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Kinetics of pectinesterase inactivation during pasteurization of guyabano (*Annona muricata* L.) juice

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Abstract

The inactivation kinetics of pectinesterase (PE) in single strength guyabano (soursop) juice processed by heat pasteurization at 85°C, 90°C, and 95°C was evaluated in the study. Freshly squeezed guyabano juice having 3.5 pH value, total soluble solids of 14.8°Brix, and a titratable acidity of 0.52% malic acid had a pectinesterase activity of 5.66 ± 1.19 PEU/ml. Highest enzyme inactivation (93.52%) was observed from the isotherm curve of 95°C but values were not statistically significant from that of 90°C (89.05%). As the obtained isothermal curves showed deviation from the expected first-order kinetics, data were statistically treated applying a non-linear regression and the estimated best fit was a three-parameter multicomponent first order model. This model enabled the estimation of PE inactivation-related *a, k_1, k_2,* and E_a values to be in the range of 0.6151–0.9170, 0.4501–0.8412 min^{-1}, 0.0015–0.0029 min^{-1}, and 75.27–86.26 kJ/mol, respectively. Processing of guyabano juice at 90°C for 10 seconds did not significantly affect the overall acceptability of the juice. Results of the sensory evaluation showed that the processed guyabano juice had a viscosity value of 5.25 cP, a creamy white color, strong guyabano taste, moderate guyabano odor, and a general acceptability rating ranging from like slightly to like very much.

Introduction

Pectinesterase (PE) or pectin methyl esterase (PME) has imperative impact on quality and stability of fruit juices. Lo and Perera (2003) stated that this enzyme causes loss of fresh juice cloudiness and gelation of pectin in juice concentrate. This is because free carboxyl groups are liberated as a result of PE de-methylating the pectin molecules. The free carboxyl groups could in turn react with one another, in the presence of divalent metal ions, especially Ca^{2+}, leading to formation of salt bridges between pectin molecules. Consequently, large aggregates formed would destabilize the cloudiness in fruit juices, resulting to undesirable sedimentation.

Fresh guyabano (English: soursop) juice contains particles in suspension giving it a "cloudy" appearance. The cloud of single strength guyabano juice is white, fine, and cottony textured. Umme (2000) reported that the cloud particles showed a continuous matrix of protein filaments in close association with lipid and pectin, as shown by the transmission electron micrograph. The researcher further reported that flocculates of insoluble pectates and aggregated particles formed by PE enzymatic action are evident in unpasteurized juice cloud. This characteristic affects the appearance and decreases the juice commercial value. To overcome this quality defect, guyabano juice must be pasteurized at temperatures necessary to inactivate the heat stable PE. Because of the low pH of guyabano (generally less than pH 4), the microorganisms occurring in the juice are less thermal resistant than PE; thus, the inactivation of this enzyme is commonly used as an indicator of the pasteurization process adequacy (Arbaisah *et al.*, 1997a).

It is therefore important to have accurate PE heat inactivation kinetic parameters to design the process. In general, PE heat inactivation is considered to follow first-order kinetics (Collet *et al.*, 2005). In the critical review on thermal inactivation of PE in citrus juices, Chen and Wu (1998) 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 stated that the use of the first order model constants could result in underestimated or super estimated processing of orange juice.

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Although several studies on fruit juice processing using pasteurization have been conducted, most of these are on PE inactivation in citrus juices. Guyabano juice contains PE enzyme and little information regarding the effect of pasteurization on PE activity in natural guyabano juice is available. Hence, the objective of this study was to evaluate the kinetics of pectinesterase inactivation during pasteurization of guyabano juice.

Materials and Methods

Materials

Mature, unripe guyabano fruits of the local variety (sour type) were obtained from farmers in Brgy. Igbical, Hamtic, Antique, Philippines. Samples were in transit for 24 hours and sent to Food Engineering Laboratory, University of the Philippines Los Baños, Laguna, Philippines through LBC. Upon receipt, the fruits were washed with distilled water and allowed to ripen at ambient conditions. Degree of ripeness of the fruit was based on the fruit firmness and on the fruit's sugar/acid ratio as determined from the fruit's total soluble solids content and titratable acidity. All the reagents used in the analyses were of analytical grade.

