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Short communication

A kinetic study on pectinesterase inactivation during continuous pasteurization of orange juice

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Abstract

Studying pectin esterase inactivation behavior is important because it is responsible for the juice cloud stability loss, is composed of several isoenzymes and occurs naturally in orange. Freshly squeezed juice of 'pera' orange (*Citrus Sinensis (L) Osbeck)* was pasteurized at temperatures of 82.5 °C, 85.0 °C and 87.5 °C using ARMFIELD laboratory plate heat exchanger. At least five runs with different holding times were conducted for each temperature. As the obtained isothermal curves showed deviation from the expected first-order kinetics, data was statistically treated applying a non-linear regression and the estimated best fit was a three-parameter-multicomponent-first-order model. At 82.5 °C, the isothermal curves showed a non-zero asymptote of inactivation indicating that at this temperature the most heat resistant isoenzyme could not be totally inactivated. The 87.5 °C isotherm showed the highest inactivation among the temperatures studied. These facts agree with the batch inactivation data found in literature, but the holding time required for a satisfactory inactivation was significantly smaller than the found in literature, suggesting that the proposed model can be used to design continuous process with more accuracy.

Keywords: Orange juice; Pectinesterase; Heat inactivation kinetics; Pasteurization; Isoenzymes

1. Introduction

Pectinesterase or pectin methyl esterase (PME) is the enzyme responsible for the hydrolysis of the pectin present in citrus fruit juices that causes loss of fresh juice cloudiness and gelation of pectin in concentrate juice (Basak & Ramaswamy, 1996). It occurs naturally in orange and is composed of several isoenzymes. Versteeg, Rombouts, Spaansen, and Pilnik (1980) described the existence of three isoenzymes in orange, one of them having greater molecular weight and thermal stability. Snir, Koehler, Sims, and Wicker (1996) reported that two of them are located in the albedo and the third one is in the flavedo. They also showed that the most heat resistant isoenzyme is located in the albedo and juice sacs membrane, therefore it would be impossible to avoid its presence in the fresh juice.

Cameron, Baker, and Grohmann (1998) isolated four isoenzymes in *valentia* orange and studied the effects of each one in juice cloud stability, concluding that the most heat resistant form, although being only 7.9% of the total enzyme, had the major influence on the juice cloud stability loss at storage conditions (5–10 °C). They also reported that these heat resistant isoenzymes were located in the albedo and the juice sacs membrane.

As PME is more thermal resistant than the pathogenic microorganisms that can be present in orange juice and is responsible for the cloud stability loss, its inactivation is commonly used as an indicator of the pasteurization process adequacy (Basak & Ramaswamy, 1996);

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therefore, it is important to have accurate PME heat inactivation kinetic parameters to design the process. In general, PME heat inactivation is considered to follow first-order kinetics. Ülgen and Özilgen (1991), Versteeg et al. (1980) and Kim, Tadini, and Singh (1999) used this approach to study the PME heat inactivation kinetics. The heat exposition times in these studies were all greater than 30 s (most of them in minutes), for this range of holding time the more heat labile isoenzymes are already inactivated, so for smaller holding times this approach could lead to an overestimated design. Chen and Wu (1998) in their critical review stated that first order model does not give the best fit for historical data studied and proposed a two component first order model as an alternative. They also state that the use of the first order model constants could result in underestimated or super estimated processing of orange juice.

Fujikawa and Itoh (1996) presented heat inactivation kinetics model for microorganisms and enzymes with three parameters that takes into account the existence of more than one microorganism and/or enzyme with different heat stability. Polakovic and Vrábel (1996) described several models for enzyme heat inactivation kinetics, ranging from two to five parameters with the necessary methodology to decide which model should be adopted, using selected experiments from the literature, but none of them was PME. Among these models presented by Polakovic and Vrábel (1996) was the three-parameter model presented by Fujikawa and Itoh (1996).

The aim of this research was to study the kinetics of pectinesterase inactivation during continuous pasteurization of single strength orange juice. This is part of a research project to obtain a minimally processed orange juice preserving the fresh juice quality attributes.

2. Material and methods

2.1. Orange juice pasteurization

Orange (*Citrus sinensis (L.) Osbeck)* was purchased at the local market, selected and washed, then it was squeezed in a Fresh'n Squeeze® Multi-Fruit Juicer (FMC Food Tech. Citrus Systems, Lakeland, FL).

Pasteurization at different temperatures and holding times was accomplished using an ARMFIELD FT43A pasteurization unit, composed of a plate heat exchanger, heating and cooling systems and a data logger. The chosen pasteurization temperatures were 82.5 °C, 85.0 °C and 87.5 °C. For each temperature the following holding times were chosen: 11.1, 12.3, 13.9, 15.8, 18.4, 35.1, 43.9, 50.1 and 58.5 s. The setting of these holding times was allowed by the previous calibration of the product pump speed dial and the use of two different holding tubes.

Fresh orange juice was pumped through the system until steady state was established for the chosen condition. The pasteurized orange juice samples were collected at the cooling section outlet and immediately frozen, in order to preserve any residual enzyme activity. Samples of unprocessed orange juice were also collected and frozen to measure the native enzyme activity.

2.2. Analytical measurements

In order to characterize the fresh and pasteurized orange juice produced and to detect possible changes caused by the pasteurization, the following analytical measurements were performed:

- pH was measured directly using a pH-STAT (model PHM290 with autoburette ABU901, Radiometer, France).
- Soluble solids content was determined by a refractometer (model 711849, Carlzeiss Jena, Germany) and corrected with acidity and temperature values according to Kimball (1991).
- Citric acid was determined according to the AOAC (1995) method, conducted in the pH-STAT (model PHM290 with autoburette ABU901, Radiometer, France).
- PME activity was determined according to Rouse and Atkins (1955), using citrus pectin (Sigma P9436) as substrate in the pH-STAT mentioned, for fresh and pasteurized juice.

One unit of enzyme activity was defined as the amount of enzyme which liberates 1 μ equivalent of acid from pectin per minute at pH 7.50 and 30 °C. The unit was PMEU/ml °Brix and the results were reported in the normalized form: PMEU/$PMEU_O$, where $PMEU_O$ is the fresh juice enzyme activity and PMEU is the pasteurized juice enzyme activity.

All statistical and mathematical treatment was done using Statgraphics Plus v. 4.0 for Windows program (Manugistics, 1999).

3. Results and discussion

Unprocessed orange juice presented pectinesterase activity $6.5 \times 10^{-4} \pm 3.3 \times 10^{-4}$ PMEU/ml °Brix, soluble solids content 11.4 ± 0.7% and the pH ranged from 3.5 to 4.4. There was no significant difference between unprocessed and processed orange juice pH, the same occurred to soluble solids and acidity.

Multifactor ANOVA was applied to the analytical of processed orange juice measurements results (Table 1) indicating that the enzyme inactivation was influenced