EFFECT OF FROZEN STORAGE ON THERMAL INACTIVATION KINETICS OF ORANGE JUICE PECTINESTERASE

A.F. Molinari[1] and C.L.M. Silva[2]

1 Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto, Portugal, molinari@morango.esb.ucp.pt

2 Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto, Portugal, crislui@esb.ucp.pt

ABSTRACT

During the harvest season the orange juice industry squeezes the fruits to extract the juice and freezes it for further storage at low temperatures until processing. Due to its pH, the orange juice requires, only a pasteurization to reach the stabilization of the product at ambient temperature. The pasteurization aims at the inactivation of the enzyme pectinesterase (PE). This enzyme causes the loss of the juice cloud and its thermal resistance is greater than that of common bacteria and yeasts in citrus juices. Therefore, the objective of this work was the study of the effect of frozen storage on thermal inactivation kinetics of PE in orange juice.

The degradation kinetics of PE in fresh and frozen stored (at -20°C for 5, 12, 19 and 27 days) orange ('Dalmau') juice were determined. The PE activity was analysed using the method described by Köner [1] and adaptations suggested by Amstalden and Montgomery [2]. To evaluate the kinetics, seven isothermal experiments were carried out at temperatures ranging from 55°C to 85°C.

As observed by other authors, it was found the presence of two main enzyme fractions, one more heat resistant than the other. In average the more heat sensitive fraction had 75% of the total PE activity in the original samples. At a given constant temperature both fractions follow a first order inactivation reaction kinetics. The Arrhenius model describes well the temperature effect on the kinetics of both enzyme fractions. A non-linear regression to all data was performed using the software STATA to calculate simultaneously the activation energies and reaction rates at the reference temperature of 75°C of the two fractions. In order to investigate the effect of frozen storage time a stepwise regression to all data was carried out. It was concluded that this parameter has a significant influence on the pectinesterase inactivation within 95% confidence.

1. INTRODUCTION

Fresh orange juice contains particles in suspension giving it a "cloudy" appearance. After juice extraction the cloud losses stability and forms an unattractive two-phase system. This characteristic affects the appearance and decreases the juice commercial value. The cloud loss occurs due to the pectinesterase (PE) enzyme activity ([1], [2], [3], [4], [5], [6], [7],[8]). Thermal treatment is used commercially to inactivate this enzyme. The adequacy of a pasteurization treatment depends on the extent of the PE inactivation. Because of the low orange pH (generally pH < 4) the microorganisms occurring in the juice are less thermal resistant than the enzyme PE ([9], [10], [11], [12], [13]). However, the relatively

high temperatures necessary for PE inactivation produce undesirable "cooked" off-flavor and degrades juice aroma ([8], [14], [15]). People have become more aware about quality, therefore there is need to develop alternative methods that allow maximum retention of natural fruit properties. Seymour et al. [8] observed some grapefruit PE inactivation during frozen storage. Therefore, in order to preserve the quality attributes of the juice the use of freezing and frozen storage as a pre-treatment before a less severe pasteurization can be proposed.

Different forms of PE in citrus juices have been found. The thermostable (PE I), thermolabile (PE II) and high molecular weight PE (HMW) are present in all citrus species already studied. These isoenzymes have different inactivation kinetic properties and temperature stabilities. PE I and PE II account for almost 90% of the total PE activity and the HMW activity is variable in the different species of citrus. Furthermore, the main agents causative of the cloud loss are HMW and PE I ([4], [8], [16]).

Several authors carried out research about inactivation kinetics of PE ([1], [2], [3], [4], [5], [6], [7], [8], [17]). Particularly the effect of pH and solids concentration has been analysed. However, a relatively fewer number of research works exist in the field of modelling and quantification of inactivation kinetics.

Table 1. Fruit characteristics and pectinesterase (PE) inactivation kinetic data [18].

Fruit	Medium	pH	Soluble solids (°Brix)	$D_{85°C}$ (min)	z (°C)	T range (°C)	Reference
papaya	puree	3.5	7 - 9	4.8	14.8	75 - 85	[17]
		3.8		5.7	14.7		
		4.0		7.2	14.2		
papaya	acidified pulp	4.0	10 - 12	3.9	15.0	82 - 102	[19]
papaya	nectar	3.8	14	5.0	15.1	75 - 85	[17]
mandarin orange	juice	3.6	12	2.2	11.4	82 - 94	[10]
		4.0	12	3.6	10.1		
Old South Brand orange	juice	3.7	11.7	0.11 Heat Sensitive 5.48 Heat resistant	17.6 31.1	60 - 90 60 - 90	[13]

Furthermore, the individual inactivation kinetics of the two PE fractions (heat sensitive and heat resistant) was studied only by Tajchakavit and Ramaswamy [13].

