J. of Supercritical Fluids 92 (2014) 257–263

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Supercritical carbon dioxide combined with high power ultrasound: An effective method for the pasteurization of coconut water

Martina Cappelletti, Giovanna Ferrentino, Sara Spilimbergo*

Department of Industrial Engineering, University of Trento, via Sommarive 9, 38123 Trento, Italy

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 5 June 2014
Accepted 7 June 2014
Available online 14 June 2014

Keywords:
Supercritical carbon dioxide
High power ultrasound
Pasteurization
Salmonella enterica
Natural microbial flora
Coconut water
Synergistic effect

ABSTRACT

Industrially, thermal treatments are extensively used to inactivate microorganisms in foods. However, the demand for new pasteurization methods with reduced impact on the nutritional content and overall food quality is increasing. In this context, this study investigated and compared the effect of supercritical carbon dioxide (SC-CO_2) alone or in combination with high power ultrasound (HPU) on both the natural microbial flora (mesophilic, lactic acid bacteria and yeast and molds) of coconut water and the pathogenic Gram-negative bacteria *Salmonella enterica* inoculated in the product. Inactivation kinetics were obtained at 12 MPa, by means of batch apparatuses, at different times (from 1 up to 60 min) and temperature conditions (from 25 up to 45 °C). The synergistic effect of SC-CO_2 + HPU was evident and a higher microbial reduction was achieved compared to SC-CO_2 alone: at 12 MPa and 40 °C about 5 log reductions were achieved for natural microbial flora in about 15 min while about 30 min were needed for SC-CO_2 treatment. The storage study highlighted that SC-CO_2 treated coconut water resulted microbiologically unstable and showed heavy regrowth phenomena during the storage, while, a full shelf life of 4 weeks was assured for SC-CO_2 + HPU treated samples.

1. Introduction

Conventional thermal methods are the most currently used to inactivate microorganisms in food products. During the last few years innovative preservation technologies have been developed driven by the constant pursuit to reduce the degree of thermal damage to the quality of the processed foods in terms of nutritional, sensorial and physical/chemical attributes [1]. The new preservation techniques could pasteurize the products, reducing or eliminating the amount of heat required. These processes are, for the most part, less energy-intensive, therefore more cost-efficient, and environmentally friendly than conventional thermal processing. Some of the most common non-thermal alternatives include pulse-electric field, microfiltration, pulse-light, high hydrostatic pressure and ultrasonication. Among others, a promising alternative to the traditional pasteurization processes is the use of supercritical carbon dioxide (SC-CO_2) technology.

Since the 1980s, SC-CO_2 has been increasingly investigated as a technique able to induce the inactivation of the natural microbial flora but also pathogens occurring in solid and liquid matrices [2–5]. CO_2 used in this process is relatively inert, inexpensive, nontoxic, nonflammable, recyclable and readily available in high purity leaving no residues when removed after the process. Furthermore, it is considered a GRAS (*Generally Recognized as Safe*) substance, which means it can be used for food products. The critical temperature (31.1 °C) is compatible with the thermal stability of most materials, and the critical pressure (7.3 MPa) is easily reached in industrial processes [6]. Theories explaining the inactivation mechanism of SC-CO_2 involve the diffusion and solubility of SC-CO_2 in the culture medium, the decrease of the pH in the medium, the increase of the membrane fluidity and permeability, the diffusion of CO_2 into the cells, the cell membrane rupture caused by the increase of the internal pressure, and the resultant changes in the cellular environment, such as a decrease in pH, the inactivation of key enzymes, and the extraction of critical intracellular materials [7,8]. Nevertheless, long treatment times and temperatures are needed to guarantee the safety and stability of some food products, limiting the efficiency of SC-CO_2 inactivation processes [9,10]. That is the reason why there is increasing scientific interest in combining SC-CO_2 processes with synergistic techniques to enhance SC-CO_2 inactivation rates [11].

Higher power ultrasounds (HPU) at low frequencies (20–100 kHz) have the potential to be used for the inactivation of bacterial populations. The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals [1,12,13]. When sound energy meets a

* Corresponding author. Tel.: +39 0461 282485; fax: +39 0461 281945.
E-mail address: sara.spilimbergo@unitn.it (S. Spilimbergo).

http://dx.doi.org/10.1016/j.supflu.2014.06.010
0896-8446/

liquid medium, longitudinal waves are generated creating alternating compression and expansion regions [14,15]. These regions with different pressures cause cavitation and formation of gas bubbles with large surface area in the medium. When the provided ultrasonic energy is not sufficient to retain the vapour phase in the bubble, a rapid condensation occurs: the condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching up to 5500 °C and 50 MPa. The pressure changes resulting from these implosions are the main bactericidal effect in ultrasounds [1]. The advantages of ultrasounds over heat pasteurization include the minimizing of flavor loss with greater homogeneity and significant energy savings during the process [1,16]. Unfortunately, very high intensities are needed if ultrasound alone is used for permanent pasteurization. However, the use of ultrasounds coupled with other decontamination techniques, such as pressure, heat or low pH is promising.

