

Correlation of non-linear model parameters and pH on pectinesterase thermal inactivation in minimally pasteurized orange juice.

Tadini C. C.(1), Tribess T.B.(2), Silva Jr P.A.(3)

(1) São Paulo University, Escola Politécnica, Chemical Eng. Dept., Food Eng. Laboratory,
P.O.Box 61548, Zip Code 05424-970, São Paulo, SP, Brazil

catadini@usp.br

(2) São Paulo University, Escola Politécnica, Chemical Eng. Dept., Food Eng. Laboratory,
P.O.Box 61548, Zip Code 05424-970, São Paulo, SP, Brazil

tatiana.tribess@poli.usp.br

(3) São Paulo University, Escola Politécnica, Chemical Eng. Dept., Food Eng. Laboratory,
P.O.Box 61548, Zip Code 05424-970, São Paulo, SP, Brazil

paulo.andrade@poli.usp.br

Abstract

A three parameter model can be used to describe pectinesterase inactivation kinetics. Thermal inactivation kinetics was obtained for six pH values, at three temperatures and at least six holding times for each condition. This study showed that PE isoforms fraction (a) and velocity constant (k_1) were influenced by pH and processing temperature.

Key words: pectinesterase, orange juice, pasteurization.

Introduction

Orange juice is an important product for Brazilian agriculture and economy. Brazil is responsible for almost 90% of the oranges produced in South America, which corresponds to 34% of the worldwide production of this fruit. Brazil is the greatest orange juice producer in the world [1].

Fresh orange juice has a good acceptance in the market. However, since its shelf-life is of a few days (one or two days) when stored at 8 to 10°C, its potential for commercialization is limited [2]. Minimally processed orange juice, stored in aluminum cans during 57 days and in polyethylene bottles during 21 days under refrigeration, was microbiologically analyzed. The total plate count and yeast and mold count of minimally processed orange juice were significantly lower than unprocessed orange juice after 21 days under refrigeration, a reduction of 4.35×10^6 to 1.2×10^3 and 2×10^3 to 500 CFU/mL, respectively [3].

According to Badolato (2000) [4], the thermal processing of orange juice at a minimum temperature and time provides a product of better acceptance to the consumer if compared to the commercial pasteurized juices. The sensorial attributes of the minimally processed orange juice presented a small difference when compared to unprocessed orange juice.

Pectinesterase or pectin methyl esterase (PME) is the enzyme responsible for catalysing the hydrolysis of pectin present in citrus fruit juices into pectic acid and methanol. This implies a loss of fresh juice cloudiness and gelation of pectin in concentrate juice [5]. It occurs naturally in orange and is composed of several isoenzymes. Versteeg *et al.* (1980) [6] described the existence of three isoenzymes in orange, one of them having greater molecular weight and thermal stability. Snir *et al.* (1996) [7] 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 *et al.* (1998) [8] 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 representing only 7.9 % of the total enzyme, had the major influence on the juice cloud stability loss at storage conditions (5°C – 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 [5]; 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) [9], Versteeg *et al.* (1980) [6] and Kim *et al.* (1999) [10] used this approach to study the PME heat inactivation kinetics. Nevertheless, the heat exposition times in these studies were all greater than 30 s (most of them in minutes) when the more heat liable isoenzymes are already inactivated, however, for smaller holding times this approach could input error to an adequate design. Recently Collet *et al.* (2001) [11] reported that the heat inactivation of pectinesterase during continuous pasteurization of orange juice did not follow first order kinetics and the pH of natural orange juice influenced the thermal inactivation of PE.

Fujikawa and Itoh (1996) [12] presented an enzyme heat inactivation kinetics model with three parameters that takes into account the existence of more than one isoenzyme with different heat stability. Polakovick and Vrabel (1996) [13] 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. Among these, the model presented by Fujikawa and Itoh (1996) [12], is figured.

The aim of this research was to study the kinetics of pectinesterase inactivation during continuous minimal pasteurization of single strength orange juice from blends with controlled pH. According to pH, a multicomponent model was used to fit the obtained residual pectinesterase activity data. The values of the model parameters were fitted to obtain a correlation according to pH and processing temperature.

