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Preliminary characterization of the enzyme polyphenol oxidase and rheological behavior from *Averrhoa carambola* juice

Caracterización preliminar de la enzima polifenol oxidasa y comportamiento reológico del jugo de *Averrhoa carambola*

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ABSTRACT

Averrhoa carambola fruit represents a potential as an agro-industrial production line. A restriction on the use of this exotic fruit is the susceptibility to enzymatic browning, affecting nutritional and visual acceptance. The aim of this study was to determine the physical and chemical composition of *A. carambola* at three stages of maturity. The polyphenol oxidase enzyme has also been characterized on the fruits and juices. Also, the enzymatic effect on the ripening stages and the fruit juices flow behavior were equally determined using different rheological models. The increasing in degrees Brix (unripe (UR) 6.63 ± 0.25, intermediate (IN) 6.8 ± 0.10 and ripe (R) 8.26 ± 0.37) and the decreasing of the pectinic content (UR 4.35% ± 0.98, IN 3.6% ± 1.26 and R 2.25% ± 0.76) could be considered as indicators of fruit ripening. The protein content and levels of organic acids decreased during the ripening of fruit was observed, indicate a high metabolic rate during this process. For all stages, the polyphenol oxidase exhibited a maximum activity at pH 8 and 40 °C. In the fruit aqueous fraction, the enzyme would be an appropriate indicator in industrial handling (temperatures below 20 °C and pH less than 7), which would control enzymatic browning. According to the rheological study, the viscosity variation (η: UR (13.4 to 1.1), IN (15.4 to 1.4), R (69.6 to 2.9)) with temperature changes (10 to 50 °C) is adjusted to the Arrhenius equation, whereas the effect of soluble solids content on the samples viscosity was accurately described by an exponential equation. This appears to be the first work to study the rheological properties and polyphenol oxidase enzyme of *A. carambola* fruit in our country, the information provided from this study could be helpful to the successful development of new food functional products.

Key words:
Carambolo
Enzymatic browning
Rheology
Tropical fruits
Agroindustry
Peroxidase enzyme

RESUMEN

Averrhoa carambola representa un potencial como una línea de producción agroindustrial. Una restricción en el uso de esta fruta exótica es la susceptibilidad al pardeamiento enzimático, que afecta su aceptación nutricional y visual. En este estudio se determinó la composición física y química de *A. carambola* en tres estados de madurez; además se caracterizó la enzima polifenol oxidasa de fruta y zumos. De igual manera, se evaluó el efecto enzimático y el comportamiento al flujo del jugo en las etapas de maduración de la fruta utilizando diferentes modelos reológicos. El incremento en los grados Brix (verde (V) 6,63 ± 0,25, intermedio (IN) 6,8 ± 0,10 y maduro (M) 8,26 ± 0,37) y la disminución del contenido pectínico (V 4,35% ± 0,98, IN 3,6% ± 1,26 y M 2,25% ± 0,76) podrían considerarse como indicadores de la maduración del fruto. Se observó que el contenido de proteína y los niveles de ácidos orgánicos disminuyeron durante la maduración de la fruta, indicando una alta tasa metabólica durante este proceso. Para todas las etapas, la máxima actividad de la polifenol oxidasa se encontró a pH 8 y 40 °C. En la fracción acuosa de la fruta, la enzima es un indicador apropiado en el manejo industrial (temperaturas por debajo de 20 °C y pH inferior a 7), lo que controlaría el pardeamiento enzimático. El estudio reológico mostró que la variación de la viscosidad (η: V (13,4 a 1,1), IN (15,4 a 1,4), M (69,6 a 2,9)) con los cambios de temperatura (10 a 50 °C) se ajusta a la ecuación de Arrhenius, mientras que el efecto del contenido de sólidos solubles sobre la viscosidad de las muestras fue descrito con precisión por una ecuación exponencial. Este parece ser el primer trabajo para estudiar las propiedades reológicas y la enzima polifenol oxidasa de *A. carambola* en nuestro país; la información proporcionada aquí podría ser de utilidad para el desarrollo exitoso de nuevos productos funcionales alimentarios.

