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Influence of moderate electric field on inactivation kinetics of peroxidase and polyphenol oxidase and on phenolic compounds of sugarcane juice treated by ohmic heating

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ABSTRACT

This study investigated the non-thermal effects of electricity on enzymatic inactivation kinetics of peroxidase and polyphenol oxidase and on degradation of total phenolic and total flavonoid compounds during ohmic heating of sugarcane juice. A kinetic modeling was carried out from the adjustment of various models applicable to enzyme inactivation, and the Weibull distribution model was the most suitable to describe the inactivation of peroxidase. The presence of an electric field with low intensity significantly influenced the biochemical reactions occurred during peroxidase activation at 60 °C and inactivation at 80 °C. Polyphenol oxidase, a more thermolabile enzyme, was almost totally inactivated before reaching the target temperature (holding phase) of the experiments. The maximum total phenolic and total flavonoid degradation were approximately 23 and 39%, respectively. Statistical analyses showed no differences between conventional and ohmic heating for total phenolic and total flavonoid compounds.

1. Introduction

Sugarcane (*Saccharum officinarum* L) is an important crop of the Brazilian economy, and the country is the world's largest producer of this vegetable, holding the global leadership in ethanol and sugar production technology. By-products and residues (such as bagasse, the filter cake, molasses and vinasse, among others) are also used for power cogeneration, animal feed manufacturing and fertilizer for crops (CONAB, 2015).

The juice, obtained through sugarcane grinding, is popular in many countries and is a sweet and cheap drink; however it is not industrialized and its selling takes place predominantly in the informal market, which makes difficult the access to statistics about its consumption (Kunitake, 2012). According to Oliveira, Spoto, Canniatti-Brazaca, Sousa, and Gallo (2007), the juice is characterized as an energy drink, non-alcoholic, with sweet and pleasant taste, low acidity, opaque, with color ranging from dun to dark green. Regarding its nutritional composition, it contains vitamins (especially B_6 and C), minerals (Ca, Fe, P, Mg, Mn, K, and Zn) (TACO, 2011) and phenolic compounds, mainly phenolic acids and flavonoids (Payet, Shum Cheong Sing, & Smadja, 2006). These compounds have considerable physiological and morphological features in plants. Moreover, polyphenols exhibit many biological properties, such as anti-inflammatory, antiallergic, antibacterial, antimicrobial, antioxidant and cardioprotective (Havsteen, 2002). The composition may vary depending on the variety, age and health of the cane, soil characteristics, weather conditions and agricultural practices.

Sugarcane juice processing and marketing are limited due to its rapid deterioration (Yusof, Shian, & Osman, 2000), since the juice is considered a good substrate for the development of a microbial broad spectrum. Moreover, it contains organic and inorganic nutrients and has high water activity and pH ranging from 5.0 to 5.5 (Gallo & Canhos, 1991). After 24 h of extraction, even if stored under refrigeration, the fresh juice shows sedimentation and changes in

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their sensory characteristics (flavor and appearance deterioration), mainly due to fermentation and enzymatic browning, which significantly contributes to its color changes (Qudsieh, Yusof, Osman, & Rahman, 2002; Yusof et al., 2000).

The development of effective treatments and procedures to maintain the quality of sugarcane juice would allow a broader market and increase their quality and safety (Mao, Xu, & Que, 2007). Ohmic heating (OH) is an emerging technology in the food industry, which consists of passing an alternating electric current (a moderate electric field) through a food with the main purpose of warming it by internal power generation. Due to its inherent electrical resistance, food transforms electrical energy into thermal, thereby promoting a temperature rise inside (Resnick, 1996). This technology is preferable over conventional processes, as it allows nutritional and sensory quality maintenance and makes possible greater efficiency and speed of heating (Castro, Macedo, Teixeira, & Vicente, 2004; Wang & Sastry, 2002).

The activity of naturally occurring enzymes in sugarcane juice, such as polyphenol oxidase (PPO) and peroxidase (POD), promotes undesirable changes in color, texture, flavor, aroma and nutritional composition. POD (EC 1.11.1.7) is an enzyme naturally present in vegetables with high thermal resistance and is, therefore, used for determining patterns of pasteurization parameters (Jakób et al., 2010). PPO (EC 1.14.18.1) is an oxidoreductase enzyme which, in the presence of oxygen, catalyses the oxidation of *o*-phenolic to *o*-quinones substrates, which are subsequently polymerized to dark-colored pigments (Icier, Yildiz, & Baysal, 2008).

Enzymes are charged molecules and thus respond to external electric fields. At the present time, information concerning to the non-thermal effects of moderate electric field (MEF) treatments on food proteins and enzymes is scarce (Samaranayake & Sastry, 2016b). Castro et al. (2004) reported that lipoxygenase and polyphenol oxidase kinetics in buffer solutions were significantly affected by the electric field, reducing the time needed for inactivation, while alkaline phosphatase, pectinase, and β-galactosidase inactivation were not affected by the electric field.