Guyabano juice preparation

This study focused on single strength guyabano juice. A single strength juice is defined as a fresh juice extracted from a sound fruit, without adjustments and has not been concentrated (Bates *et al*., 2001). Guyabano fruits ripened at ambient conditions for 5 days postharvest were washed under running water, sliced, decored, hand peeled, deseeded, and the pulp was subjected to hydraulic screw pressing method to obtain the fruit juice. The extracted juice was filtered through double-layer cheesecloth and pasteurized immediately under temperatures 85°C, 90°C, and 95°C. For each temperature, the following holding times were used: 10, 20, 30, 40, 50, and 60 s.

Thermal inactivation experiments

Aliquots (10 ml) of guyabano juice were transferred to 0.06 mm thick aluminum tubes having a capacity of 15 ml (10.44 mm i.d. x 96.2 mm length) and fitted with a rubber stopper having a small plastic tube in it to allow for the insertion of a 24 AWG (American Wire Gage) Type T thermocouple. A perforation on the plastic tube was made immediately below the rubber stopper to allow trapped air to escape hence preventing outflow of juice when rubber stopper is fitted to the aluminum tube. The aluminum tubes were placed in a customized container made from welded wire mesh to ensure stability during heat processing (See Figure 1). Samples were heated in a circulating water bath set at the desired temperature. The temperature of the water bath, controlled to ± 0.3°C, was verified with a thermocouple connected to an analog-to-digital converter (Model ADC-1, Remote Measurement Systems, Inc., Seattle, WA), which in turn is connected to a computer (see Figure 2). The breadboard and relay box (Model G3NA-210B, Omron Corporation, Japan) served as switches for the heaters of the water bath. These relays were connected to a regulated DC power supply (locally fabricated, 5V DC output). Come-up times, the times necessary for the juice to reach the temperature of the surrounding water bath, were determined to be 107 s, 125 s, and 165 s for 85°C, 90°C, and 95°C, respectively. After pasteurization, samples were immediately cooled in ice water bath, transferred to polyethylene bag, quick-frozen in liquid nitrogen at -196°C, and stored at -18°C until analysis. Sample of freshly extracted guyabano juice was also collected and quick-frozen to measure the initial enzyme activity. Thawing was carried out by keeping the samples overnight at 4°C. Freeze-thaw process was also employed in tomato juice samples by Terefe *et al.* (2009) and the authors mentioned that freezing

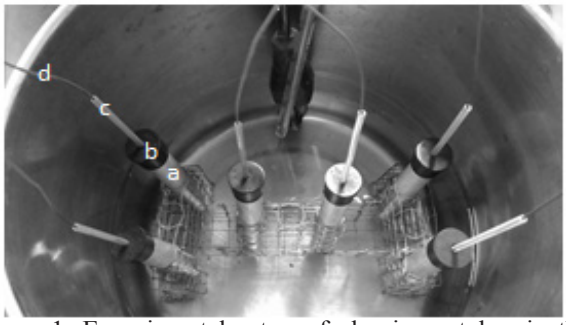


Figure 1. Experimental setup of aluminum tubes in the water bath during guyabano juice pasteurization. Setup includes (a) aluminum tube, (b) rubber stopper, (c) plastic tube, and (d) thermocouple.

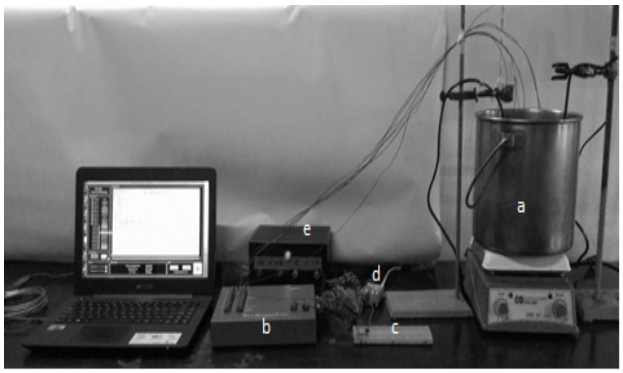


Figure 2. Experimental setup for the pasteurization of guyabano juice. Setup includes (a) water bath, (b) ADC-1, (c) breadboard, (d) relay box, and (e) DC power supply

and subsequent thawing did not have any measurable impact on the activity of the enzyme. Two independent experiments for each condition were performed.

