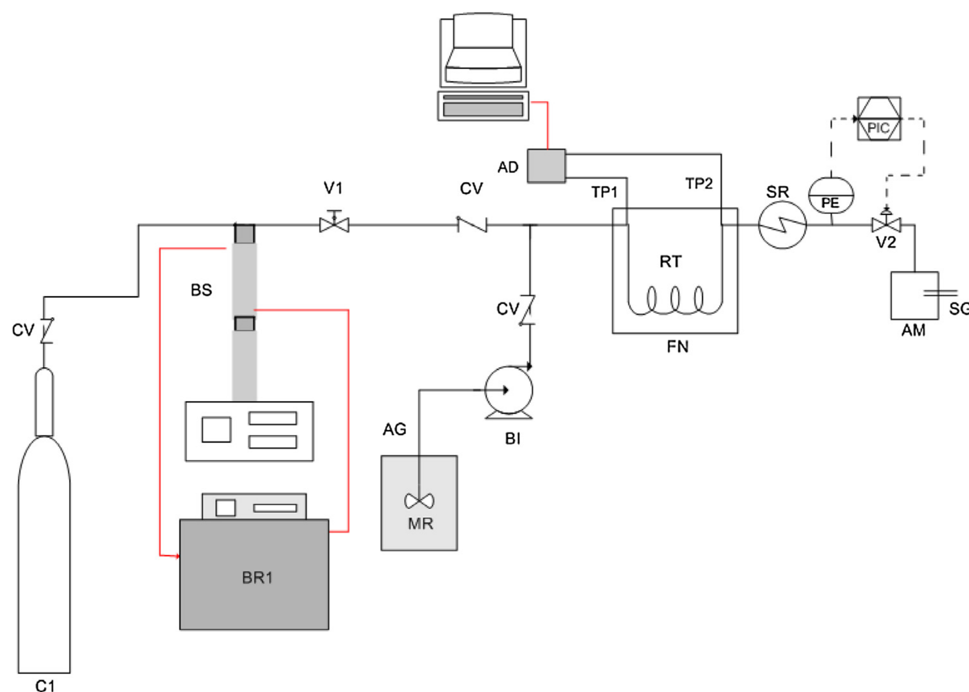

Fig. 1. Schematic diagram of the experimental apparatus used in the inactivation tests. MR—reactional mixture; AG—mechanical stirring device; BI—high-pressure liquid pump; CV—check-valve; C1—solvent reservoir; BR1—thermostatic bath; BS—syringe pump; FN—furnace; RT—tubular reactor; TP1—temperature indicator at the reactor inlet; TP2—temperature indicator at the reactor outlet; AD—data acquisition system; SR—cooling system; V1—feed valve; PE—pressure indicator; PIC–controller; V2—pressure control valve; AM—glass collector; SG—gas output.

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