Innovative Food Science and Emerging Technologies 33 (2016) 416–421

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Apple juice preservation through microbial adsorption by nano/micro-tubular cellulose

Angelika-Ioanna Gialleli [a], Argyro Bekatorou [a], Maria Kanellaki [a], Poonam Nigam [b], Athanasios A. Koutinas [a,*]

[a] Food Biotechnology Group, Department of Chemistry, University of Patras, Patras 26500, Greece
[b] School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, Co., Londonderry BT52 1SA, United Kingdom

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 27 October 2015
Accepted 18 November 2015
Available online 24 November 2015

Keywords:
Apple juice
Tubular cellulose
Saccharomyces cerevisiae
Lactobacillus plantarum
Filtration
Preservation

ABSTRACT

A novel continuous preservation process of apple juice using a nano/micro-porous cellulosic material (or "tubular cellulose", abrrev. TC), is presented in this study. This investigation aims to the development of a non-thermal system for microbial stabilization, avoiding the degradation of food quality caused by heat. TC was used as filter in a packed-bed type bioreactor supplied with commercial apple juice contaminated with *Saccharomyces cerevisiae* or *Lactobacillus plantarum* cells at 4 °C. The effect of the filter size on the microbial load removal and chemical/sensory properties of the juice was evaluated. The system presented good operational stability during 55 and 30 days for the removal of yeast and bacteria, respectively. The increase of filter size improved the microbial removal yield and the system effectiveness. The organoleptic parameter values decreased after TC regeneration but then reached almost initial levels. The proposed process is a low-cost and promising alternative to existing thermal pasteurization technologies.

Industrial relevance: Conventional thermal pasteurization treatments are applied industrially for microbial stabilization of foods but significantly affect the organoleptic characteristics of the products. This study evaluated a novel non-thermal preservation method of contaminated apple juice that meets the consumer's demand for less processed products with high nutritional value. Commercial apple juice was contaminated with *S. cerevisiae* or *L. plantarum* cells and then was continuously pumped through tubular cellulose. The increased size of the filter leads to improved microbial removal yields. Despite the decrease of volatile compounds and colour at the start up of the process and after each filter regeneration with hot water, almost initial parameter values were achieved as the process evolved. The proposed technology can be a promising alternative of industrial pasteurization techniques for food applications.

1. Introduction

Preservation and prevention of spoilage have always been a major concern of food industries. According to official surveys, in 2010 the total food waste was 87 Mt. for the European Union and microbial spoilage was the main cause of food loss in the production line (Lin et al., 2013).

Apple juice is often spoiled by yeasts (*Saccharomyces*, *Zygosaccharomyces* and *Candida* spp.), various bacilli (*Alicyclobacillus* spp., *Lactobacillus plantarum*), and the heat resistant fungus *Byssochlamys fulva* (De Souza Sant'Ana, Dantigny, Tahara, Rosenthal, & De Massaguer, 2010; Bevilacqua, Campaniello, Speranza, Sinigaglia, & Corbo, 2013), which may lead to turbidity, off-flavours, and packaging distortion due to carbon dioxide production. At industrial scale, apple juice production involves mashing, thermal treatment, depectinization, filtration, pasteurization and packaging (Savatović, Tepić, Šumić, & Nikolić, 2009). Traditionally, apple juice spoilage is prevented by thermal processing. Industrial pasteurization of apple juice involves the HTST (High Temperature Short Time) treatment at 77–88 °C for 25–30 s (Rupasinghe & Yu, 2012). According to U.S. Food and Drug Administration, juice pasteurization is based on a 5-log reduction of the microorganisms that can cause spoilage and public health problems (Food and Drug Administration (FDA), 2001).

Despite thermal processing is effective to preserve fruit juices, the biochemical and nutritious changes that occur cannot be avoided. Nowadays, the increasing consumer demand for healthier, tastier and less processed foods has raised the industrial interest to innovative, non-thermal pasteurization techniques. Emerging technologies have to be able to deliver microbiologically stable products with elongated shelf-life and high sensory, nutritional characteristics. New non-thermal technologies such as pulsed electric fields (Charles-Rodríguez, Nevárez-Moorillón, Zhang, & Ortega-Rivas, 2007), ultraviolet light (Noci et al., 2008), high hydrostatic pressure (Valdramidis et al., 2009), supercritical CO_2 or N_2O (Gasperi et al., 2009), have been assessed for their effectiveness on microbiological stability of the final product as well as on their impact on sensory characteristics.

In addition, membrane filtration comprises an important non-thermal process that is implemented in apple juice for clarification, concentration, microbial stabilization and depectinization by removal of

* Corresponding author. Tel.: +30 2610 997104; fax: +30 2610 997105.
E-mail address: a.a.koutinas@upatras.gr (A.A. Koutinas).

http://dx.doi.org/10.1016/j.ifset.2015.11.006
1466-8564/

suspended solids (Girard & Fukumoto, 2000). Various materials such as polysulfones, polypropylene, polyamide, nylon or cellulose acetate have been employed in membrane configuration for dead-end or cross-flow filtration (Grandison, 2003; Fuenmayor, Lemma, Mannino, Mimmo, & Scampicchio, 2014). According to the pore size, membrane filtration can be classified as micro (0.2–2 μm), ultra (0.01 μm) or nanofiltration (0.5–1 nm) leading to improved product quality and reduced energy consumption (Salehi, 2014). The system performance depends on the chemical composition of the liquid, the temperature and the flow rate. However, membrane fouling and its cleaning methods are crucial factors for the wide industrial application of this technology (Van der Bruggen, Mänttäri, & Nyström, 2008).