The objectives of this work were to determine the inactivation kinetic parameters, activation energy (Ea - value) and rate constant at a reference temperature (k - value), for PE inactivation by thermal treatment in fresh and frozen stored orange juice and to determine if the freezing and frozen storage influences the time/temperature relationship required for PE inactivation.

2. MATERIALS AND METHODS

Orange juice preparation

'Dalmau' oranges (*Citrus sinensis* L.), grown in Portugal, were bought in the local market and squeezed. The juice (pH 3.6 and 12.6°B) was filled into plastic bags, quick frozen in a blast freezer (Armfield) and stored at -20°C. At time intervals (5, 12, 19 and 27 days), samples were taken out of the cold store and thawed in a water-bath at 25°C.

Pectinesterase assay

PE activity was determined by the method described by Körner et al. [1]. Basically, the method consisted of a titrimetric measurement of the rate of carboxyl group liberation from

a 1% pectin (Unipectin Up Slow Set 150), 0.15 M NaCl solution at pH 7.0 and 30°C. The activity was expressed in PEU, that corresponds to the miliequivalents of acid liberated per min per ml at pH 7.0 and 30°C. Some modifications, suggested by Amstalden and Montgomery [2] were done. A 20 ml of juice sample was added to 40 ml of pectin solution (previously adjusted to pH 7.0 and heated until 65°C to reach 55°C after adding the juice) with constant stirring and quickly adjusted to pH 7.0 with 0.1 N NaOH. When the pH 7.0 was reached the chronometer was put in action simultaneously with the addition of 1 ml of 0.05 N NaOH. The chronometer was stopped when the pH was back to 7.0. A constant temperature of 55°C was maintained during the titration. PE activity was calculated by the equation:

$$PEU = \frac{(1\text{ml NaOH} * \text{N of NaOH} * 10^4)}{(\text{time (min)}) * (\text{ml of sample})} \quad (1)$$

where PEU - unit of pectinesterase/ml of sample.

In order to take into account the effect of pectin degradation by the alkali which causes a pH decrease, an analysis was made with the pectin solution heated with distillated water instead of juice. The value determined was subtracted from the PE activity calculated by equation (1). The accuracy of the method was ± 0.98 PEU.

Thermal inactivation of pectinesterase

Pyrex glass tubes of 100 mm length, 13 mm OD and 10 mm ID were used in order to reduce the temperature come-up-time inside the tubes. Six ml of sample were pipetted into each tube and heated in a well agitated thermostatic water-bath (Thermomix B, B. Braun Melsungen AG, Germany) for known times. Immediately after heating, they were cooled by plunging in an ice-water-bath. Four tubes were used for each time and temperature. Residual enzyme activities were then measured. Heating temperatures varied from 55 to 85°C in increments of 5°C.

Inactivation kinetics

In the Food Engineering field first order inactivation kinetics are commonly use to model isothermal degradation kinetics. If the two enzyme fractions (heat sensitive - PEII, and heat resistant, PEI) follow a first order inactivation kinetics the total PE activity can be calculated as:

$$C = C_{PEI}\, e^{-k1\ t} + C_{PEII}\, e^{-k2\ t} \quad (2)$$

where C_{PEI} and C_{PEII} are the initial concentrations (for time zero) of PEI and PEII, respectively, k_1 and k_2 are the rate constants for PEI and PEII, respectively, and t is the time.

The Arrhenius model usually describes mathematically well the effect of temperature on the reaction rate constant:

$$k = k_{ref} * e^{-\frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \quad (3)$$

where k_{ref} is the reaction rate constant at the reference temperature (T_{ref}), E_a the activation energy and R the universal gas constant.

