Thermal inactivation of mango *(Mangifera indica* L., variety Palmer) puree peroxidase

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Abstract. *Mango (Mangifera indica L.) is a very important crop in tropical countries and Brazil is the seventh greatest producer in the world. In Brazil, mango is mainly consumed as fresh fruit, but consumption of processed products is increasing.*

Peroxidase is commonly associated with deterioration in flavor, color, texture and nutritional qualities of raw fruits and vegetables and is one of the most thermostable enzymes present in mangoes. Heat treatment of mango products is important to inactivate enzymes and to maintain the product quality.

Two different heat exchangers were used in the pasteurization of 'Palmer' mango puree: a plate heat exchanger (holding tube volume: 75.8 cm^3) and a double-pipe heat exchanger (heating section area: 0.339 m2; holding tube volume: 71.6 cm^3). Different temperatures were tested: 75, 80 and 85 ^{o}C in the plate heat exchanger and 65, 70 and 75 ^{o}C in the double-pipe heat exchanger. It was not observed peroxidase activity in mango puree pasteurized at 80 and 85 ^{o}C; at 65, 70 and 75 ^{o}C, residual enzyme activity varied from 78.0 to 4.3 %.

To complete this study, physical, chemical and sensorial analyses should be conducted to correlate peroxidase residual activity and mango puree quality.

Keywords. Mango, peroxidase, heat inactivation.

Introduction

Mango *(Mangifera indica* L.) is one of the most important tropical fruits. It grows throughout the tropics and it is the second most produced tropical fruit. Due to its excellent flavor, attractive fragrance and delicious taste, mango is considered one of the best fruits (Ctenas, Ctenas & Quast, 2000; Marin & Cano, 1992).

Brazil is the seventh greatest producer in the world and around 30 varieties are produced in the country. The most important ones are *Tommy Atkins, Palmer* and *Haden* (Ctenas, Ctenas & Quast, 2000; FAO, 2004).

In Brazil, mango is consumed mainly as fresh fruit and the losses of production are extremely elevated because of its highly perishable nature, susceptibility to disease and to physical injury (Lizada, 1993).

Processing the fruit is an alternative to increase its shelf life, as pasteurization, that inactivates enzymes, reduces the microbial count and maintains the product quality.

Enzymes could be used as bioindicators to pasteurization process. Peroxidase, one of the most thermostable enzymes, is commonly associated with deterioration in flavor, color and texture of raw fruits and processed products. Due to its thermostability, peroxidase is frequently used as bioindicator to blanching treatment (Khan & Robinson, 1993).

Heat exchangers could be used to pasteurize fruit purees and juices. Plate heat exchangers have been used frequently to pasteurize these products (Lewis & Heppell, 2000), but double-pipe and scrapped-surface heat exchangers could be also used. Brekke, Cavaletto & Stafford (1968) used a plate heat exchanger to pasteurize mango puree, for 1 minute at 91 - 93 °C. This puree was then frozen at - 23 °C and held at - 18 °C for eight months. Puree showed no enzyme activity and sensory quality was preserved after storage. Isaacs (1991) used a scrapped-surface heat exchanger to heat the puree and a double-pipe exchanger to cool it. Puree maintained its microbial, physical, chemical and sensory quality for eight months in ambient storage.

The aim of this work was to study the thermal inactivation of mango puree peroxidase. Nineteen different pasteurization conditions were studied and two types of heat exchangers were used in this work.

Materials and Methods

Ripe mangoes *(Mangifera indica* L., var. *Palmer)* were harvested from commercial orchards from Monte Alto *(São Paulo, Brazil).* Fruits were sorted, washed and hand peeled. Seeds were removed and fruits were sliced. Mango slices were put through a fruit pulper with a 1 mm openings screen to remove fibers. Mango puree was then acidified to pH < 4.5 and pasteurized. Pasteurization process was conducted at temperature range 65 - 85°C, at different holding times, totalizing nineteen different conditions.

It was used an Armfield plate heat exchanger *(model FT43A, England)* to pasteurize mango puree at three different pasteurization temperatures (75, 80 and 85°C), constanlty monitored by a temperature controller. This exchanger consisted of three sections: regeneration (10 x 1/10x1), heating (6 x 1/6 x 1) and cooling (4 x 1/4 x 1), and a holding tube of 75.8 cm^3 volume.

