Food Research International 51 (2013) 474–481

Contents lists available at SciVerse ScienceDirect

Food Research International

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

# Application of high power ultrasound in the supercritical carbon dioxide inactivation of *Saccharomyces cerevisiae*

Carmen Ortuño, María Teresa Martínez-Pastor, Antonio Mulet, Jose Benedito *

*Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camí de Vera s/n, E46022, Valencia, Spain*

ARTICLE INFO

*Article history:*
Received 31 October 2012
Accepted 16 January 2013

*Keywords:*
Supercritical carbon dioxide
High power ultrasound
*Saccharomyces cerevisiae*
Inactivation
Synergistic effect

ABSTRACT

The objective of the study was to analyze the influence of high power ultrasound (HPU) on the supercritical carbon dioxide (SC-$CO_2$) inactivation kinetics of *Saccharomyces cerevisiae* and to determine the effect of the temperature (31–41 °C), pressure (100–350 bar) and composition of the medium (YPD Broth, apple and orange juice) on the process of inactivation. Using a batch-mode SC-$CO_2$ at 350 bar and 36 °C, a reduction of 6.7 log-cycles was obtained after 140 min of treatment. However, when HPU (40 W ± 5 W and 30 kHz) was applied during the SC-$CO_2$ treatments, a reduction of 7 log-cycles was achieved after 2 min of treatment for all pressures and temperatures applied. The effect of increasing pressure (from 100 to 350 bar, 36 °C) or temperature (from 31 to 41 °C, 225 bar) did not significantly influence this inactivation level. The application of ultrasound leads to a vigorous agitation and cavitation which could accelerate the SC-$CO_2$ dissolving in the medium. This accelerates the penetration of $CO_2$ into cells and its inactivation mechanisms. In batch operations the application of HPU increases the speed of reaching saturation solubility of $CO_2$ in many liquid media and significantly reduces microbial inactivation times.



## 1. Introduction

Non-thermal processing alternatives have been developed by the food industry in response to an increasing consumer demand for fresh, high quality food products. These preservation technologies aim to preserve the nutrients and functionality of food, extending food shelf-life and minimizing the changes in natural color, taste, flavor and texture (Bermúdez-Aguierre & Corradini, 2012; Rawson et al., 2011), while at the same time being energy-saving, and environmentally safe processes.

The use of supercritical carbon dioxide (SC-$CO_2$) continues to attract attention as a non-thermal technique for pasteurization processes since the low temperatures used permit the preservation of natural flavors and nutrients of foods (Ferrentino, Bruno, Ferrari, Poletto, & Balaban, 2009; Kincal et al., 2006; Spilimbergo & Ciola, 2010). SC-$CO_2$ has been shown to be efficient at inactivating a large variety of microorganisms, such as *Escherichia coli*, *Enterococcus faecalis* or *Saccharomyces cerevisiae* (Garcia-Gonzalez et al., 2007; Ortuño, Martínez-Pastor, Mulet, & Benedito, 2012a,b).

SC-$CO_2$ has a density close to liquid $CO_2$, while its diffusivity and solubility are similar to those of a gas which results in an improved dissolving capacity (Tomasula, 2003). Theories explaining the inactivating mechanism of SC-$CO_2$ involve the diffusion and solubility of SC-$CO_2$ in the culture medium, the decrease in the medium pH, the increase in the membrane fluidity and permeability, the diffusion of $CO_2$ into the cells, the cell membrane rupture caused by the increase in the internal pressure, and the resultant changes in the cellular environment, such as a decrease in pH, the inactivation of key enzymes, and extraction of critical intracellular materials (Garcia-Gonzalez et al., 2007; Pataro, Ferrentino, Ricciardi, & Ferrari, 2010).

Nevertheless, long treatment times and, in some cases, high pressures or temperatures needed to guarantee the food's safety and stability, limit the efficiency of SC-$CO_2$ inactivation processes (Garcia-Gonzalez et al., 2009; Liu, Hu, Zhao, & Song, 2012). That is the reason why there is increasing scientific interest in process intensification, which focuses on combining SC-$CO_2$ processes with synergistic techniques that enhance the SC-$CO_2$ inactivation mechanisms.