3. RESULTS AND DISCUSSION

Figure 1 presents the inactivation kinetic data for orange juice stored at -20°C during 12 days. Similar results were obtained for fresh and other frozen stored samples (5, 19 and 27

days of storage). It can be observed that there are at least two enzyme fractions, one more heat sensitive (PEII) and another more heat resistant (PEI). In average the more heat sensitive fraction had 75% of the total PE activity in the original samples.
It was observed that the effect of temperature on the reaction rate for the two enzyme fractions was reasonably well described by the Arrhenius model.

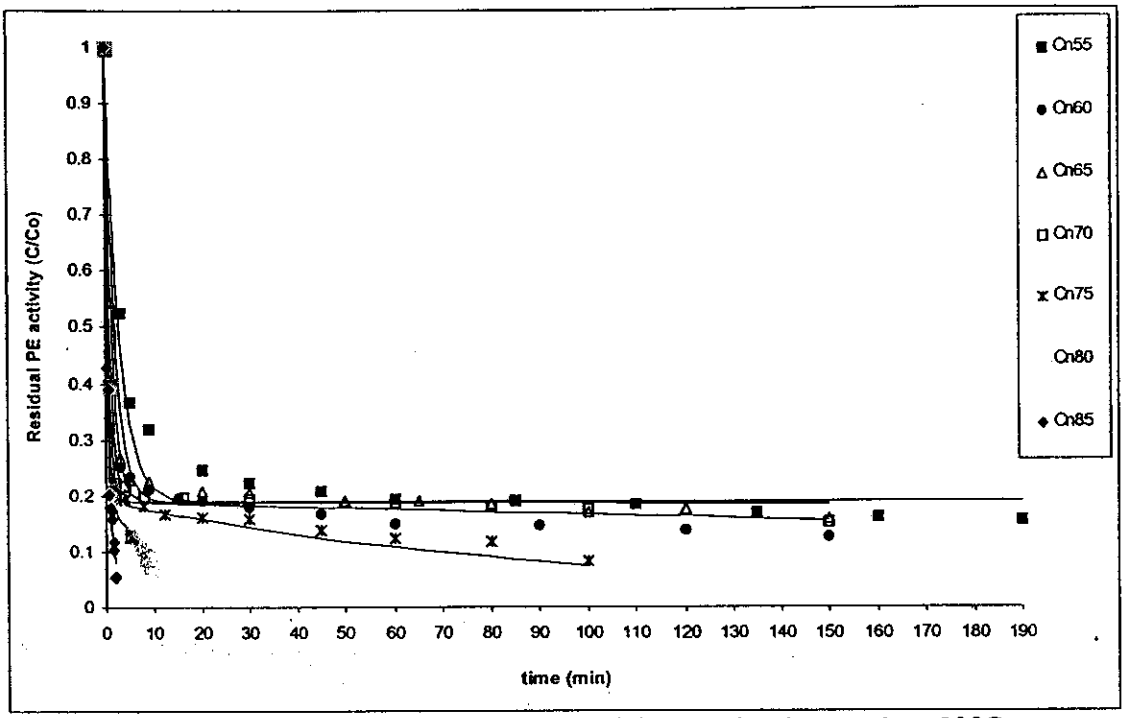

Figure 1. Inactivation kinetic data for orange juice previously stored at -20°C during 12 days.

Using the software Stata, a one-step non-linear regression to all data was carried out in order to calculated the inactivation kinetic parameters for fresh and frozen stored samples. Table 2 presents the results together with the corresponding standard deviations.

Table 2. Parameters for PE inactivation kinetics in 'Dalmau' orange juice.

PARAMETERS	TIME OF STORAGE (days)				
	0	5	12	19	27
$C_{PEII}^{(*)}$	0.73 ±0.03	0.79 ±0.02	0.81 ±0.13	0.71 ±0.03	0.72 ±0.03
$C_{PEI}^{(*)}$	0.26 ±0.01	0.22 ±0.01	0.189 ±0.005	0.29 ±0.01	0.29 ±0.01
k1 (min^{-1})	0.61 ±0.05	1.3 ±0.1	1.66 ±0.08	1.21 ±0.01	0.84 ±0.06
k2 (min^{-1})	0.0015 ±0.005	0.019 ±0.004	0.0094 ±0.0019	0.009 ±0.003	0.096 ±0.003
E1 (kJ/mol)	76 ±6	31 ±7	74 ±3	72 ±6	51 ±6
E2 (kJ/mol)	1033 ±157	325 ±40	398 ±30	388 ±40	1127 ±134
R^2	0.97	0.98	0.99	0.98	0.98

