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# Combined use of thermo-ultrasound and cinnamon leaf essential oil to inactivate *Saccharomyces cerevisiae* in natural orange and pomegranate juices

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ABSTRACT

The inactivation of *Saccharomyces cerevisiae* in natural orange and pomegranate juices untreated (control) and treated with mild temperature (50 °C), cinnamon leaf essential oil, thermo-ultrasound (24 KHz; 33.31 W $mL^{-1}$; 30 min; 50 °C), alone or in combination, and its survival under refrigerated conditions (5 °C, 28 days) were studied. Additionally, the maximum concentration of cinnamon leaf essential oil accepted by consumers in both juices was determined by sensory analysis. A significant reduction in the yeast population was obtained compared to controls. The most effective treatment to inactive yeast cells was the combination of ultrasound and cinnamon leaf essential oil during 30 min at 50 °C, which, from an initial population of 5.13 log cfu $ml^{-1}$, achieved significant 2.81 ± 0.19 and 2.52 ± 0.26 log-reductions in natural pomegranate and orange juices respectively. During refrigerated storage (5 °C, 28 days), the *S. cerevisiae* population in orange and pomegranate juices, treated with ultrasound and cinnamon leaf essential oil at 50 °C for 30 min, decreased by 1.55 ± 0.19 and 0.68 ± 0.16 log respectively, while the controls kept the yeast population without significant changes.



## 1. Introduction

Fresh juices are an important source of beneficial bioactive compounds for human beings. Orange and pomegranate juices are highly valued by consumers for their nutritional content and refreshing taste. Orange juice (OJ) is an important source of compounds of antioxidant relevance (carotenoids, phenols and vitamin C). The principal phenolic compounds of OJ are hydroxycinnamic acids and flavonoids (Stinco et al., 2015). Pomegranate juice (PJ) is rich in polyphenols and ascorbic acid. Pomegranate polyphenols include flavonoids (anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (Li et al., 2015).

Yeasts such as *Saccharomyces cerevisiae*, are the most important microorganism responsible for juice spoilage in the industry and produce severe economical losses (Marx, Moody, & Bermúdez-Aguirre, 2011). Thermal pasteurization is the most common method to prolong juices shelf-life. But, heat treatments may alter their organoleptic and nutritional properties (Soria & Villamiel, 2010). The demand of the consumers has increased the effort of juice industry to offer products that preserve their nutritional value through the development of technologies which cause the minimal damage on juice composition.

Among these emergent technologies, special attention has been paid to the application of ultrasound (UST) in beverages conservation (Chemat, Huma, & Khan, 2011; Jabbar et al. 2014; Soria & Villamiel, 2010; Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008; Valero et al., 2007). UST at frequencies from 20 to 100 kHz causes cavitation, resulting in a rapid creation, growth, and abrupt breakdown of bubbles, yielding localized extremely high temperatures (5,500 °C) and pressures (50 MPa). Cavitation erodes the cell wall with fine membrane fragmentation, produces disruption of sub-cellular particles, as well as structural and functional

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components up to cell lysis, causing inactivation of enzymes and microbial death (Guerrero, López-Malo, & Alzamora, 2001a; Guerrero, Tognon, & Alzamora, 2005; López-Malo, Palou, Jiménez-Fernández, Alzamora, & Guerrero, 2005b; Marx et al., 2011; Piyasena, Mohareb, & McKellar, 2003).

Essential oils (EOs) and their components have being proposed as natural preservatives (Tserennadmid et al., 2011; Unlu, Ergene, Unlu, Zeytinoglu, & Vural, 2010), and have been used in milk (Cava-Roda, Taboada, Palop, López-Gómez, & Marin-Iniesta, 2012a; Cava-Roda, Taboada-Rodríguez, Valverde-Franco, & Marín-Iniesta, 2012b), fruits (Lanciotti et al., 2004; Prakash, Singh, Mishra, & Dubey, 2012) and fruit juices (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009; Tserennadmid et al., 2011). *Cinnamomum zeylanicum* leaf EO (CLEO) is recognized as a safe additive and it is possible to use it or their main component (eugenol) to extend the shelf life of selected foods considering their GRAS status (Prakash et al., 2012) and their antimicrobial activity (Burt, 2004; Suhr & Nielsen, 2003).

UST and EOs have been assayed for their combined application (Gastélum, Avila-Sosa, López-Malo, & Palou, 2012; Guerrero et al., 2001a; López-Malo et al., 2005b), to obtain high-quality beverages with "fresh-like" characteristics and novel functionalities (Bevilacqua, Speranza, Campaniello, Sinigaglia, & Corbo, 2014).

The aim of this research was to study the inactivation effect of the combined application of UST, CLEO and mild heat treatment on *S. cerevisiae* in juices and its survival after treatments during 28 days of storage at 5 °C.