A double-pipe heat exchanger was also used to pasteurize puree, at three different temperatures (65, 70 and 75°C). For each temperature, at least three different holding times were tested. The double-pipe heat exchanger, designed by our laboratory specifically for fruit puree pasteurization processes, consisted of a heating section and a holding tube (71.6 cm^3 volume). The heating section consisted of 10 double tubes (1.80 m long each one, outer tube diameter 3.0×10^{-2} m, inner tube external diameter 6.0×10^{-3} m, inner tube internal diameter 4.5×10^{-3} m), totalizing 0.339 m^2 of area, and a temperature controller monitored the pasteurization temperature.

Physical chemical analyses

The pH was directly measured using the pH-Stat PHM-290 *(Radiometer Analytical S.A., France)* (AOAC, 1995).

Titratable acidity, expressed as citric acid percentage, was determined according to AOAC (1995) method. Titration was carried out in the pH-Stat PHM-290 (Radiometer Analytical S.A., France), until pH 8.2 was reached.

Total and soluble solids were determined according to AOAC (1995) and Instituto Adolfo Lutz (1976) methods. Soluble solids, expressed as °Brix at 20 °C, were determined with a Carlzeissjena refractometer *(model I, Germany).*

Peroxidase activity determination

Samples of untreated and pasteurized mango puree were colected of each pasteurization condition. After collection, samples were immediately cooled in icy water. Mango puree peroxidase was extracted according to the method described by Khan & Robinson (1993) and the enzyme activity was assayed with o-dianisidine (McLellan & Robinson, 1981). Changes in absorbance were recorded at 460 nm, at 25 °C, using a UV-VIS spectrophotometer, *(Femto, model 700 PLUS, Brazil).* One unit of peroxidase activity was defined as an increase of 1.0 optical density at 460 nm $mL^{-1}.min^{-1}$ and the residual activity was the relation of peroxidase activity of mango puree after and before the pasteurization.

Kinetic parameters D and z

Kinetic parameter D was calculated based on residual peroxidase activity and equivalent heating time.

The equivalent heating time *(t_{equiv})* was calculated based on mininum holding time *(t_{min}),* pasteurization temperature (T), reference temperature *(T_{ref})* and z value (Toledo, 1991):

$$t_{equiv} = \int_0^{t_{min}} 10^{\left(T - T_{ref}/z\right)} dt \qquad (1)$$

First, an estimated value of z was used in calculation of equivalent heating time. Then, D values were obtained from linear regression of log residual peroxidase activity versus equivalent time. The z value was then obtained from a linear regression of log D value versus temperature. Calculations of D and z values were repeated until convergence was reached *<fiz <* 0.5%) (Toledo, 1991; Tajchakavit & Ramaswamy, 1997).

The minimum holding time was the residence time of the fastest-flowing portion of mango puree (Toledo, 1991):

- ^ - (2)
max

Where L is the holding tube length and v_{max} is the maximum velocity, calculated by Equation 3:

$$v_{max} = \frac{(3n+1)}{n+1} v_{avg} \quad (3)$$

Where n is the flow index (for power law fluids) and v_{avg} is the average velocity, calculated by Equation 4:

$$v_{avg} = \frac{Q}{A} \quad (4)$$

Where Q is the volumetric flow rate and A is the cross-sectional area of the holding tube.

Results and Discussion

Table 1 shows physical chemical characteristics of untreated mango puree from different fruit batches.

Table 1. Physical chemical characteristics of untreated mango puree from different fruit batches.