(*) Normalized concentrations

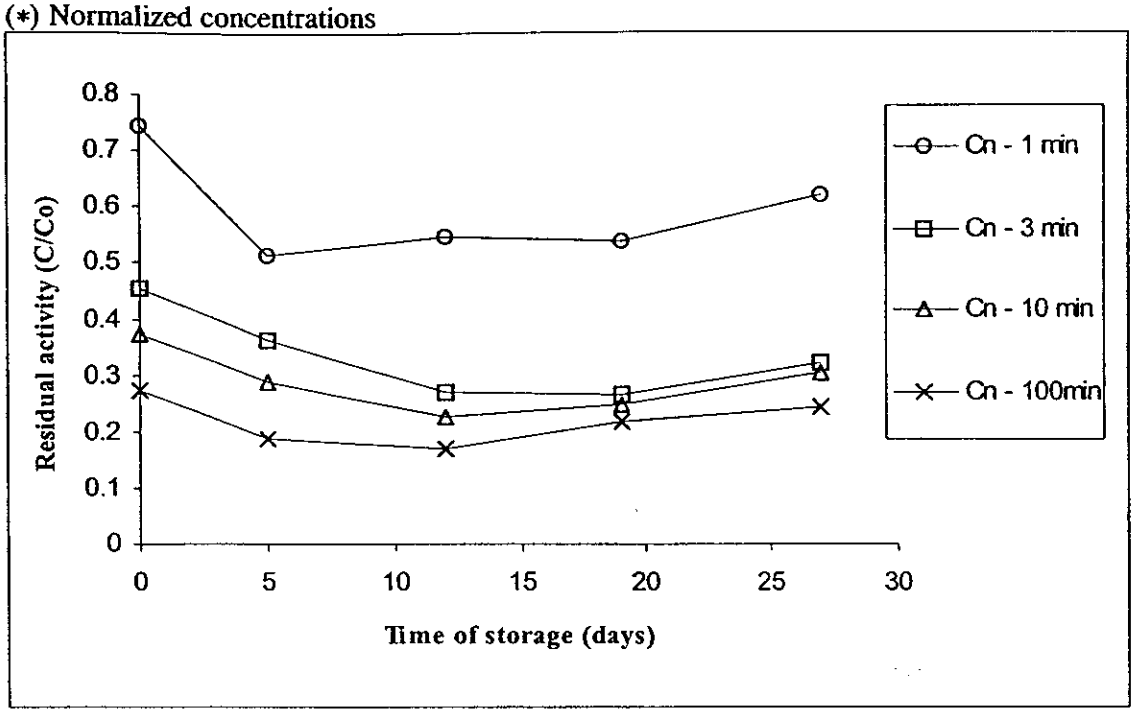

Figure 2. Effect of frozen storage on the inactivation kinetics. Data obtained at 65°C (1, 3, 10 and 100 min) for all intervals of frozen storage (0, 5, 12, 19 and 27 days)

In order to investigate the effect of time of frozen storage on the inactivation kinetics of PE a stepwise regression to all data was carried out. It was concluded that this parameter has a significant influence on the pectinesterase inactivation within 95% confidence. Figure 2 presents the effect of frozen storage on the residual PE activity for different times of thermal processing at 65°C. Similar results are observed for other temperatures of processing. It was not possible to model mathematically this behaviour. Furthermore, more experiments have to be carried out to confirm that the PE of frozen stored orange juices can be more easily inactivated.

4. CONCLUSIONS

It was found the presence of two main enzyme fractions, one more heat resistant than the other. In average the more heat sensitive fraction had 75% of the total PE activity in the original samples. At a given constant temperature both fractions follow a first order inactivation reaction kinetics. The Arrhenius model describes well the temperature effect on the kinetics of both enzyme fractions. A non-linear regression to all data was performed using the software STATA to calculate simultaneously the activation energies and reaction rates at the reference temperature of 75°C of the two fractions. In order to investigate the effect of frozen storage time a stepwise regression to all data was carried out. It was concluded that this parameter has a significant influence on the pectinesterase inactivation within 95% confidence.

5. ACKNOWLEDGMENTS

The authors acknowledge the research financial support of European Union, contract STD n° ERB-TS3-CT94-0300, project "Etude Pluridisciplinaire de Transformations de Fruits Amazoniens en vue de leur Valorisation Commerciale par les Organisation Paysannes Existantes".