It is known that high power ultrasound (HPU) technology accelerates and improves mass transfer processes (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). In fact, it has been demonstrated 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 (Riera et al., 2010). When ultrasound travels through a medium, it produces effects, such as alternating compressions, cavitation, vibration, streaming and agitation, which enhance mass transfer. Riera et al. (2010) reported that the yield of almond oil was increased by 20% in the presence of ultrasound compared to traditional SC-$CO_2$ extraction. Ortuño et al. (2012b) showed the advantages of simultaneously applying SC-$CO_2$ with the HPU treatment when inactivating a Gram-negative bacterium, *E. coli*, compared with the use of SC-$CO_2$ alone.

The microorganisms investigated by means of SC-$CO_2$ treatments ranged from Gram-negative bacteria like *Salmonella* typhimurium,

* Corresponding author. Tel.: +34 96 3879147; fax: +34 96 3879839.
*E-mail address:* jjbenedi@tal.upv.es (J. Benedito).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.01.041

*E. coli* or *Yersinia enterocolitica*, to Gram-positive bacteria or yeasts, like *Listeria innocua*, *Listeria monocytogenes* or *S. cerevisiae* (Bermúdez-Aguierre & Corradini, 2012; Garcia-Gonzalez et al., 2007). Most of the studies dealing with inactivation techniques, including SC-$CO_2$, have indicated that gram-positive cells are more resistant than gram-negative ones due to the fact that their cell wall is thicker (Ramírez Santos, Contreras Ferrat, & Gómez Eichelmann, 2005). Moreover, it is known that *S. cerevisiae* has a thicker cell wall, which makes it similar to gram-positive bacteria (Villas-Boas, Nielsen, Smedsgaard, Hansen, & Roessner-Tunali, 2006). In this regard, Ortuño et al. (2012a) showed that to inactivate *S. cerevisiae* and *E. coli* using SC-$CO_2$ under 350 bar and 36 °C, 140 and 25 min are needed to reach a reduction of 7 log, respectively, which would support the connection between wall thickness and inactivation resistance.

A previous study has been performed to analyze the advantages of coupling SC-$CO_2$ with HPU for the inactivation of a Gram-negative bacterium, *E. coli* DH1 (Ortuño et al., 2012b). This study showed that the combination of both techniques accelerated the death of *E. coli* compared with the use only of SC-$CO_2$. In this regard, it is of great interest to know how a microorganism with a higher resistance than *E. coli*, such as *S. cerevisiae* (Ortuño et al., 2012a), responds to SC-$CO_2$ + HPU treatment.

The objective of this study was to evaluate the effect of high power ultrasound on the SC-$CO_2$ inactivation kinetics of *S. cerevisiae* and to determine the effect of the temperature, pressure and composition of the medium on the inactivation process.

## 2. Materials and methods

### 2.1. Microbial preparation

#### 2.1.1. Microorganisms

The microbial strain used in this study was *S. cerevisiae* T73 (*S. cerevisiae*). It is a natural strain isolated from wine fermentation in Alicante (Spain) (Querol, Barrio, & Ramon, 1992), and it is commercialized as Lalvin T73 (Lallemand Inc., Montreal, Canada).

#### 2.1.2. Sample preparation and growth conditions

*S. cerevisiae* was grown in Yeast Peptone Dextrose Broth (YPD Broth, Sigma-Aldrich, USA) overnight at 30 °C, using an incubation chamber (J.P. SELECTA, Model 3000957, Barcelona, Spain) and an orbital shaker at 120 rpm (J.P. SELECTA, Rotabit Model 3000974, Barcelona, Spain). For each experiment, a subculture was prepared by inoculating 50 μL from the starter in 50 mL of sterilized medium and incubating at 30 °C for 24 h to obtain cells in the early stationary phase. Growth curves were determined in advance by both plating and the measurement of absorbance at 625 nm (data not shown).

*S. cerevisiae* stock cultures were maintained in Yeast Peptone Dextrose Agar (YPD Agar, Sigma-Aldrich, USA), stored at 4 °C and transferred monthly to new plates.