Palabras claves:
Carambolo
Pardeamiento enzimático
Reología
Frutos tropicales
Agroindustria
Enzima peroxidasa

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The fruit world market is mainly supplied by developed northern hemisphere countries (USA, Canada and some European countries), with species such as apple, pear, plum, and cherry, among others, or subtropical regions by producers of orange, tangerine, lemon, etc.; there are some southern hemisphere countries included such as Argentina, Chile and Brazil, which export grape, pear, apple, cherry, among others (Giacinti, 2001). The only tropical fruit with which Colombia competes in that market is banana, which has a prominent place in its production (Orduz-Rodríguez and Mendoza, 2002).

However, the geostrategic position of the Colombian Andes confers them different climatic zones and land availability, nominating them as an area with potential for developing agricultural business with a diversity of fruit products, among which, carambolo (*Averrhoa carambola* L.), cape gooseberry (*Physalis peruviana* L.), small pineapple (*Bromelia karatas*) and the purple mangosteen (*Garcinia mangostana*) may be mentioned, just to name a few examples. Nevertheless, the main restriction on the use of exotic Colombian fruits is the lack of technical information on many of them, underestimating their potential. Another limitation is the fruit susceptibility to enzymatic browning, which affects both nutritional and visual acceptance (Ma *et al*., 2010).

The carambolo (*Averrhoa carambola* L., Oxalidaceae), is a small tree that grows best in the hot humid tropic. This species is established in smallholder farms in the foothill region of Meta (Colombia) for household consumption and sale in small supermarkets. Owns a fruit with good nutritional content and multiple uses both fresh and processed. There exist the acids and sweets types, in which it finds a lot of varieties. The acid type is smaller, very sour, richly flavored and more oxalic acid; the sweet type is larger, mild-flavored, rather bland and with less oxalic acid (Manda *et al*., 2012). Like most fruit, carambolo has deterioration problems during storage or when processed. Cutting and other mechanical procedures damage the walls of the fruit and allow oxygen to penetrate, resulting in darkening, which is better known as enzymatic browning reaction (Márquez *et al*., 2015). The color change that leads to golden brown is a consequence of enzymatic reactions involving the polyphenolic compounds (Sellés-Marchart, 2007), specifically caused by browning enzymes, generically known as polyphenoloxidase (Fang, 2007).

Moreover, in both the processing and the storage stages, juices suffer continuous changes in soluble solid contents, because they undergo a water removal stage through evaporation, where they are subjected to different temperatures. They continuously change their properties such as viscosity, given that the conditions depend on concentration and temperature. Knowledge of these changes can be of prime importance in the manipulation, calculation and design of all the unit operations involved in the juice processing, mainly those based on heat transfer and motion.

Some reports are available on the physical (Bezerra *et al*., 1989), physical chemical characterization of the ripening (Mitcham and McDonald, 1991) and chemical composition (Herrman, 1994) of carambolo fruit. The mineral, amino acids, volatile flavors and carotenoid compositions of the fruit have been reported (Becerra *et al*., 1992). Manda *et al*. (2012), reviewed data on the taxonomy, botanical description of the plant, its distribution and ecological requirement of this fruit. Also, the phytochemical and pharmacological profile were reviewed by Thomas *et al*. (2008) and Gheewala *et al*. (2012), whereas Saghir *et al*. (2013), investigated on traditional uses to pharmacological activities from star fruit and Dasgupta *et al*. (2013), reviewed and compile all the updated information on botany, phytochemical and pharmacological properties, drug interaction, contraindication and toxicity studies of *A. carambola*.