Analytical measurements

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

Fruit firmness was determined with the use of AIKOH SX digital penetrometer bench-mounted on a fixed, rigid drill stand and fitted with a pointed (38.4 µm) plunger. The fruit was allowed to rest on a plate at the base of the stand and the tip of the plunger was lowered until it touched the skin of the fruit ensuring at the same time that the penetrometer display a zero value. A steady downward pressure was applied, i.e., three rotations of the mounting head, until the plunger has penetrated the flesh of the fruit up to the depth mark on the plunger. The reading on the penetrometer display was noted, and the plunger was removed. Six measurements were taken on each fruit: at each midpoint, 1/3 from the apical, and 1/3 from the stem end of two sides diametrically opposite each other. Determination of firmness was done without removal of fruit skin sections from each measurement sides.

pH was measured directly using a pH meter (pH610, Eutech Instruments) calibrated with standard buffer pH 4.0 and counterchecked with pH 7.0 buffer. A 50-ml freshly extracted juice sample was prepared and allowed to equilibrate at 20°C before measurements were made. pH meter electrode tip was immersed in the sample, and the reading was allowed to stabilize before the displayed pH value was recorded. Measurement was made in triplicate.

Total soluble solids (TSS) content was measured as °Brix using the digital hand-held ATAGO PAL-1 pocket refractometer equipped with automatic temperature compensation (ATC). Around 2-3 drops of the prepared fruit juice were placed onto the refractometer prism surface and the displayed °Brix value was noted. Measurement was made in triplicate.

Titratable acidity was determined by titration using phenolphthalein as indicator, following the procedure given by Nielsen (2010). A 10 ml aliquot of the freshly-squeezed juice was added with 10 ml distilled water and 1 to 2 drops of phenolphthalein indicator. The solution was titrated with standard 0.1N NaOH to a faint pink color. Titratable acidity was determined as follows:

$$\%\ TA = \frac{V_{NaOH} \times N_{NaOH} \times \text{meq. weight of predominant acid}}{\text{weight equivalent of aliquot in g}} \times 100 \quad (1)$$

where VNaOH and NNaOH is the volume and normality of NaOH used in titration, respectively. Titratable acidity was expressed as % malic and measurement was made in triplicate.

Viscosity of the guyabano juice processed under optimum pasteurization conditions was measured using Brookfield Viscometer (digital model DV-I Prime) with 20 rpm setting. Sixteen (16) ml juice sample was placed in the sample jar and the spindle attached to the viscometer was immersed into the sample. The viscometer was started and the displayed viscometer reading was noted. Measurement was made in triplicate.

Color measurement of the fresh and processed guyabano juice was made using a chromameter (Konica Minolta CR-10 Tristimulus Colorimeter). Color values of the sample through lightness *(L),* coordinate red/green *(a)*, and coordinate yellow/blue *(b)* were measured. Sample was placed on an optically clear disposable petri plate positioned on a flat surface. The receptor head of the chromameter was then positioned on the surface of the petri plate. Measuring button was pressed and L, a, and b values were read from the display. The procedure was done in triplicate.

PE activity measurements

PE extracts were prepared following the method proposed by Hagerman and Austin (1986). Twenty (20) ml of thawed juice sample was homogenized in 40 ml of 8.8% sodium chloride (NaCl) at a high speed at 4°C for 15 seconds. The homogenate was stirred with a magnetic stirrer for 15 minutes and then centrifuged at 4,000 x *g* for 50 minutes at 2°C using cooling centrifuge (HERMLE model Z326K). The supernatant obtained was assayed for PE activity.

PE activity was assayed by an acid base titration of free carboxylic acid groups produced by PE during hydrolysis of a pectin solution at pH 7.5 and at 30°C following the procedure described by Lee and MacMillan (1968) as provided by Siwach (2012). A 30 ml aliquot of solution containing 0.15 M NaCl and 0.5% (w/v) pectin was equilibrated to 30°C and pH was adjusted to about pH 7.5 – 7.55 using 1N sodium hydroxide (NaOH). Following the addition of 600 µl of sample, the pH was readjusted to 7.5 – 7.55 by 1 N NaOH and maintained for 10 minutes by addition of 0.01 N NaOH during enzymatic hydrolysis by use of a pH meter (pH610, Eutech Instruments). The volume of base added (VNaOH) was monitored as a function of time. All samples were measured in triplicate. Pectin and all reagents used were of analytical grade.