The novel preservation method presented in this study is a promising alternative to ensure microbial stability in apple juice and maintain the sensory qualities as well. The system involves the use of a porous cellulosic material (tubular cellulose, abbrev. TC), containing nano/micro-pores and tubes produced after wood sawdust delignification. TC has been successfully used as solid carrier for cell immobilization in many wine making and brewing studies improving the fermentation rates and the quality of the products mainly regarding the formation of volatile congeners (Koutinas et al., 2012; Kourkoutas, Bekatorou, Marchant, Banat, & Koutinas, 2004). It is a low-cost, abundant material and in combination with the simple technology involved, the proposed pasteurization technique shows a good potential to encourage scale-up applications. Therefore, the aim of this investigation was to study the efficiency of TC material for apple juice preservation.

Fig. 1. Experimental set-up using a packed-bed bioreactor with tubular cellulose.

2. Materials and methods

2.1. Microbial strains and media

In this study, the alcohol-resistant and psychrotolerant yeast strain *Saccharomyces cerevisiae* AXAZ-1 (Argiriou et al., 1996) and the mesophilic lactic acid bacterium *Lactobacillus plantarum* DSM 20174 (DSMZ, Germany) were used. *S. cerevisiae* was grown in a synthetic medium containing (% *w*/*v*) 0.1 NH_4SO_4, 0.1 KH_2PO_4, 0.5 $MgSO_4$, 4 glucose, and 0.4 yeast extract. *L. plantarum* was cultivated at 30 °C in MRS broth (LabM, Lancashire, UK) (55 g/L). All media were autoclaved at 120 °C for 15 min prior to use. The cell biomass was harvested by centrifugation at 5000 rpm for 10 min. Commercial clarified apple juice (pH 3.7; total sugars 121 g/L; no preservatives) was used in the experiments, after being deliberately contaminated with 70×10^3 cfu/mL *S. cerevisiae* AXAZ-1 or *L. plantarum*.

2.2. Filter preparation

The TC material used as filter for the cold pasteurization process was prepared by delignification of pine sawdust (*Pinus strobus*). A quantity of 300 g of sawdust was treated with 3 L NaOH 1% for 3 h as described by Papafotopoulou-Patrinou et al. (2015). Tubular cellulose was tightly wrapped in a perforated nylon fabric to form a cylindrical filter, which was and then placed in a glass cylindrical bioreactor to form a well-packed column.

2.3. Cold pasteurization of apple juice

The system used consisted of a cylindrical glass bioreactor of 2 L (60 cm; 7 cm i.d.) packed with the TC filter, a high accuracy peristaltic pump (Masterflex L/S 7016, Cole-Parmer, USA) and a magnetic stirrer for the inflow (Fig. 1). The upstream flow rate used was 2 L/day and the contaminated apple juice was continuously pumped through the bioreactor at 4 °C. Two sizes of TC filter were used; 1348 cm^3 (F_1) and 1733 cm^3 (F_2). The microbial removal yield was evaluated by standard plate counting of the inlet and outlet liquid stream. When the microbial removal was reduced below 90%, regeneration of the filter using hot water (70–80 °C). Water was pumped downstream through TC using flow rate 200 mL/min and measuring the optical density of the outflow. When the absorbance at 700 nm for *S. cerevisiae* or 600 nm for *L. plantarum* was zero, the cold pasteurization process continued with contaminated apple juice.

2.4. Analytical methods

The polyphenols concentration was determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) as gallic acid equivalents. Malic acid was analysed on a Jasco LC-2000 Series HPLC system (Jasco Inc., Japan) equipped with a size-exclusion organic acid analysis column (Aminex HPX-87 H, 300 × 7.8 mm i.d., 9 μm particle size, Bio-rad, France) fitted in a CO-2060 PLUS column oven, a PU-2089 pump, a AS 2050 PLUS autosampler and a MD-2018 Photodiode array detector operated at 210 nm. Isocratic separation at 50 °C with 0.008 N H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min, was performed. The detector signals were recorded and analysed by ChromNav software. Aliquots of the samples were filtered through 0.2 μm nylon filters. For quantitative analysis, standard solutions of acid (Sigma-Aldrich Ltd) in pure water were prepared at various concentrations. Finally, samples of the TC filter were examined in a scanning electron microscope (SEM) model JSM-6300 (Jeol, USA). Samples were dried overnight at 40 °C and then were coated with gold in a Balzers Sputter Coater SCD 004 (Balzers, Switzerland) for 3 min before analysis.

2.5. Headspace SPME-GC/MS

The volatile constituents of the commercial apple juice before and after the cold pasteurization process were detected by gas chromatography/mass spectroscopy (GC/MS). The volatiles were isolated by the headspace solid-phase microextraction (SPME) technique. The fibre used was a 2 cm fibre coated with 50/30 mm divinylbenzene/carboxen on poly(dimethylsiloxane) bonded to a flexible fused silica core (Supelco, Bellefonte, PA, USA). The conditions of headspace SPME sampling were as follows: 10 mL of liquid sample, 3 g of NaCl, and internal standard (4-methyl-2-pentanol) were transferred into a 20-mL headspace vial fitted with a Teflon-lined septum sealed with an