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Lemon juice clarification using fungal pectinolytic enzymes coupled to membrane ultrafiltration

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A B S T R A C T

Lemon juice was treated with *Penicillium occitanis* pectinase at various enzyme concentrations (0–1200 U/L), temperatures (25–50 °C) and times (0–90 min). The effect of these enzymatic treatments on the viscosity of the juice was evaluated. The optimum treatment conditions were: enzyme concentration 600 U/L, time 45 min and temperature 30 °C. Their application led to a 77% and 47% reduction of viscosity and turbidity, respectively. The enzymatic treatment was followed by ultrafiltration (cutoff value = 15 kDa). Analysis of the clarified juice indicated that enzyme depectinization permitted a higher permeate flux and a higher juice quality. The lemon juice obtained was clear, stable and characterized by viscosity = 0.7 mPa s, turbidity = 0.17 NTU, clarity (A_{650nm}) = 0.063 and color (A_{420nm}) = 0.232. Microbiological study showed that lemon juice was free from aerobes, molds, enterobacteriaceae and coliforms and was microbiologically stable during 3 months storage. Results suggested that enzymatic treatment coupled to ultrafiltration could be used for production of lemon juice with high commercial value.

Keywords: Lemon juice; Pectinase-treatement; Ultrafiltration; Clarification; Penicillium occitanis

1. Introduction

Fruit and vegetable juices are of a high nutritional value since they are enriched with minerals, vitamins and other beneficial components for human health. Unfortunately, some juices have after their extraction a cloudy appearance often unwanted by consumers as is the case of lemon juice. Among the constituents involved in lemon juice opalescence (Klavons et al., 1991), is pectin (0.1%) (Robards et al., 1999). Pectin is a long chain of α-D-galacturonic acid linked by alpha 1-4 bonds. This polymer has a viscous appearance when in solution and high gelling power (Masmoudi et al., 2008). In order to minimize the turbidity of the juice while maintaining its organoleptic and sensory characteristics, a clarification step is necessary.

The use of membrane processes, such as microfiltration (MF) and ultrafiltration (UF) in the clarification of juices has lately gained importance over some conventional treatments including diatomaceous earth, paper filters, bentonite (Todisco et al., 1998; Wu et al., 1990; Jiao et al., 2004; Cassano et al., 2007). This is due to the fact that membrane processes have the advantage that separation occurs at room temperature and consequently without loss of aromatic volatile substances. In addition, the possibility of microbial contamination in the permeate stream is minimized (Tallarico et al., 1998). However, the major disadvantage of this process is membrane fouling during permeation caused by the retention of some components over the surface of the membrane, leading to a rapid decrease of flux (Cassano et al., 2003; Espamer et al., 2006). This problem can be overcome by an enzymatic treatment of the juice, in which the colloidal particles are first degraded before the step of ultrafiltration. This degradation is carried out by adding hemicellulases, phenol oxidase and in particular pectinases. They enable the reduction of the

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viscosity of the juice by depolymerization of insoluble pectin (Kilara and Van Buren, 1989). Commercial preparations of pectinases are often used and include several enzymes: pectin lyases, polygalacturonase and pectin esterase (Liew Abdullah et al., 2007; Belafi-Bako et al., 2007).

The depectinization of fruit juices through the use of pectinases was described as an efficient alternative to reduce turbidity in many studies (Kashyap et al., 2001; Landbo and Meyer, 2007; Vaillant et al., 1999). It depends on several physicochemical factors such as incubation time, enzyme concentration and incubation temperature (Lee et al., 2006; Liew Abdullah et al., 2007).

In the present work efficacy of a fungal pectinolytic enzyme has been analyzed for clarification of lemon juice. The pectinase-treated juice was then subject to UF for the production of lemon juice with high nutritional and organoleptic values.

2. Materials and methods

2.1. Materials

2.1.1. Fungal strain

Penicillium occitanis Pol6, provided by Professor Tiraby, Toulouse, France was used for pectinase production. This fungus was already characterized by the unusual secretion of high amount of pectinase (Jain et al., 1990).

2.1.2. Lemon juice

Lemon juice was obtained by squeezing fresh fruit (*Citrus limon*) supplied from a local market in Tunisia and filtering it with a 50-mesh sieve.