Materials and methods

Orange juice pasteurization

The juice was squeezed from two orange varieties, *Citrus aurantium L.* and *Citrus aurantifolia*, whose pH values were respectively 3.6 - 4.1 and 5.1 - 5.5. The proportion of the two varieties was chosen in order to meet pre-established pH values (3.6, 3.7, 3.8, 3.9, 4.0 and 4.1). These pH values were set by observation of São Paulo orange pH variation in the 1999/2000 harvest. The fruits used in this study were purchased from March to December 2002.

Pasteurization was accomplished using an ARMFIELD FT43A pasteurization unit, composed of a plate heat exchanger, heating and cooling systems and a data logger as illustrated in Figure 1. The heat treatments were done using three different temperatures (82.5°C, 85.0°C and 87.5 °C), in at least six holding times (151 different treatments).

Fresh orange juice was pumped through the system until steady state was established for the condition chosen. 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.

Analytical measurements

- pH was measured using a pH-STAT (model PHM290 with autoburette ABU901, Radiometer, France).
- Soluble solids were determined by a refractometer (CARLZEISS JENA, mod. 711849, Germany) and corrected with acidity and temperature values according to Kimball, 1991[14].
- Citric acid was determined according to the AOAC method [15], conducted in the pH-STAT (model PHM290 with autoburette ABU901, Radiometer, France).
- PME activity was determined according to Rouse & Atkins (1955) [16], 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/PMEU₀, where PMEU₀ is the fresh juice enzyme activity and PMEU is the pasteurized juice enzyme activity.

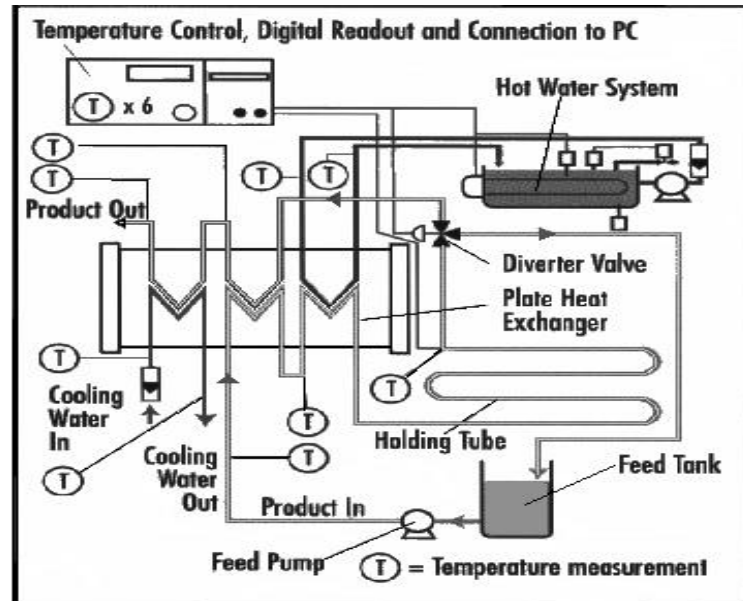


Figure 1: Flow Diagram of Orange Juice Pasteurization Unit.

All statistical and mathematical treatment was done using Statgraphics Plus v. 4.0 for Windows program [17].

Results and discussion

The processed orange juice from oranges harvested from March to December 2002, presented total acidity from 0.38 to 1.02 % as citric acid and soluble solids from 8.60 to 13.68 °Brix. The native PE activity of orange juice varied from 2.031×10^{-4} to 4.276×10^{-4} PEU/g °Brix, similar to the values found for *valentia* and *pineapple* varieties by Rouse and Atkins(1953) [18] (from 2×10^{-4} to 4×10^{-4} PEU/g Brix) and by Badolato (2000) [4] for *pera* variety (from 4.3×10^{-4} to 2.5×10^{-3} PEU/mL °Brix).

The obtained data were fitted in a model chosen presented by Fujikawa & Itoh (1996)[12], that takes into account the presence of several isoenzymes and is as follows:

Considering as in the case of orange juice, a sample of enzymes that consists of 2 components which are thermally inactivated independently following first order kinetics under certain conditions, and arranging each component according to the magnitude of the inactivation rate constant, denote G_1 , G_2 the first and second components and k_1 , k_2 the rate constant of G_1 and G_2 , respectively; where $k_1 > k_2 > 0$. Let A_{0i} and A_i be the enzyme activity for G_i ($i=1,2$) per unit of volume at times zero and t respectively.