The slope $S = dV_{NaOH}/dt$ was determined in the linear part of the titration curve by linear regression

analysis. The slope is directly proportional to the PE activity of guyabano juice sample, PEU, which is calculated by equation 2 and expressed as microequivalent of acid from pectin per minute and ml juice sample, under the aforementioned conditions (Reed, 1975; Siwach, 2012).

$$PEU = \frac{S \times N_{NaOH}}{V_{sp}} \quad (2)$$

where S is the slope of titration curve (ml/min), N_{NaOH} is the normality (μequivalent/ml) of standard NaOH used during titration, V_{sp} is the volume (ml) of PE solution added into the reaction mixture.

Sensory evaluation

Following a randomized (complete) block design, guyabano juice processed under pasteurization time-temperature combination resulting to highest PE inactivation was subjected to general acceptability test and descriptive ranking of color and flavor against the unprocessed guyabano juice.

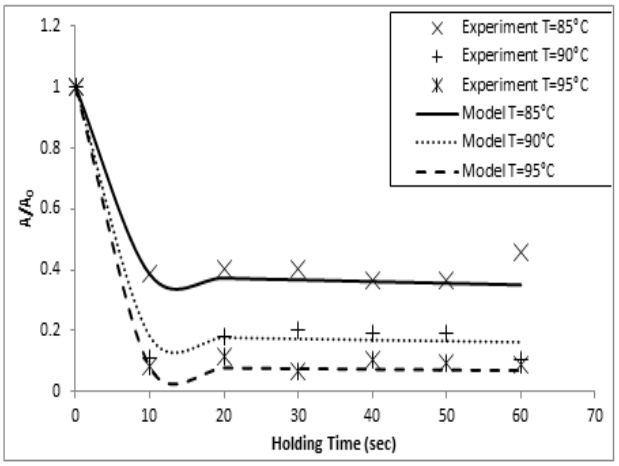
Data analysis

The results of analytical and sensory tests were subjected to Analysis of Variance (ANOVA) in Randomized Complete Block Design (RCBD) with significance of differences defined at $P < 0.05$. Least Significant Difference (LSD) test was used to determine the significant difference among the samples. All statistical and mathematical treatment was done using Statistical Tool for Agricultural Research (STAR) version 2.0.1 (STAR, 2014) which was developed by the Biometrics and Breeding Informatics group of the International Rice Research Institute (IRRI).

Results and Discussion

Residual PE activity

Freshly squeezed guyabano juice for pasteurization treatment had pH value of 3.5, TSS of 14.8°Brix, and a titratable acidity of 0.52% malic acid. At these physicochemical characteristics, unprocessed guyabano juice had a pectinesterase activity of 5.66 ± 1.19PEU/ml. Residual PE activity decreased as pasteurization temperature is increased. Lowest residual PE activity (6.5%) was measured from juice sample processed to pasteurization temperature of 95°C, while the highest PE activity (47.7%) was measured from juice sample processed to a pasteurization temperature of 85°C. This relationship, i.e., decrease in PE activity at higher temperature, was also observed by Rabelo *et al.* (2016) in their study on the thermostability of PE enzyme in soursop juice. This observation is in agreement with the fact that stability of an enzyme is a function of temperature. At higher temperatures, there is a decrease in the total amount of enzyme present because it is being denatured (Whitaker, 1994). Enzymes are proteins and although protein content is only slightly affected by temperature (Ohdaira *et al.*, 2010), the native, three-dimensional protein structure of the enzyme becomes destabilized at high temperature causing denaturation, thus, a decline in activity (Bisswanger, 2014).



Model: $A/A_0 = a \bullet \exp(-k_1 t) + (1 - a) \bullet \exp(-k_2 t)$

Figure 3. Thermal inactivation curves (model and experimental) of PE in guyabano juice at different temperatures.