Batch	pH[1]	Titratable acidity[2]	Soluble solids[3]	Total solids[4]
1	4.32 ±0.01	0.22 ± 0.00	18.3 ±0.2	18.9 ±0.0
2	4.36 ±0.01	0.24 ±0.01	18.3 ±0.2	19.3 ±0.0
3	4.40 ±0.04	0.22 ± 0.00	17.1 ±0.0	18.0 ±0.1
4	4.43 ±0.01	0.21 ±0.00	13.4 ±0.0	15.7 ±0.0
5	4.47 ±0.01	0.21 ±0.00	12.6 ±0.0	15.1 ±0.0
6	4.48 ±0.01	0.22 ± 0.00	14.0 ±0.0	16.7 ±0.0

1. *After acidification.*
2. *Expressed as citric acid percentage*
3. *Expressed as °Brix at 20 °C*
4. *Percentage (%).*

Pasteurization conditions and mango puree peroxidase activity, before and after pasteurization, are given in Table 2.

Raw mango puree peroxidase activity varied from 0.37 to 0.66 UAbs.mL^{-1}.min^{-1}. These values are lower than those reported in literature: 4.15 UAbs.mL^{-1}.min^{-1} for *Tommy Atkins* mangoes (Azevedo & Pastore, 2004) and 3.50 UAbs.mL-^{1}.min^{-1} for *Chaunsa*mangoes (Khan & Robinson, 1993). It was not detected peroxidase activity in mango puree pasteurized at 80 and 85°C. At 75°C, residual peroxidase activity varied from 4.1 to 24.4 %. Longer holding times at 65 and 70°C resulted in an inactivation of enzyme of 40 and 9%, respectively.

There were some difficulties to process mango puree in the plate heat exchanger. Despite the use of a fruit pulper to remove fibers, there was accumulation on the plate surface that led to blockage of the flow. Consequently, the flow rates were too low (see Table 2, Batch 5). To avoid the fibers accumulation, a pump of higher capacity was tested and in consequence, pressure between plates increased and mango puree poured out.

Table 2. Pasteurization conditions (pasteurization temperature, flow rate and holding time) and peroxidase (POD) activity, before and after pasteurization.

Batch	Pasteurization temperature[1] (°C)	Flow rate ($m^3.s^{-1}$)	Holding time (s)	POD activity ($UAbs.mL^{-1}.min^{-1}$)	Residual POD activity[2] (%)
1[3]	Raw sample	–	–	0.37 ± 0.01	100.0
	65.3 ± 0.3	1.46E-05	3.4	0.27 ± 0.01	73.0
	64.7 ± 0.5	7.58E-06	6.5	0.27 ± 0.01	71.6
	64.6 ± 0.5	3.75E-06	13.1	0.15 ± 0.02	39.2
2[3]	Raw sample	–	–	0.66 ± 0.03	100.0
	70.1 ± 0.2	1.49E-05	3.3	0.52 ± 0.02	78.0
	69.3 ± 0.2	7.61E-06	6.4	0.48 ± 0.03	73.2
	69.6 ± 0.1	3.65E-06	13.4	0.06 ± 0.01	8.6
3[3]	Raw sample	–	–	0.38 ± 0.04	100.0
	75.3 ± 1.1	3.78E-06	12.9	Not observed	
	75.0 ± 1.1	6.17E-06	7.9	0.02 ± 0.00	4.3
	76.2 ± 1.4	9.33E-06	5.2	0.06 ± 0.00	14.9
	77.2 ± 0.8	1.21E-05	4.1	0.04 ± 0.00	10.5
4	Raw sample	–	–	0.43 ± 0.02	100.0
	73.9 ± 0.4	1.5E-06	51.7	0.03 ± 0.01	6.7
	75.3 ± 1.1	2.1E-06	36.6	0.02 ± 0.00	4.1
	73.9 ± 0.4	2.7E-06	28.6	0.10 ± 0.01	24.4
5	Raw sample	–	–	0.57 ± 0.02	100.0
	79.2 ± 0.6	7.3E-07	103.5	Not observed	
	81.2 ± 1.5	1.4E-06	53.9	Not observed	
	78.2 ± 0.7	6.0E-07	125.5	Not observed	
6	Raw sample	–	–	0.60 ± 0.12	100.0
	85.9 ± 0.5	1.7E-06	44.8	Not observed	
	85.9 ± 0.3	1.9E-06	40.7	Not observed	
	86.0 ± 0.6	2.5E-06	30.8	Not observed	

1. *Mango puree temperature at the end of holding tube*
2. *Related to POD activity of untreated mango puree*
3. *Mango puree pasteurized in double-pipe heat exchanger*

In view of these results, lower pasteurization temperatures were studied in a double-pipe heat exchanger. This type of exchanger has a relatively larger flow channel, compared to plate heat exchanger, and could be used to pasteurize liquids containing high levels of pulp or fibers (Lewis & Heppell, 2000).