By definition we have:

$$A_i = A_{0i} \times \exp(-k_i \times t) \quad (2)$$

$$A = A_1 + A_2 \quad (3)$$

$$A_0 = A_{01} + A_{02} \quad (4)$$

Dividing (3) by (4) and substituting (2) we have:

$$\frac{A}{A_0} = \frac{A_{01} \times \exp(-k_1 \times t) + A_{02} \times \exp(-k_2 \times t)}{A_{01} + A_{02}} \quad (5)$$

Making:

$$\frac{A_{0_1}}{A_{0_1} + A_{0_2}} = a \quad (6)$$

We have:

$$\frac{A}{A_0} = a \times \exp(-k_1 \times t) + (1 - a) \times \exp(-k_2 \times t) \quad (7)$$

where a is the activity fraction of enzyme 1 in relation to total enzyme activity

The criterion used to select the most appropriated model is described by Polakovic & Vrabel (1996) [13] and is based on the premise that the simplest model providing reasonably accurate fit should be considered the best. The obtained data were well fitted, with all R^2 values above 0.987. The results of the non-linear regression for the chosen model are listed on Table 1.

Table 1: Model parameters obtained for kinetics inactivation of PE of orange juice at pHs 3.6, 3.7, 3.8, 3.9, 4.0 and 4.1, processed at 82.5°C, 85.0°C and 87.5 °C.

pH	Temperature (°C)	Model parameters ¹			
		a^2	k_1^3	k_2^4	R^2
3.6	82.5	0.985	0.501	0.006	1.000
	85.0	0.999	0.472	0.017	1.000
	87.5	0.996	0.560	0.002	1.000
3.7	82.5	0.944	0.182	0.010	0.987
	85.0	0.999	0.681	0.001	1.000
	87.5	0.999	0.711	0.001	1.000
3.8	82.5	0.904	0.414	0.002	0.998
	85.0	0.970	0.310	0.003	0.998
	87.5	0.977	0.343	0.028	1.000
3.9	82.5	0.957	0.238	0.001	0.999
	85.0	0.977	0.392	0.011	1.000
	87.5	0.996	0.455	0.007	1.000
4.0	82.5	0.958	0.452	0.011	1.000
	85.0	0.987	0.330	0.005	1.000
	87.5	0.996	0.537	0.011	1.000
4.1	82.5	0.947	0.471	0.018	1.000
	85.0	0.981	0.358	0.009	1.000
	87.5	0.998	0.740	0.009	1.000

¹ model: $\frac{A}{A_0} = a \times \exp(-k_1 \times t) + (1 - a) \times \exp(-k_2 \times t)$

² a : activity fraction of isoenzyme 1 in relation to total enzyme inactivation.

³ k_1 is the reaction rate constant (s^{-1}) of isoenzyme 1.

⁴ k_2 is the reaction rate constant (s^{-1}) of isoenzyme 2.

It has been observed that at pH 3.6 a greater inactivation was obtained at 85.0 °C than at 87.5 °C. While at pH 3.7 an increase in process temperature from 85.0 °C to 87.5 °C did not provide additional inactivation.

The highest inactivation rates were achieved at pH 3.6 and 85.0 °C process temperature and at pH 3.7 with 85.0 °C and 87.5 °C. In all cases 99.9% inactivation was reached in less than 15 seconds of holding time.

Multifactor ANOVA ($p < 0.05$) was applied to verify the influence of pH and pasteurization temperature on PE thermal inactivation parameters and the results are presented on Tables 2 and 3. The parameters a and k_1 were significantly influenced by pH, while only a was affected by pasteurizing temperature. Although the ANOVA did not indicate a significant difference, there was a tendency of increasing k_1 with increasing temperature, as expected.

Table 2: PE thermal inactivation parameters in relation to orange juice pH.

pH	3,6	3,7	3,8	3,9	4,0	4,1	Tukey HSD 5%
a	0,994 ^a	0,981 ^{a,b}	0,950 ^b	0,977 ^{a,b}	0,980 ^{a,b}	0,975 ^{a,b}	0,035
k_1	0,511 ^{a,b}	0,684 ^b	0,356 ^a	0,362 ^a	0,440 ^{a,b}	0,523 ^{a,b}	0,266
k_2	0,0082 ^a	0,0036 ^a	0,0012 ^a	0,0062 ^a	0,0090 ^a	0,0092 ^a	0,0180

Averages with same letter, in the same row, are not significantly different at a 95% confidence interval

Table 3: PE thermal inactivation parameters in relation to pasteurization temperature.