The curves for heat-induced inactivation of guyabano juice PE at different temperatures are presented in Figure 3. As can be seen from the graph, heat-inactivation of PE is nonlinear. This nonlinear enzyme activity-time relationship has also been observed in various studies (Nath, 1996; Tadini *et al.,* 2004; Collet *et al*., 2005; Aghajanzadeh *et al.,* 2016; Díaz-Cruz *et al.,* 2016). In those studies, the nonlinear relationship was interpreted as a result of the formation of enzyme groups with differing heat stabilities or the presence of stable/labile isoenzymes, or could be due to a series-type enzyme inactivation kinetics. The presence of two isoforms of enzyme PE had been determined by Arbaisah *et al.* (1997b) when they carried out the purification and characterization of PE in soursop fruit. The illustration in Figure 3 also shows an abrupt decline in enzyme activity at 10s pasteurization treatment followed by a slight increase and an almost constant activity value at succeeding holding times. This slight increase in activity could be due to differences in proportion of the two isoenzymes present in guyabano juice. Figure 3 further shows that an increase in holding time results to a negligible change in enzyme activity. This observation is similar to that observed

Table 1. Multifactor analysis of variance for A/A_0 (mean residual activity after heat treatment related to initial enzyme activity) of pasteurized guyabano juice.

Source of variation	DF	Sum of squares	Mean square	F-value	Pr (>F)
Pasteurization temperature	2	0.3685	0.1843	156.19	0.0000
Holding time	5	0.0028	0.0006	0.47	0.7930
Error	10	0.0118	0.0012		
Total	17	0.3831			

ANOVA at 95% confidence level.

by Aghajanzadeh *et al*. (2016), who investigated the kinetics of PME thermal inactivation and cloud stability in sour orange juice. Their study showed that the high sensitive fraction of PME was rapidly inactivated during thermal treatment come-up time. After that, the authors observed that the slope of the PME inactivation curve decreased attributed mainly to the presence of heat resistant enzyme fraction wherein the extension of the heat treatment had no special effect on PME inactivation of heat resistant fraction. Similarly, in this study, the negligible increase in enzyme inactivation at prolonged holding times could be due to the presence of stable/labile isoenzymes. According to Nath (1996), at longer heating times, the contribution of the heat-labile isoenzyme to the total enzyme activity would have decayed to zero leaving only the heat-stable isoenzyme that is hardly inactivated by temperature applied.

Analysis of Variance (ANOVA) applied to the percent residual activity indicated that the enzyme inactivation was significantly influenced by pasteurization temperature (see Table 1). Least Significant Difference (LSD) test indicated that a significant difference existed between pasteurization temperatures of 85°C and 90°C and 85°C and 95°C but no significant difference between pasteurization temperatures of 90°C and 95°C. There was no statistically significant difference among the pasteurization time at each temperature.

Thermal inactivation kinetics of guyabano juice PE

Thermal inactivation of enzymes can often be described by a first-order kinetic model. This model is based on the assumption that inactivation of enzyme is brought about by a disruption in a single bond or structure (Gökmen, 2010). The equation model, adapted from Whitaker (1994), is shown in equation 3:

$$\ln\left(\frac{A}{A_0}\right) = -kt \qquad (3)$$

where k is the reaction rate constant at given temperature (s^{-1}) and t is the holding time in seconds.

Fitting of the above model to the experimental data resulted to a standard error of estimation (SEE) ranging from 0.13-0.22, r^2 value in the range of 0.72 to 0.93, and mean absolute deviation (MAD) ranging from 0.099-0.194. Although the model gave low MAD values, SEE and r^2 values were high thus another model was chosen to try a better fit. The alternative was based on the model presented by Fujikawa and Itoh (1996). The model takes into account the presence of several isoenzymes that can be separated in two groups, one more thermal resistant than the other and both thermally inactivated according to the first order decay kinetics. The equation model is shown in equation 4.

$$\frac{A}{A_0} = a \cdot \exp(-k_1 t) + (1 - a) \cdot \exp(-k_2 t) \qquad (4)$$

where k_1 is the rate constant of thermal inactivation of the thermal labile group of isoenzymes, k_2 is the rate constant of thermal inactivation of the thermal resistant group of isoenzymes, a is the activity fraction of the thermal labile isoenzyme group in relation to total enzyme activity.