About the double-pipe heat exchanger, there were no difficulties to process mango puree. It indicates that this type of exchanger was more appropriate to pasteurize mango puree.

Kinetic parameters D and z

Kinetic parameters D and z were calculated based on results of residual peroxidase activity (batches 1, 2 and 3) and the equivalent heating time.

For each batch, the maximum temperature was considered as reference temperature. Table 3 shows pasteurization and reference temperatures, maximum velocity, minimum holding time and equivalent time for each pasteurization condition. In the literature, fruit purees are refered as power law fluids, presenting a pseudoplastic behavior. To calculate maximum velocity, a flow index nof 0.3 was considered (Sugai, 2002).

Table 3. Reference temperature (T_{ref}), pasteurization temperature (T), maximum velocity (v_{max}), minimum holding time (t_{min}) and equivalent time (te_{quiv}) of mango puree pasteurized in double-pipe heat exchanger.

Reference temperature (°C)	Pasteurization temperature ($m.s^{-1}$)	Maximum velocity (s)	Minimum holding time (s)	Equivalent Batch time (°C)
3.4	3.4 1	65.3	64.7 ±0.5	0.70 6.5
5.9 64.6 ±0.5	0.34	13.1	11.7 70.1 ±0.2	1.37
3.3	3.3 2	70.1	69.3 ±0.2	0.70 6.4
5.7 69.6 ±0.1	0.34	13.4	12.4 75.0 ±1.1	0.57
7.9	5.6 3	77.2	76.2 ±1.4	0.86 5.2
4.5 77.2 ±0.8	1.11	4.1	4.1	

The log of residual activity was plotted against equivalent time (Figure 1), D values at different reference temperatures were determined and the log of these D values was then plotted against the temperature (Figure 2) for z value determination.

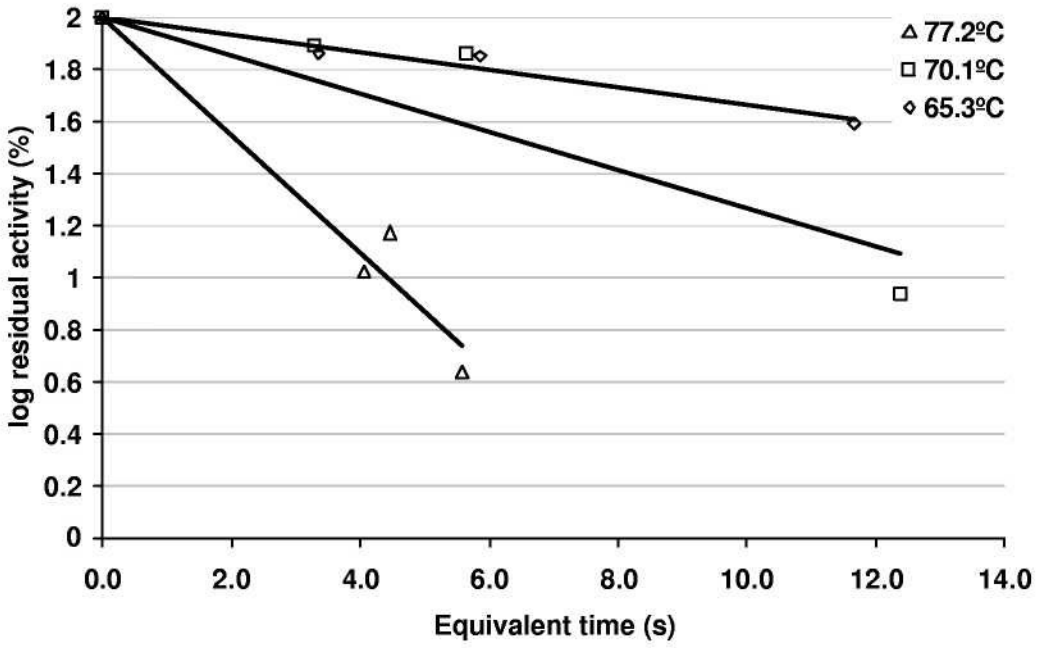

Figure 1. Residual activity of mango puree peroxidase at different temperatures: 77.2ºC (Δ); 70.1ºC ( ); 65.3ºC (◊).