Temperatura (°C)	82,5	85,0	87,5	Tukey HSD 5%
a	0,949 ^a	0,986 ^b	0,994 ^b	0,019
k_1	0,456 ^a	0,424 ^a	0,558 ^a	0,155
k_2	0,0079 ^a	0,0075 ^a	0,0033 ^a	0,0099

Averages with same letter, in the same row, are not significantly different at a 95% confidence interval

From the kinetics data it is possible to observe an inflexion point of PE inactivation at pH 3.8, with the minimum inactivation. Eagerman and Rouse (1976) [19] Körner *et al.* (1980) [20] and Badolato (2000) [4] also cited pH as an influential parameter on PE inactivation (smallest value of a). It means that the thermo resistant isoenzyme fraction at this pH was the greatest found (average 5.0%). At this pH, the velocity constant k_1 was also smaller than at other studied pHs and corresponds to the slower inactivation of thermo labile isoenzyme fraction. The velocity constant k_1 values varied from 0.182 to 0.740 s⁻¹ and the velocity constant k_2 varied from 0.001 to 0.028 s⁻¹. The ANOVA shows that pasteurization temperature and pH did not significantly influence ($p < 0.05$) the velocity constant k_2 .

As can be observed in Table 2 thermo resistant and thermo labile isoforms can be obtained with a and $a - 1$ values, respectively. The values of a varied from 0.904 to 0.999, this represents the thermo labile fraction and corresponds to the presence of almost 9.6% of the thermo resistant isoforms, in the studied interval. Previous studies presented similar values, Han; Nielsen; Nelson (1998) [21] reported that thermo resistant PE isoenzyme fraction was 1.5% and Versteeg *et al.* (1980) [6] and Cameron; Niedz; Grohmann (1994) [8] found 5% of thermo resistant PE isoforms in OJ.

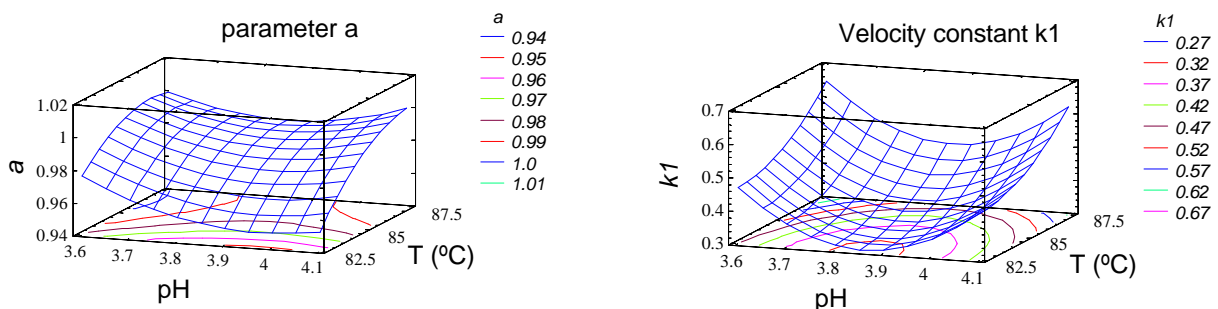


Figure 2: Surface responses of parameters a and k_1 according to process temperature and pH

In Figure 2, it is possible to observe distinct behaviors in the variation of parameter a with pH at extreme temperatures. For each pH studied, the parameter was also influenced by process temperature, with an increase of thermolabile isoenzyme fraction with temperature increase. The velocity constant k_1 varied in a similar way in relation to pH and temperature, presenting minimum points (0.29 and 0.34 s⁻¹) relatives to pHs 3.8 and 3.9 at a temperature of 85.0 °C. The highest values for the velocity constant k_1 are found at the extremities of pH and processing temperature.

Conclusion

The kinetics model parameters obtained in this study confirmed pH and process temperature as important factors on PE inactivation study. Both pH and process temperature have significant influence on isoenzyme fractions. pH also presented significant influence on the kinetic velocity constant k_1 . By following the kinetics equations obtained and knowing the pH of the juice, it is possible to prevent an overprocessing, minimize nutritional and sensorial characteristics loss.

Acknowledgements: Authors acknowledge the financial support from FAPESP - The State of São Paulo Research Foundation and from CNPq - National Research Foundation of Brazil.