In proposing the model, Fujikawa and Itoh (1996) considered a sample of enzymes consisting of two components which are thermally inactivated independently following first order kinetics under certain conditions. Each component is arranged according to the magnitude of the inactivation rate constant k_1, k_2 (the rate constant of component 1 and 2, respectively) where $k_1 > k_2 > 0$.

By definition:

$$A_i = A_{0i} \cdot \exp(-k_i t) \qquad (5)$$

where A_0 is the enzyme activity at time 0 and A is the enzyme activity at time t, and k_i is the rate constant of thermal inactivation of the ith component of enzyme.

$$A = A_1 + A_2 \qquad (6)$$

$$A_0 = A_{01} + A_{02} \qquad (7)$$

Dividing (6) by (7) and substituting (5), then:

$$\frac{A}{A_0} = \frac{A_{01} \cdot \exp(-k_1 t) + A_{02} \cdot \exp(-k_2 t)}{A_{01} + A_{02}} \qquad (8)$$

As explained by Fujikawa and Itoh (1996), when

Table 2. Estimated kinetic parameters for the inactivation of guyabano juice PE by heat treatment.

Pasteurization temperature (°C)	Model estimates[a,b]			R^2	Mean absolute deviation	Standard error of estimation[c]
	a	k_1 (min^{-1})	k_2 (min^{-1})			
85	0.6151	0.4501	0.0015	0.9696	0.0318	0.0131
90	0.8144	0.6707	0.0022	0.997	0.0345	0.0106
95	0.9070	0.8012	0.0029	0.9985	0.0201	0.0020
E_a (kJ/mol)		86.26	75.27			

[a] Model: $A/A0 = a \cdot \exp(-k_1 t) + (1 - a) \cdot \exp(-k_2 t)$
[b] Graphically determined as described in Fujikawa and Itoh (1996)
[c] 95% confidence level.

t increases A/A_0 approaches the value of $A_{02}\exp(-k_2t)/(A_{01} + A_{02})$ because the value of $A_{01}\exp(-k_1t)$ approaches zero more rapidly than that of $A_{02}\exp(-k_2t)$. Thus, as t increases, $\ln(A/A_0)$ approaches an asymptote expressed by the following equation:

$$\ln\left(\frac{A}{A_0}\right) = \ln\left(\frac{A_{02}}{A_{01} + A_{02}}\right) - k_2 t \qquad (9)$$

The value of k_2 in (9) can be graphically estimated from the slope of the straight portion of the inactivation curve, or by using a linear regression method when there is a high correlation coefficient for linearity. An intercept z (< 0) of the $\ln(A/A_0)$-axis by the asymptote can also be evaluated graphically or by linear regression method. Equation 9 is now transformed to:

$$z = \ln\left(\frac{A_{02}}{A_{01} + A_{02}}\right) \qquad (10)$$

If a is denoted as the activity fraction of the thermal labile isoenzyme group in relation to total enzyme activity,

$$\frac{A_{01}}{A_{01} + A_{02}} = a \qquad (11)$$

Then (10) becomes:

$$z = \ln(1 - a) \qquad (12)$$
$$a = 1 - \exp(z) \qquad (13)$$

To find the value of a, the value of z is substituted to (13). When the estimated values of k_2 and a and a value of A/A_0 for a given t are substituted into (4), a value of k1 can be evaluated.

Fitting the multi-component model to the experimental data resulted to a standard error of estimation (SEE) ranging from 0.0156 to 0.0520, r^2 value in the range of 0.9696 to 0.9985, and mean absolute deviation (MAD) ranging from 0.020-0.034. When compared to that of the first-order kinetic model shown in equation 3, it can be noted that deviations from fitting multi-component kinetic model to the experimental data were considerably lower. According to Weiers (2011), when evaluating alternative models, the best-fit model is the one having the lowest value of MAD. From the inactivation curves presented in Figure 3, it can be deduced that at temperatures below 95°C, only the labile PE fraction was inactivated as shown by the dispersion of data points from the model curve. This observation indicates that PE in guyabano juice has high heat stability but longer heating times at temperatures above 90°C would result to inactivation as shown by the concentration of data points along the model curve.