Enzymes inactivation is desirable in most preservation processes, and hence their inactivation kinetics is of great interest (Corradini & Peleg, 2004). Considering the possibility of the presence of isozymes at the beginning of the inactivation process, the kinetic models used in the literature are based on different mechanisms: first order, consecutive or parallel reactions (Table 1) (Chen & Wu, 1998; Rudra Shalini, Shivhare, & Basu, 2008; Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1998).

The aim of this study was to evaluate the influence of MEF on phenolic compounds degradation and POD and PPO enzymes inactivation of sugarcane juice treated by ohmic heating. An additional objective was to establish the best kinetic model of enzymatic inactivation for both enzymes at temperatures ranging from 70 to 80 °C.

2. Materials and methods

2.1. Samples

The sugarcane juice was donated by a rural property located in Veranópolis (Rio Grande do Sul State/Brazil). The juice was homogenized and frozen in plastic bags in individual volumes of 300 mL. Samples were thawed in a water bath immediately prior to use.

The fresh juice was characterized according to its pH (Digimed, MD-22 model), soluble solids (refractometer portable SZJ-A model), electrical conductivity (Digimed, MD-3P model), water activity (measured in an electric hygrometer at 25 °C; Novasina, Labmaster-aw model), titratable acidity index (method 016/IAL, 2008), POD and PPO enzymatic activities, total phenolic and total flavonoid contents. All analyzes were performed in triplicate.

2.2. Ohmic and conventional heating processes

The ohmic heating apparatus used to conduct the experiments is described elsewhere (Mercali, Schwartz, Marczak, Tessaro, & Sastry, 2014a). The ohmic cell consisted of a 500 mL jacketed glass vessel. The electrodes were made of titanium and were curved to conform to the reactor dimensions. The maximum inter-electrode gap was 7.5 cm and the minimum gap was 5.7 cm. Electrode height was 5 cm and reactor height was 9.9 cm.

Kinetic assays were conducted at temperatures of 60, 70, 75 and 80 °C using 25 V applied to the electrodes (electric field intensity varying from 3.57 to 4.39 V/cm because of the curvature of the electrode) and hot water through the jacket of the cell during 12 min. Two heating baths were used: one set at 98 °C to promptly increase the temperature up to the temperature of the study, and another set 1 K below the target temperature used during the isothermal phase. The electric field was turned on at time zero, the time at which the sample reached the working temperature. For conventional treatment, the same procedure was followed without the application of the electric field and with the second heating bath set at the target temperature. This was necessary to match the temperature profiles of the conventional and ohmic heating, enabling the assessment of the non-thermal effects of electricity during the heat treatment. Fig. 1 shows temperature over time for conventional and ohmic heating experiments, indicating identical profiles. As can be seen, the come-up period was approximately 2.0, 3.0, 3.5 and 4.0 min for 60, 70, 75 and 80 °C, respectively.

The experiments were carried out with 250 g of sugarcane juice. The juice was agitated on a magnetic stirrer device (IKA, model C-MAG HS10, Germany) at the rate of 750 rpm. Samples were collected in seven heating times (0, 2, 4, 6, 8, 10 and 12 min), immediately placed on an ice bath to stop the effect of heating, and subsequently analyzed for POD and PPO enzymatic activity. All experiments were performed in duplicate.

Table 1
Kinetic equations used to predict enzymatic inactivation.

Model	Equation[a]
First-order	$\ln(A/A_0) = -k.t$
Distinct isozymes	$A/A_0 = A_L.\exp(-k_L t) + A_R.\exp(-k_R.t)$
Two-fraction	$A/A_0 = a.\exp(-k_L.t) + (1-a).\exp(-k_R.t)$
Multicomponent first-order	$A/A_0 = \frac{[\exp(-k_1 \cdot t)+r \cdot \exp(-k_2 \cdot t)]}{(1+r)}$
Fractional conversion	$A/A_0 = A_r+(A_0 - A_r).\exp(-k.t)$
Weibull distribution	$A/A_0 = \exp(-b.t^n)$
*n*th order	$A/A_0 = [A_0^{1-n} + (n-1).k.t]^{1/(n-1)}$
Series	$A/A_0 = \alpha_2 + [1 + (\alpha_1 k_1/k_2 - k_1) - (\alpha_2 k_2/k_2 - k_1)]\exp(-k_1.t) - [(\alpha_1 k_1/k_2\text{-}k_1) - (\alpha_2 k_1/k_2 - k_1)] \exp(-k_2.t)$

[a] *A* represents enzyme activity at time *t*; A_0 is the initial enzyme activity; *k* is the reaction rate constant at a given temperature (s^{-1}).