6. REFERENCES

[1] Köner, B., Zimmermann, G. and Berk, Z. (1980). Orange pectinesterase: purification, properties, and effect on cloud stability. *J. Food Sci.*, **45**:1203-1206.

[2] Amstalden, L. C. and Montgomery, M. W. (1994). Pectinesterase em suco de laranja: caracterização. *Ciênc. Tecnol. Aliment.*, **14**(1):37-45.

[3] Atkins, C. D., Rouse, A. H. (1953). Time-temperature relationships for heat inactivation of pectinesterase in citrus juices. *Food Technol.*, 7:489-491.

[4] Versteeg, C., Rombouts, F. M. Spaansen, C. H. and Pilnik, W. (1980). Thermostability and orange juice cloud destabilizing properties of multiple pectinesterase from orange. *J. Food Sci.*, **45**:969-971, 998.

[5] Marshall, M. R., Marcy, J. E. and Braddock, R. J. (1985). Effect of total solids level on heat inactivation of pectinesterase in orange juice. *J. Food Sci.*, **50**:220-222.

[6] Wicker, L., Bradock, R. J. and Vassalo, M. (1987). Effect of assay temperature on activity of citrus pectinesterase in fresh orange juice. *J. Food Sci.*, **52**:378-380.

[7] Seymour, T. A., Preston, J. F., Wicker, L., Lindsay, J. A., Wei, C. and Marshall, M. R. (1991). Stability of pectinesterases of Marsh White grapefruit pulp. *J. Agr. Food Chem.*, **39**:1075-1079.

[8] Seymour, T. A., Preston, J. F., Wicker, L., Lindsay, J. A., and Marshall, M. R. (1991). Purification and properties of pectinesterases of Marsh White grapefruit pulp. *J. Agr. Food Chem.*, **39**:1080-1085.

[9] Eagerman, B. A. and Rouse, A. H. (1976). Heat inactivation temperature-time relationship for pectinesterase inactivation in citrus juices. *J.Food Sci.*, **41**:1396-1397.

[10] Nath, N. and Ranganna, S. (1977). Time/temperature relationship for thermal inactivation of pectinesterase in mandarin orange (*Citrus reticulata* Blanco) juice. *J. Food Technol.*, **12**:411-419.

[11] Irwe, S. and Olsson, I. (1994). Reduction of pectinesterase activity in orange juice by high pressure treatment. In: *Minimal processing of foods and process optimization - an interface (Eds.* R. P. Singh and F. A. R. Oliveira) CRC Press, Inc., USA, 35-42.

[12] Argáiz, A. and López-Malo, A. (1995). Kinetcs of first change on flavour, cooked flavour development and pectinesterase inactivation on mango and papaya nectars and purees. *Rev. Esp. Cienc. Tecnol. Aliment.*, 35(1):92-100.

[13] Tajchakavit, S. and Ramaswamy, H. S. (1997). Thermal vs. microwave inactivation kinetics of pectin methylesterase in orange juice under batch mode heating conditions. *Lebnsm. Wiss. u.-Technol.*, **30**: 85-93.

[14] Kiefer, F. (1961). A new oxidative mechanism in the deteriorative changes of orange juice. *Food Technol.*, **6**:302-305.

[15] Baker, R. A. and Bruemmer, J. H. (1972). Pectinase stabilization of orange juice cloud. *J. Agr. Food Chem.*, **20**(6):1169-1173.

[16] Rombouts, F. M., Versteeg, A., Karman, H. and Pilnik, W. (1982). Pectinesterase in component parts of citrus fruits related to problems of cloud loss and gelation in citrus products. In: *Use of Enzymes in Food Technology, Symposium International*, Versailles, 483-487.

[17] Argáiz, A. (1994). Thermal inactivation kinetics of pectinesterase in acidified papaya nectar and purees. *Rev. Esp. Cienc. Tecnol. Aliment.*, 34(3):301-309.

[18] Silva, F. V. M., and Silva, C. L. M. (1997). Quality optimization of hot filled pasteurized fruit purees: container characteristics and filling temperatures. *J. of Food Engineering*. Accepted for publication.

[19] Nath, N and Ranganna, S. (1981). Determination of thermal schedule for acidified papaya. *J. Food Technol.*, **46**:201-206, 211.