### 2.2. Experimental design

To determine the effect of the process conditions (temperature and pressure) on the SC-$CO_2$ inactivation of *S. cerevisiae*, YPD Broth was selected as a medium. To determine the effect of temperature, samples were exposed to SC-$CO_2$ at 31 °C, 36 °C and 41 °C at a constant pressure of 225 bar. The temperatures chosen were higher than the critical one for $CO_2$ and lower than lethal temperatures for *S. cerevisiae*. To determine the effect of pressure, samples were treated by SC-$CO_2$ at 100, 225, 290 and 350 bar at a constant temperature of 36 °C. The pressures chosen were higher than the critical one for $CO_2$ (73.8 bar) and lower than 350 bar according to a previous study about inactivation of *E. coli* using SC-$CO_2$ and SC-$CO_2$ + HPU where it was observed that higher pressures of 350 bar was not necessary to reach an acceptable level of inactivation (7 log) using SC-$CO_2$ + HPU (Ortuño et al., 2012b). In the literature, the hydrostatic effect of pressure on microorganisms is negligible below about 200 MPa (Corwin & Shellhammer, 2002), therefore the possible inactivation effect found in the pressure range considered in this study should be attributed to the aforementioned effects of SC-$CO_2$ on vegetative cells rather than to pressure.

In order to determine whether the combined use of SC-$CO_2$ and HPU affected the inactivation kinetics, experiments to sonicate the treatment medium (YPD Broth) were conducted under the same process conditions of temperature and pressure as the treatments using only SC-$CO_2$.

In addition, to evaluate the possible combination effect between SC-$CO_2$ and HPU, an inactivation experiment using HPU (40 W ± 5 W, 36 °C, 30 kHz) on YPD Broth medium was carried out and compared with the SC-$CO_2$ and SC-$CO_2$ + HPU inactivation treatments at 36 °C and 225 bar (intermediate conditions).

In order to evaluate the effectiveness of this novel technique (SC-$CO_2$ + HPU) in food matrices, apple and orange juice were selected to compare them with the inactivation kinetics on YPD Broth. These juices were chosen due to the fact that they are used extensively for microbial inactivation using SC-$CO_2$ (Ferrentino et al., 2009; Garcia-Gonzalez et al., 2007; Kincal et al., 2006).

Fruit was purchased from a local market and kept at 4 °C until juice extraction. Apple and orange juices were obtained according to the following steps: washing, peeling and extraction (Ultra Juicer, Robot Coupe J80, USA). The apple juice (pH = 5.4; °Brix = 15.6) and orange juice (pH = 3.8; °Brix = 11.6) produced was sealed in plastic containers and stored at −18 °C and thawed at 4 °C (overnight) before the treatment. The juice samples were exposed to SC-$CO_2$ and SC-$CO_2$ + HPU treatments, at 225 bar and 36 °C (intermediate conditions). All experiments were done in triplicate.

### 2.3. Supercritical fluid equipment and processing procedure

#### 2.3.1. Apparatus

The SC-$CO_2$ experiments were carried out in a laboratory scale SC-$CO_2$ batch system which permits the treatment of liquid foods (Fig. 1). The maximum pressure at which the apparatus may be operated was 1000 bar. The system included a $CO_2$-tank (1, Fig. 1) and a $N_2$-tank (2, Fig. 1), which were kept at room temperature; a chiller reservoir stored at −18 °C (3, Fig. 1); a pump (4, Fig. 1) and a thermostatic bath (5, Fig. 1) to keep the inactivation vessel (6, Fig. 1) at the desired temperature. The inactivation vessel (500 mL), as well as the different connections and valves in contact with SC-$CO_2$, were made of stainless steel, type 316. The inactivation vessel was 3 cm in thickness, to assure that the process temperature was reached inside the treatment vessel, a temperature probe was installed at the inner vessel surface (7, Fig. 1). This probe was connected to a digital controller (E5CK, Omron, Hoofddorp, Netherlands) which used an electric resistance to heat the bath and keep the temperature in the vessel at the desired value before to start of the treatment. On average, the bath temperature was 5–6 °C higher than that inside the vessel. A pressure gauge was installed in the inactivation vessel to confirm that only a short time (2.5 min) was needed for the supercritical conditions to be reached and subsequently maintained during processing. In addition, the ultrasound equipment (Benedito, Martínez-Pastor, Mulet, Ortuño, & Peña, 2011) was embedded in the supercritical fluid vessel. The transducer (>1 W/$cm^2$) was inserted in the inactivation vessel and included two commercial ceramics (10, Fig. 1; 35 mm external diameter; 12.5 mm internal diameter; 5 mm thickness; resonance frequency of 30 kHz) and a sonotrode (8, Fig. 1), which was specially constructed to concentrate the highest amount of acoustic energy on the application point. The power generator unit (11, Fig. 1) supplied constant energy to the transducer during the SC-$CO_2$ process. The piezoelectric ceramics were insulated by means of a polypropylene joint (9, Fig. 1) covered with teflon in order to avoid possible electrical short-circuits.