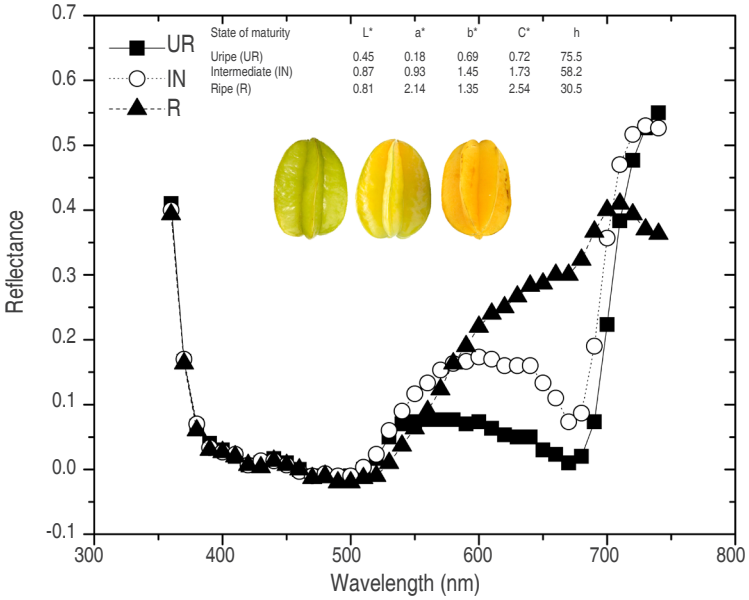
In Colombia, Grajales-Agudelo *et al*. (2011) studied the effect of freezing and heating rates on rehydration of lyophilizated fruit; and Mateus-Cagua and Orduz-Rodríguez (2015) presented a brief description of the principal zones where *A. carambola* crop has been established. Also, Gonzalez *et al*. (2001), characterized an acid variety of carambolo adapted to the Amazonian foothills conditions, through physical, chemical and physiological analyses from the setting to the commercial maturity, in function of study of fruits growth and development and to determine the appropriates parameters to indicate the harvest moment. Nevertheless, processed carambolo products are not available in our market and very little work has been done on the processing of carambolo in Colombia. To the best of our knowledge, from literature survey, no work has been carried out on the chemical composition, the characterization of the enzyme polyphenol oxidase

and rheological behavior from *A. carambola* juice in our country.

The aim of this study was to determine the physical and chemical composition, assessment of cell wall content and make the phytochemical screening on carambolo fruit at three stages of maturity. Similarly, characterizing the polyphenol oxidase enzyme and its effect on the ripening stages of *A. carambola* and flow behavior of fruit juices was also studied using different types of rheological models, all of which will help to a better understanding of fruit oxidative processes and will contribute, at least in part, to preserve good organoleptic properties of the plant material.

Materials and Methods

Harvesting of carambolo fruits was held at municipality of Lleida-Tolima (366 m, 26 °C and 100 mm rainfall per year). A plant specimen was collected with leaves, fruits and flowers, and was taxonomically determined at TOLI Herbarium in the University of Tolima, Colombia. The collected material was taken to the laboratory, where it was washed and classified depending on its ripening degree and plant health, according to the following criteria: Unripe stage (UR): completely green epicarp, intermediate stage (IN): whitish epicarp, accepting green edges, ripe stage (R): completely yellow or orange epicarp (Figure 1).



Indice	Color	Descripción
1	Yellow-Green 1	Something yellow light green color
2	Yellow-Green 2	Greenish yellow
3	Brown-orange 1	Opaque yellow
4	Brown-orange 2	Little intense orange opaque
5	Brown-orange 3	Intense orange opaque, completely colored fruit

(Gonzalez *et al.*, 2001)

Figure 1. Color table of *A. carambola* fruit at three stages of maturity (Using the standard illuminant D65 and 10^0).

From each stage (± 500 g), and through a juice extractor (Black and Decker, USA), the respective aqueous fraction was obtained, which was clarified by centrifugation (1613 g, 15 min), and from now on is called "juice;" another part of the plant material was dried in an industrial stove (70 to 80 °C for three days); nuts were ground to reduce particle size and stored in amber labeled bottles under refrigeration (4 °C) until use.

Physical and chemical composition of *A. carambola* fruits and juice at three stages of maturity

Twenty-five fruits of each maturity group were individually analyzed for physical characteristics. Length and diameter were measured with a Vernier caliper. The measurement of length was made between apex and stem. The maximum width of the fruit, measured in the direction perpendicular to the polar axis, was denominated as diameter. The measurements for the ridges were made for length and its maximum width. On the other hand, moisture, ash, lipid, total (TC) and reducing (RC) carbohydrates, hexoses, pentoses, Vitamins C, A and E; mineral elements (major and minor) contents were estimated according to the methods described by determining the nutritional content of raw plant material (AOAC, 2005). The study is complemented with the information from the cell wall content and preliminary phytochemical screening of star

fruit. The physical characterization of the juice was made by determination of density, pH, total solids, firmness (Bertuzzi penetrometer, Italia), refractive index, degrees Brix and total acids (AOAC, 2005); the pectin content was also determined (Ismail *et al*., 2012).