2.1.3. UF unit

Lemon juice was clarified using a laboratory pilot unit equipped with a cross-flow filtration system implementing tubular mineral CARBOSEP M2 membrane (stainless 31b membrane module, Tech Sep, Miribel, France) with a surface area of 75 mm^2 and a nominal MWCO of 15 kDa. The UF equipment consisted of a feed tank, feed pressure pump, a feed flow meter, a thermometer and two manometers for the measure of the inlet and outlet pressures. Transmembrane pressure (TMP) was controlled by the valve on the retentate side. The retentate and permeate were continuously recycled to the feed tank to ensure a steady state in the volume and composition on the feed (Neifar et al., 2009).

2.2. Methods

2.2.1. Pectinase production

Mandels and Weber's medium modified by Ellouz Chaabouni et al. (1995) was used for the pectinase production with 20 (g/L) of different carbon sources: orange peel, citrus pectin, sunflower seeds, barley bran and wheat bran. The cultures were incubated at 30 °C on a rotary shaker (150 rpm) for 5 days. The culture broths were clarified by centrifugation at 8000 × *g* for 15 min then filtered to remove the mycelia. The resulting clear filtrates were used as crude enzyme extracts.

2.2.2. Enzyme assays

Pectinase activity was determined by measuring the release of reducing groups using the dinitrosalicylic acid reagent DNS assay (Miller, 1959). The reaction mixture consisted of 0.5 mL of 1% citrus pectin (w/w), 0.4 mL of 0.1 M citrate–phosphate buffer (pH, 6.0) and 0.1 mL of crude enzyme extract. The reaction mixture was incubated at 60 °C for 10 min. One unit of enzymatic activity (U) was defined as 1 μmol of galacturonic acid released per minute.

2.2.3. Effect of pH and temperature on pectinase activity

The optimum pH of pectinase was studied over a pH range of 2.0–9.0. To determine the pH stability, enzyme was kept at 4 °C in different buffers (100 mM) for 24 h and the residual pectinase activity was determined under assay conditions. The buffer systems used were glycine–HCl buffer for pH 2.0; citrate–phosphate buffer for pH 3.0–7.0 and Tris–HCl buffer for pH 8.0–9.0. To analyze the effect of temperature, the activity was tested at different temperatures (20–70 °C). Thermal stability was determined after different pre-incubation times (0–90 min, at 25–50 °C).

2.2.4. Enzymatic treatment

For each of the enzymatic treatments, aliquots of juice were distributed into glass containers. The lemon juice was treated with pectinase at various enzyme concentrations (0–1200 U/L), temperatures (25–50 °C) and times (0–90 min). In order to remove most of the pectin–protein flocs formed during the enzyme treatment, a prefiltration using a metallic filter with 100 μm pore size was carried out before ultrafiltration.

2.2.5. UF treatment

Lemon juice was circulated through the membrane unit using a peristaltic pump. Experimental conditions were the following: feed temperature $T = 20$ °C, feed flow $Q = 0.9$ L/min and transmembrane pressure TMP = 0.3 MPa. The membrane module was rinsed out with distilled water after the treatment of the juices then it was cleaned with a NaOH solution (5–10 g/L; temperature = 80–85 °C; operating time = 30 min; TMP = 0.2–0.3 MPa). The system was rinsed with distilled water then cleaned by recycling 3–5 mL/L HNO_3 solution at 55–60 °C for 30 min at a TMP of 0.2–0.3 MPa. A final rinse to neutrality of the system with distilled water was carried out. After each cleaning procedure, the water flux of the membrane module was measured (Neifar et al., 2009).

2.2.6. Physico-chemical analysis

Dry matter, protein and ash were determined according to the AOAC (1990) methods. Total phenolics were determined with Folin–Ciocalteau reagent and referred to as mg/L of gallic acid (Spanos and Wrolstad, 1992). Total sugar content was measured after acid hydrolysis at 100 °C (AFNOR, 1970). The turbidity of the juice was determined using a portable turbidimeter (Model Turb 555 IR) and results were reported in nephelometric turbidity units (NTU) (Sin et al., 2006). The viscosity of juice was followed at 25 °C with a Stress Tech Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden) conducted with a steel cone-plate (C40/4) under a constant shear rate of 100 s^{-1}. The unit of measurement used for viscosity is mPa s. Clarity (as absorbance at 650 nm) and color (as absorbance at 420 nm) values were measured, after 10-fold dilution with water, by using a Shimadzu UV–vis scanning spectrophotometer (UV-mini-1240; Shimadzu, Kyoto, Japan) (Liew Abdullah et al., 2007). Pectin extraction was done as described by Thibault and Petit (1979). For pH determination a pH meter (Waterproof Combo, Hanna) was used.