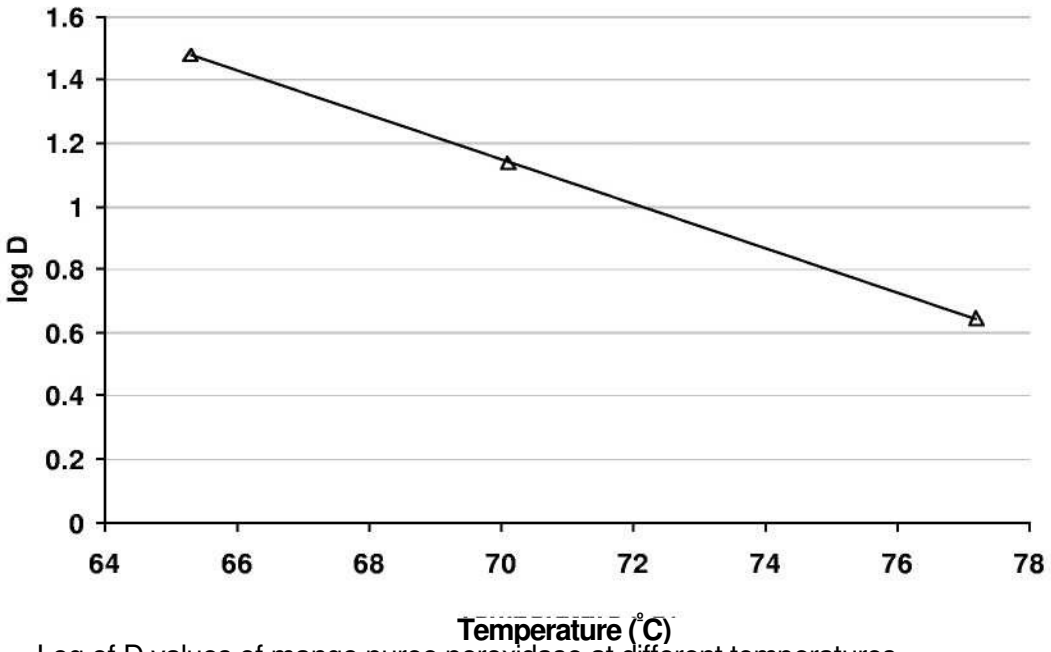

Figure 2. Log of D values of mango puree peroxidase at different temperatures.

D values determined in this study were lower than those reported by Rosenthal, Domingues & Slongo (2002). The authors determined D and z values of mango (cv. *Ubá* or *Carlotinha)* juice peroxidase: D75ºC = 284.3 s and z = 10.68°C. This difference of D values should be due to differences of the mango varieties and in the formulations of puree and juice. Table 4 presents z value and D for each reference temperature obtained in this study.

Table 4. Calculated D and z values of mango puree peroxidase.

Reference temperature (°C)	D (s)	(ºC)
65.3	30.0 (R^2 = 0.96)	14.3 (R^2 = 1.00)
70.1	13.7 (R^2 = 0.84)	
77.2	4.4 (R^2 = 0.95)	

Conclusions

It was not observed peroxidase activity in mango puree pasteurized at 80 and 85 °C; at 65, 70 and 75 °C, residual enzyme activity varied from 78.0 to 4.3 %. Kinetic parameters D and z of mango puree peroxidase were determined: $D_{77.2}$ºC = 4.4 s and z = 14.3°C.

It was observed that the double-pipe heat exchanger was adequate to pasteurize mango puree; there were no difficulties in the process. Otherwise, the use of plate heat exchanger *(Armfield, model FT43A)* was not appropriate to pasteurization of puree: there was blockage of flow channels due to the fibers of mango and consequently the flow rates were too low.

To complete this study, physical, chemical and sensorial analyses should be conducted to correlate peroxidase residual activity and mango puree quality.