Assay of polyphenol oxidase (PPO) activity from Carambolo

PPO enzyme activity was performed with a Helyos UV/VIS spectrophotometer (Thermo Fisher Scientific, UK) by measuring the initial rate of quinone formation, by an increase in absorbance at 420 nm (20 min intervals for one hour at 27 °C) by using catechol as substrate. The assay reaction contained catechol (2.5 mL, 0.5 M in a sodium citrate buffer 0.1 M/sodium phosphate 0.1 M, pH 7) and 1 mL of freshly prepared crude enzyme extract (Falguera *et al*., 2012). A unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 1.00 min^{-1} (U min^{-1}), under the conditions of the assay.

pH and temperature optimums

To find the best activity conditions of the PPO enzyme, pH scan (range of 7 to 8.5, room temperature) was performed using catechol in two buffer solutions: sodium citrate buffer (0.1M, pH range of 4.5 to 6), and sodium phosphate buffer 0.1 M, pH 7. The optimum temperature obtained from this study was used in other determinations. The effect of temperature on enzyme activity was evaluated changing the variable from 10 to 60 °C (intervals of 10 °C for 60 min, at optimum pH). The reaction mixture contained 3.9 mL of phosphate buffer (pH 7.0), 1.0 mL of 50 mM substrate and 0.1mL of crude PPO extract (Calan *et al*., 2010). The optimum temperature obtained from this study was used in other determinations

Quantification of protein

The quantification of protein by the method of Lowry (Pavel *et al*., 2013), complements the information. Under alkaline conditions the divalent copper ion forms a complex with peptide nitrogen in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. Absorbance readings were interpolated on the curve made with bovine serum albumin (BSA) at different concentrations (200 to 800 µg mL^{-1}).

Peroxidase enzyme activity (POD)

POD activity was estimated by the Andrade-Cuvi *et al*. (2010) method, with some modifications. The measuring cell, to a final volume of 2 mL, contained juice (200 µL) and a guaiacol mixture 1.8 µL (60 mM:H_2O_2 100 mM) in phosphate buffer 20 mM, pH 8.0; this mixture was kept at 25 °C. The blank solution was previously boiled in water bath (92 °C, 10 min), which ensures the enzyme denaturation and the not reaction with the substrate. One unit of enzyme activity was defined as the change in absorbance per min. G fresh tissue.

Rheological measurements

The rheological measurements of the samples tested were carried out with a Rheometer Haake RS 80 RheoStress, equipped with a concentric cylinder measuring system Z40-DIN (1.0847 radius ratio). For temperature control, there was used a programmable thermostat Thermo Haake C25 P, which allows setting temperature with a variation of ± 0.2 °C. To evaluate rheological behavior, samples were sheared at a constant rate (100 s^{-1}), and measured the variation over time. In the study of the flow behavior, the samples were previously sheared at 400 s^{-1} (3 min), followed by a ramp shear rate values of ascending and descending. To study the flow behavior of the carambolo juice, there were used different rheological models at different temperatures (10, 20, 30, 40, 50 °C):

Newton equation: $\sigma = \eta \cdot \dot{\gamma}$ (1)

Bingham equation: $\sigma = \sigma_0 + \eta' \cdot \dot{\gamma}$ (2)

Power law (Ostwald de Waele equation): $\sigma = K \cdot (\dot{\gamma})^n$ (3)

Herschel-Bulkley equation: $\sigma = \sigma_0 + K \cdot (\dot{\gamma})^n$ (4)

Where, σ is shear stress (Pa), σ_0 threshold stress (Pa), $\dot{\gamma}$ shear rate (s^{-1}), η viscosity (Pa s), *K* consistency index (Pa s^n), and *n* flow behavior index (dimensionless).

Statistical Analysis

Each of the characterization tests of the PPO was individually analyzed and data was reported as the means of three determinations (n=3x3) ± SD. For the analysis of enzyme kinetics, a multivariate analysis of variance MANOVA was applied, consisting of three factors: enzyme kinetics, ripening stage, and pH-temperature. Additionally, multiple comparisons Fisher's

LSD post hoc tests were then applied to identify noted differences among ripening stages. A P value of less than 0.05 was considered statistically significant. The statistical program InfoStat/Professional® Version1.2, was used for these analysis. Experimental results of carambolo juice were adjusted to different rheological models using the statistical data processing software Statgraphics (STSC Inc. Rockville, Md, USA, version Plus 5.1). Both the settings and estimates of the parameters were calculated at a significance level of 95%.