References

1. FAO. FAOSTAT – *Statistics Database*. Available on <http://www.fao.org>, access in: April, 2002.
2. Shomer, R., Cogan, U., Mannheim, C.H. (1994) Thermal death parameters of orange juice and effect of minimal heat treatment on carbon dioxide on shelf-life. *Journal of Food Processing and Preservation*, 18, p. 305-315.
3. Sugai, A. Y., Shigeoka, D. S., Badolato, G. G., Tadini, C. C. (2002) Análise físico-química e microbiológica do suco de laranja minimamente processado armazenado em lata de alumínio. *Ciência e Tecnologia de Alimentos*, 22 (3), p. 233-238.
4. Badolato, G.G. (2000) Tratamento térmico mínimo do suco de laranja natural: cinética da inativação da pectinesterase. *Master of Science Thesis*. São Paulo University, São Paulo.
5. Basak, K. & Ramaswamy, H.S. (1996). Ultra high pressure treatment of orange juice: a kinetic study on inactivation of pectin methyl esterase. *Food Research International*, 26(7), 601-607.
6. Versteeg, C., Rombouts, F.M., Spaansen, C.H. & Pilnik, W. (1980). Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. *Journal of Food Science*, 45, 9690-9697.
7. Snir, R., Koehler, P.E., Sims, K.A., & Wicker, L. (1996). Total and thermostable pectinesterases in citrus juices. *Journal of Food Science*, 61(2), 379-382.
8. Cameron, R.G., Baker, R.A. & Grohmann, K. (1998). Multiple forms of pectinesterase from citrus peel and their effects on juice cloud stability. *Journal of Food Science*, 63(2), 253-255.
9. Ülgen, N. & Özilgen, M. (1991). Kinetic compensation relations for ascorbic acid degradation and pectinesterase inactivation during orange juice pasteurization. *Journal of Science of Food and Agriculture*, 57, 93-100
10. Kim, H. B., Tadini, C. C. & Singh, R. K. (1999). Effect of different pasteurization conditions on enzyme of orange juice in pilot scale experiments. *Journal of Food Process Engineering*, 22(5), 395-403.
11. Collet, L. S. F. C. A., Shigeoka, D. S., Badolato, G. G., Tadini, C. C. (2001) A kinetic study of pectinesterase (PME) during continuous pasteurization of orange juice. In: *11th World Congress of Food Science and technology*, Seoul, South Korean.
12. Fujikawa, H. & Itoh, T. (1996). Characteristics of a multicomponent first-order model for thermal inactivation of microorganisms and enzymes. *International Journal of Food Microbiology*, 31, 263-271.
13. Polakovic, M. & Vrábel, P. (1996). Analysis of the mechanism and kinetics of thermal inactivation of enzymes: critical assessment. *Process Biochemistry*, 31(8), 787-800.
14. Kimball, D. A. (1991) *Citrus processing quality control and technology*, New York, Chapman & Hall, 473 p.
15. AOAC - Association of Official Analytical Chemists. (1995) *Official Methods of Analysis*, V. 2, 16ed., Washington D. C..
16. Rouse, A.H. & Atkins, C.D. (1955). Pectinesterase and pectin in commercial citrus juice as determined by methods used at the citrus experiment station. 19p. *Fra. Agr. Exp. Sta. Bull. No.570*, University of Florida, Gainesville, Fl.
17. Manugistics (1999) *Statgraphics Plus for Windows - version 4* Standard Edition, Rockville, MD, USA.
18. Rouse, A.H. & Atkins, C.D. (1953). Distribution of pectinesterase and total pectin in component parts of citrus fruits. *Food Technology*, v.&, n.9, p. 360-362.
19. Eagerman, B.A. & Rouse, A.H. (1976). Heat inactivation temperature-time relationships for pectinesterase inactivation in citrus juices. *Journal of Food Science*, 41, 1396-1397.
20. Kórner, B.; Zimmermann, G. Berk, Z. (1980) Orange pectinesterase: purification, properties and effect on cloud stability. *Journal of Food Science*, v. 45, p. 1203-1206.
21. Han, Y.; Nielsen, S. S. & Nelson, P. E. Isolation of thermostable and thermolabile orange pectinesterases. In: *IFT'S 1998 Annual Meeting*. Atlanta, Georgia, 1998. Resumos. Atlanta, 1998.