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Nomenclature

A	Cross sectional area of holding tube [m^2]
D	Decimal reduction time [s]
L	Holding tube length [m]
n	Flow index [dimensionless]
Q	Volumetric flow rate [$m^3.s^{-1}$]
T	Pasteurization temperature [$^{\circ}C$]
T_{ref}	Reference temperature [$^{\circ}C$]
t_{equiv}	Equivalent heating time [s]
$t_{mir?}$	Minimum holding time [s]
v_{avg}	Average velocity [$m.s^{-1}$]
V_{max}	Maximum velocity [$m.s^{-1}$]
z	D temperature dependence [$^{\circ}C$]

References

AOAC (Association of Official Analytical Chemists). 1995. *Official Methods of Analysis ofAOAC International.* 16th ed. Arlington, USA: AOAC.

Azevedo, A. C. S. & Pastore, G. M. 2004. Estudo das enzimas polifenol oxidase e peroxidase em manga *(Mangifera indica* L.) Tommy Atkins do Brasil. In: *Congresso Brasileiro de Ciência e Tecnologia de Alimentos 19,* 914. Recife, Brazil.

Brekke, J., Cavaletto, C. & Stafford, A. E. 1968. Mango puree processing. Honolulu, USA> (Hawaii Agricultural Experiment Station. Technical Progress Report N º 167).

Ctenas, M. L. B., Ctenas, A. C. & Quast, D. 2000. Manga. In: *Frutas das Terras Brasileiras,* pp. 130-135. M. L. B. Ctenas, A. C. Ctenas & D. Quast, ed. Brazil: C2 Editora e Consultoria em Nutrição.

FAO. FAOSTAT Agricultural Data 2004. Available at: faostat.fao.org/faostat/collections?subset=agriculture. Accessed September 2005.

Instituto Adolfo Lutz. 1976. *Normas Analíticas do Instituto Adolfo Lutz - Métodos Químicos e Físicos para Análise de Alimentos.* 2nd ed. São Paulo, Brazil: IAL.

Isaacs, A. R. 1991. Aseptic packaging of mango puree. In: III *International Mango Symposium,* Darwin, Australia. Available at: www.actahort.org/books/291/291_63.htm. Accessed June 2005.

Khan, A. A. & Robinson, D. S. 1993. Purification of an anionic peroxidase isoenzyme from mango *(Mangifera indicaL.* var. *chaunsa). Food Chemistry* 46:61-64.

Lewis, M. & Heppell, N. 2000. *Continuous Thermal Processing of Foods: Pasteurization and UHT Sterilization.* Maryland, USA: Aspen Publishers, Inc.

Lizada, C. 1993. Mango. In: *Biochemistry of Fruit Ripening;* pp. 255-271; G. Seymour, J. Taylor & G. Tucker, ed. London, England: Chapman and Hall.

Marin, M. A. & Cano, M. P. 1992. Patterns of peroxidase in ripening mango *(Mangifera indica,* L.) fruits. *Journal of Food* Science57(3): 690-692;734.

McLellan, K. M. & Robinson, D. S. 1981. The effect of heat on cabbage and Brussels sprout peroxidase enzymes. *Food Chemistry* 7:257-266.

Rosenthal, A., Rodrigues, A. M. & Slongo, A. P. Cinética de inativação térmica de peroxidase de suco de manga. Available at: www.ufpel.tche.br/sbfruti/anais xvii cbf/tecnologia de alimentos/759.htm. Accessed March 2005.

Sugai, A. Y. 2002. Processamento descontínuo de purê de manga *(Mangifera indica* Linn.), variedade Haden: estudo da viabilidade do produto para pronto consumo. MS thesis. São Paulo, São Paulo, Brazil. Universidade de São Paulo, Chemical Engineering Department.

Tajchakavit, S. & Ramaswamy, H. S. 1997. Continuous-flow microwave inactivation kinetics of pectin methyl esterase in orange juice. *Journal of Food Processing and Preservation* 21: 365-378.

Toledo, R. T. 1991. *Fundamentals of Food Process Engineering.* 2nd ed. New York, USA: Chapman & Hall.