RESULTS AND DISCUSSION

Physical and chemical composition of fresh fruit and juice characterization at three stages of maturity

The plant of interest in this paper was identified at Toli Herbarium of the Tolima University with the name of *Averrhoa carambola* Linnaeus (COL 10121), and it was described as belonging to the Oxalidaceae family. This is a very cold-susceptible plant species, its ideal temperature is between 26 and 28 °C. It is not very demanding on soil conditions, but it grows best in soils with plenty of organic matter. The pH can range from 5.5 to 6.5 (Mateus-Cagua and Orduz-Rodríguez, 2015).

The current study reveals a large variation in the physical and chemical composition of the carambolo fruit during maturation from green mature stage to ripe fruits. Table 1 presents some certain physical parameters of the star fruit juices obtained, which complement the physical and chemical characteristics about the fruit of this plant species. It reveals that fruits become more turgid as ripening progresses, substituting biomass for water content. In relation to the decrease in firmness with ripening progress in carambolo, this behavior is typical of fleshy berries, in which the parenchymal tissue accumulates large amounts of water, making it a very succulent fruit. Similar results were reported by other authors (Narain *et al*., 2001; Navarro, 2011). On the other hand, the fruit equatorial and longitudinal diameters suggest that growth occurs initially by longitudinal cell division and later by cell elongation mainly in the mesocarp. While the accumulation of water improves physical fruit appearance and decreases its acid concentration, also makes it prone to pathogen attack, just as its mechanical strength decreases, exposing it to physical deterioration from bruises.

Also, the values specified in the table show that the refractive index, density and pH do not seem to be related to the maturation stages, which is possible to see through the values of total solids, degrees Brix and the content of pectic substances. Furthermore, organic acids are a direct respiratory power source both in animal and plant cells. The final product of ß-oxidation (Acetyl-CoA) is converted into CO_2 and H_2O, preferably via the Krebs cycle (Cheftel *et al*., 1989); thus, the fruit cells are able to use them as a respiratory substrate and convert them into sugars. This explains, at least in part, that the levels of organic acids descend during the ripening of carambolo: UR 0.234%, IN 0.174% and R 0.099%; indicate a high metabolic rate during this process, and that the fruit cells are capable of using them as a source of breathing energy during the high respiratory rate required with ripening advance. Many of them are really contributing to the flavor (Da Conceicao-Neta *et al*., 2007). The increasing in degrees Brix and the decreasing of the pectinic content could be considered as indicators of fruit ripening. The progressive decrease of the pectinic contents could be a response to the decrease in fruit firmness and in the acidity. During ripening of the fruit, protopectins are depolymerized and solubilized strongly as a result of the action of pectolytic enzymes, pectin methylesterases, polygalacturonases and glycosidases located in the middle lamella of the cell wall (Cheftel *et al*., 1989).

The titratable acidity, reducing sugars, vitamin A and tannin contents of the fruits were significantly different in fruits among all stages of maturity, and calcium and sodium contents of the fruits were higher in the rape stage than the fruits at green mature or half-ripe stages. The presence of polyphenols, flavonoids, saponins, phenylpropanoids and terpenoids was also observed.

Assay of polyphenol oxidase (PPO) activity from carambolo

Effect of pH and temperature. As seen in Figure 2, optimal pH for carambolo PPO was found from 7.5 to 8.0 for catechol substrate. The peak for all stages was reached at pH 8. There is dependence between enzyme activity and the increase of pH. The increased activity is observed in the UR, but is not significantly different to the other (P>0.05). Similar results were achieved in apple (Manohan and Wai, 2012). Nevertheless, the common range of pH for PPO activity in other fruits is between pH 6.0 and 7.0 (Fang, 2007).

Table 1. Physical parameters of carambolo fruit juice in three stages of maturity.