## 2. Materials and methods

### 2.1. Samples preparation

The oranges were harvested in a local orchard (Beniaján, Murcia, Spain). The pomegranates were purchased at local supermarket in Murcia (Spain). The fruits were kept at 5 °C for 1 day before juice extraction. Damaged fruits were discarded. Fruits were washed in cold tap water before drained. OJ was obtained by squeezing (Citro New 100 W, Solac) in aseptic conditions. To obtain PJ, the top and bottom of the pomegranate husks were removed with a disinfected sharp stainless steel knife to prevent microbial contamination. After, pomegranates were cut in halves and arils were hand-separated from the pith. Juice was immediately obtained of the arils with a blender (Ariete Centrika metal 173) in aseptic conditions. Both juices were pasteurized and stored at −20 °C in the dark until treatments.

### 2.2. Measurement of °Brix and pH

pH was measured using a digital pH meter (pH meter 507, Crison) and °Brix were measured using a refractometer (pocket PAL-1, Atago, Japan). Measurements were performed at 25 ± 0.5 °C. All measures were carried out in triplicate.

### 2.3. Cinnamon leaf essential oil (CLEO)

CLEO ($\rho = 1.0524$ g ml$^{-1}$; GC-FID: 74.32% eugenol, 2.98% benzyl benzoate, nominal values) was supplied by Destilerías Muñóz Gálvez, SA (Murcia, Spain). Following the procedure described by Ait-Ouazzou, Espina, García-Gonzalo, and Pagán (2013), a vigorous shaking method by stirrer (TQTECH, multipoint magnetic stirrer, Spain) agitation was used to prepare CLEO suspension in both juices, for sensorial assessments and thermo-UST treatments.

### 2.4. Sensory assessment

In order to select the maximum concentration of CLEO accepted by consumer, OJ and PJ samples with different concentrations of CLEO (0–0.65 mg/ml) were sensorially evaluated. The tests were performed in two different days. Prior to sensory evaluation samples were refrigerated, randomly coded and served (30 ml) at 15 °C together with bottled water without gas (Lanjarón, Spain). The juice samples with different concentrations of CLEO were presented to panelists ($n = 22$) who were asked to describe differences between samples by using a 10-point hedonic scale, where 1 means very much disliked and 10 very much liked. A score of 5 was the minimum threshold for samples acceptability (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009).

### 2.5. Microorganism and inocula preparation

The strain of *S. cerevisiae* was isolated and identified by Valverde, Marín-Iniesta, and Calvo (2010) and kept at −80 °C in Microbank™ vials (Pro-labo Diagnostics, Neston, Wirrall, UK). Every two months, one of the vials was opened and the stock culture was grown in Trypticase Soy Broth (TSB; Cultimed, Barcelona, Spain) for 24 h at 25 °C. After, it was streaked onto Standard Methods Agar (SMA; Cultimed, Panreac Barcelona, Spain) containing chloramphenicol (Ch) at 0.1 g l$^{-1}$ (Tournas, Stack, Mislivec, Koch, & Bandler, 1998). The *S. cerevisiae* inocula were prepared by transferring a colony obtained in SMA-Ch plates to TSB, which was incubated for 24 h at 25 °C before being stored at −20 °C in a solution of 40% TSB and 60% glycerol until use. The fresh cultures for the experiments were made by incubating one loopful of pure culture in TSB for 24 h at 25 °C. The inocula were standardized by dilution in TSB until an optical density (OD) of 0.1 at 600 nm (Nicolet Evolution 300, Thermo Electron Corporation VIS-UV spectrophotometer) was reached to obtain a yeast concentration of $10^7$ cfu ml$^{-1}$. The yeast populations were estimated by spreading suitable diluted aliquots onto SMA-Ch plates, followed by incubation at 25 °C for 48 h.

### 2.6. Thermo-UST treatments on both juices with CLEO

Thermal and ultrasonic treatments were carried out separately and in combination in a double-wall cylindrical vessel in which water was circulated with a thermostatic bath (Digiterm 100, PSelecta®) to fix temperatures close to 50 °C in the samples. A sterile thermometer was used to measure the temperature in the samples. UST (24 kHz; 105 μm; 33.31 W ml$^{-1}$; 30 min) was continuously applied with a UP200H ultrasonic processor (Hielscher Ultrasound Technology) using a S3 probe (Hielscher). The effect of the ultrasonic treatment at 50 °C (30 min) was also tested with 0.02 mg/ml of CLEO (maximum concentration accepted by panelists). For each treatment (Table 1), a population of yeasts (~$10^5$ cfu ml$^{-1}$) was inoculated to juice samples previously heated to the desired temperature (Gastélum et al., 2012). The viable cell counts were determined immediately after 0, 5, 10, 15, 20, 25 and 30 min of treatment using the pouring method on SMA-Ch. Two plates were used for each decimal dilution and were incubated at 25 °C for 48 h. All experiments were performed by triplicate.

D-values of *S. cerevisiae* in treated juices were calculated from the survival curve slopes obtained by plotting the log reduction $\log N_0/N$ (where $N_0$ is the initial microorganism concentration and N is the surviving microorganisms) as a function of time.