The combination of HPU and SC-CO_2 and the demonstration of their synergistic effect is quite recent: it has been shown that the application of HPU to the SC-CO_2 extraction process is highly beneficial as a consequence of the mechanical effects produced in the supercritical environment, compared to SC-CO_2 extraction alone [17]. In order to obtain safe products with fresh-like quality attributes, a novel inactivation technique based on HPU embedded in a SC-CO_2 plant has been developed [18]. Ortuño et al. [11,19] showed that the population of both *S. cerevisiae* and *E. coli* microorganisms inoculated in apple juice was completely inactivated after 5 min (35 MPa, 36 °C) and 4 min (22.5 MPa, 36 °C) of treatment, respectively. On the contrary, no microbial reduction was observed if only SC-CO_2 was applied for the same treatment time and process conditions. It has been also shown that the performance of HPU treatment is affected by several factors including the type, shape or diameter of the microorganisms [20], the growth stage [21] and the medium [19].

No references have been found in the literature exploring the effect of SC-CO_2 + HPU on the inactivation of natural microbial flora, which represents the real contamination in food products, and is the required condition to exploit the technology at industrial level.

In the present work coconut water (CW), the clear liquid of the young green coconuts, highly valued and consumed in tropical areas of the world [22], has been considered as test drink sample. CW is becoming more and more popular within the athletes as “energy and healthy drink” rich in vitamin C, magnesium, calcium, potassium, vitamin B, arginine, alanine, lysine, glutamic acid, enzymes with anti-inflammatory properties, minerals and antioxidants [23]. It is nowadays processed by heat treatment to destroy the natural microbial flora occurred in the product and to prolong its shelf life for 2/3 months [24]. However, as the high process temperatures grossly alter the sensory qualities with changes in the product nutritional contents, different preservation techniques have been investigated either alone or in various combinations [23,25–27].

The objective of this work was to study and compare the effect of SC-CO_2 alone and SC-CO_2 + HPU treatment on CW and demonstrate the potential of the combined technology to inactivate both its natural microbial flora and the pathogenic *Salmonella enterica*, a gram-negative bacterium inoculated in the product. Additionally, a storage study was performed, in order to investigate the products shelf life treated with both processes for 1, 2, 3 and 4 weeks at 4 °C. Finally, some hypotheses regarding possible inactivation mechanisms associated with this novel technology have been discussed.

The investigation of the feasibility of such a pasteurization combined technology, may open the door to the exploitation of the process to different and high value drinks at industrial scale.

2. Materials and methods

2.1. Coconut water

Seventy young green coconuts (*Cocos nucifera*, *cv Nam Hom*) from Thailand were bought and sent to Trento where they were aseptically opened, the water extracted and accumulated in a 20 l plastic pail placed in ice. Once the extraction process ended, CW was homogenized, portioned in sterilized glass jars of 200 and 400 ml and immediately frozen at −20° C to prevent any microbial or enzymatic activity. CW initial natural microbial load was measured after the extraction process: the product showed about 1.5–4 log(CFU/ml) of mesophilic microorganisms and lactic acid bacteria and about 1–2 log(CFU/ml) of yeasts and molds.

2.2. Microbial contamination of CW

To evaluate the effect of the processes, CW (frozen at −20 °C after the extraction process) was thawed at 4 °C for 12 h and then aged at 30 °C for about 16 h to increase the initial microbial load. The resulting microbial load was about 7–9 log(CFU/ml) of mesophilic microorganisms and lactic acid bacteria, and about 4–8 log(CFU/ml) of yeasts and molds.

S. enterica ATCC 14023 (DSMZ, Braunschweig, Germany) strain was used to inoculate in CW. The microbial culture was grown in 10 ml Brain Heart Infusion broth (BHIB) at 37 °C overnight, then transferred to a 200 ml flask of BHIB and grown at 37 °C overnight. Cell growth was carried out in a shaking incubator (220 rpm) and carefully monitored through measurements of the optical density in order to achieve the stationary phase. The microbial suspensions were centrifuged at 6000 rpm for 10 min at 4 °C, the supernatant removed and the pellet resuspended in 100 ml of CW, reaching a final concentration of about 10^8 colony forming unit (CFU)/ml.

2.3. CW treatment with SC-CO_2

2.3.1. SC-CO_2 multibatch apparatus

SC-CO_2 treatments were carried out in a multi-batch apparatus. The vessels consisted of ten 15 ml cylinders (used for the investigation of the effect of the temperature and the treatment time on microbial inactivation) and of two 310 ml cylinders (used for the shelf life analyses), provided with a magnetic system for stirring (Vetrotecnica, micro-stirrer, Velp, about 300 rpm). A 5 ml (V_{max} = 15 ml) and a 104 ml (V_{max} = 310 ml) of CW were introduced in the cylinders, respectively. The cylinders were connected in parallel, so that each experimental run provided a set of experimental data taken at identical process conditions but different treatment times. Each reactor was connected to an on-off valve that could be used to depressurize it independently from the others. The reactors were submerged in a single temperature-controlled water bath. Liquid CO_2 (Messer, carbon dioxide 4.0, purity 99.990%) was fed into the reactors by a volumetric pump (LEWA, mod. LCD1/M910s) that increased the pressure to the desired processing levels with a rate of about 6 MPa/min. The apparatus was provided with a transducer (Endress + Hauser GmbH, Maulburg, Germany) to control the pressure values while one cover lid of the 10 reactors was equipped with a fixed thermocouple (Pt 100 Ω) to control the product temperature. At the end of the process, two micrometric valves and one on–off valve were used to depressurize and release CO_2 from the apparatus that occurred over approximately 1 min. After the treatment, the reactors were disconnected from the pressurization line and opened in a laminar flow hood. The processed samples were collected in sterile containers and cooled down immediately at 4 °C until further use [28]. The operating parameters (temperature, pressure and time) were continuously recorded by a real time acquisition data system (National Instruments, field point FP-1000