Parameters of the multi-component enzyme inactivation model shown in equation 4 were initially determined through STAR non-linear regression routine. However, the parameters a and k could not be estimated independently hence values of the parameters were calculated following the series of equations given in (9-13) with satisfactory results (MAD = 0.020-0.034). Table 2 presents the kinetic parameters for the multi-component model given in (4) as applied to the values of residual activity of pasteurized guyabano juice at unprocessed juice pH of 3.5. The heat stable PE activity was estimated to be 38.49% of total activity of guyabano juice PE based on heat treatment at 85°C. This value is higher than the heat stable PE fraction (5.11%) in orange juice estimated by Collet *et al.* (2005). However, the obtained rate constant for the heat labile PE fraction at 85°C in this study was in agreement to that obtained by the said authors. The estimated activation energy for inactivation was lower than those reported in various studies and in the range of E_a (140-629 kJ/mol) reported for the general inactivation reactions (Whitaker, 1994).

Sensory evaluation

The color description of the guyabano juice ranged from white with tinge of very light yellow for the unprocessed and creamy white for the pasteurized. Objective measurement of color showed that L value

for processed juice (45 ± 0.17) was higher than that of the unprocessed sample (40.9 ± 0.15) indicating that the processed juice has a lighter color than the unprocessed juice. This could be due to heat-linked degradation of pigments or inactivation of enzymes responsible for browning, i.e., polyphenol oxidase (PPO). In general, exposure of PPO to temperatures of 70°C-90°C destroys their catalytic activity; however, time taken for complete inactivation varies considerably with the food product (Göğüş *et al*., 2010).

Unprocessed guyabano juice had strong guyabano odor while the pasteurized sample was perceived as having a moderate guyabano odor. This indicates that processing might have destroyed some volatile components of the juice. Although there was no comparative study on the aromatic profiles of fresh guyabano juice versus the pasteurized juice, Ramesh (2007) mentioned that a small loss of volatile aroma compounds during the pasteurization of juices causes a reduction in quality. In terms of taste, ANOVA showed that there was no significant difference on the obtained scores of the juice samples. Both samples were perceived as having a strong guyabano taste.

There was no significant difference on the general acceptability of unprocessed and processed guyabano juice samples. The general acceptability of the samples ranged from like slightly to like very much. This indicates that processing of guyabano juice at 90°C for 10 s resulted to minor changes in sensory characteristics but did not significantly affect overall acceptability of the juice.

Pasteurized guyabano juice has a viscosity equivalent to 5.25 cP. This value is significantly different from the viscosity value of the unprocessed guyabano juice (3.62 cP). This decrease in viscosity could be due to the effect of pectin-degrading enzymes such as PE and polygalacturonase (PG). When present, such as in freshly squeezed juice, PE catalyzes the removal of the methyl groups from the polygalacturonic acid chain making the pectin susceptible to further degradation by PG through cleaving in the polygalacturonic acid backbone of the pectin thereby reducing the average lengths of the pectin chains. This degradation of the pectin chains reduces the viscosity of the juice (Anthon *et al.,* 2002). Heating the juice to above 90°C is believed to inactivate pectin-degrading enzymes rapidly, giving a product with high viscosity (Luh and Daoud, 1971).

Conclusion

In conclusion, processing of guyabano juice at 85°C, 90°C, and 95°C resulted to 62.74%, 89.05%, and 93.52% PE inactivation, respectively. Increasing the holding time from 10 s to 60 s did not significantly increased PE inactivation. Pasteurization at 90°C for 10 seconds did not significantly affect the overall acceptability of the juice. A distinct isoenzyme model, i.e., three-parameter multi-component first order model, was used to predict the pectinesterase inactivation of pasteurized guyabano juice. Results presented in this study will contribute to a more accurate design of guyabano juice pasteurization process to product of sensory quality and microbial stability. However, future studies on the validation of the isoenzyme model need to be conducted by running experiments with guyabano juice adjusted to standard juice pH and TSS values and verify if the observed inactivation trend occurs. With the reconstitution of the juice, it would be important to study the effect of sugar, and other agents added during standard juice pH and TSS adjustment, to the enzyme inactivation.

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