	Parameters	Ripening stage		
		Unripe	Intermediate	Ripe
Fruit	Whole fruit weight (g)	71.65 ± 2.68	44.76 ± 1.22	22.21 ± 4.22
	Edible part - pulp (%)	95.67 ± 1.08	93.23 ± 0.95	87.04 ± 1.01
	Seed (%)	0.86 ± 0.01	1.75 ± 0.01	3.08 ± 0.01
	Equatorial diameter (cm)	3.48 ± 0.02	4.09 ± 0.02	5.17 ± 0.01
	Length diameter (cm)	5.98 ± 0.01	6.99 ± 0.03	8.04 ± 0.02
	Fruit firmness (kg cm^{-1})	8.56 ± 0.30	7.20 ± 0.35	4.50 ± 0.25
	Maturity index (°Brix/acid)	28.30 ± 0.00	39.10 ± 0.00	83.40 ± 0.00
	Humidity (%)	89.56 ± 0.00	91.51 ± 0.00	96.08 ± 0.00
	Dry meterial (%)	10.44 ± 0.00	8.49 ± 0.00	3.92 ± 0.00
	Fiber (%)	8.37 ± 0.00	7.29 ± 0.00	7.80 ± 0.00
	Protein (%)	6.23 ± 0.00	5.47 ± 0.00	6.71 ± 0.00
	Lipids (%)	5.48 ± 0.00	6.01 ± 0.00	4.11 ± 0.00
	Ash (%)	3.80 ± 0.00	1.80 ± 0.00	4.50 ± 0.00
	Total carbohydrates (mgEG/g)	162.83 ± 0.00	169.90 ± 0.00	180.97 ± 0.00
	Reducing carbohydrates (mgEG/g)	53.52 ± 0.00	66.43 ± 0.00	72.38 ± 0.00
	Hexoses (mgEG/g)	44.82 ± 0.00	57.10 ± 0.00	61.18 ± 0.00
	Pentoses (mgER/g)	8.69 ± 0.00	9.32 ± 0.00	11.19 ± 0.00
	Vitamin C (mgEAA/100g)	14.83 ± 0.01	13.61 ± 0.01	8.35 ± 0.01
	Vitamin A (mgEβC/100g)	11.65 ± 0.01	5.08 ± 0.01	4.25 ± 0.01
	Vitamin E (mg/αT100g)	139.29 ± 0.01	44.76 ± 1.23	42.59 ± 0.01
	Major mineral elements (%)	Ca K Mg Na* P	Ca K Mg Na* P	Ca K Mg Na* P
		0.31 1.39 0.24 221.6 0.02	0.39 1.34 0.13 54.40 0.07	0.36 0.61 0.12 28.50 0.02
	Minor mineral elements (mg kg^{-1})	Fe Zn Cu Mn S** B	Fe Zn Cu Mn S** B	Fe Zn Cu Mn S** B
		119.00 37.20 1.59 5.80 0.18 434	72.33 22.66 2.22 2.35 0.21 371	208.33 15.33 1.91 N.D 0.30 139.50
	NDF	20.11 ± 0.00	22.54 ± 0.00	21.90 ± 0.00
	ADF	19.95 ± 0.00	18.31 ± 0.00	16.31 ± 0.00
	LDF	9.83 ± 0.00	12.20 ± 0.00	12.72 ± 0.00
	Cellulose	3.34 ± 0.00	6.23 ± 0.00	2.73 ± 0.00
	Lignin	8.98 ± 0.00	11.57 ± 0.00	12.24 ± 0.00
	Hemicellulose	6.94 ± 0.00	4.11 ± 0.00	6.45 ± 0.00
	Sílica	0.85 ± 0.00	0.63 ± 0.00	0.48 ± 0.00
	Cell content	79.89 ± 0.00	77.46 ± 0.00	78.10 ± 0.00
Juice	Density (g cm^{-3})	0.35± 0.00	0.35 ± 0.00	0.36 ± 0.01
	pH	3.75 ± 0.01	3.71 ± 0.00	3.68 ± 0.00
	Titratable acidity (% Oxalic acid)	0.23 ± 0.10	0.17 ± 0.05	0.10 ± 0.10
	Refractive index	1.34 ± 0.00	1.34 ± 0.00	1.34 ± 0.00
	Total Solids (%)	6.00 ± 7.30	6.12 ± 7.37	5.08 ± 1.32
	°Brix	6.63 ± 0.25	6.80 ± 0.10	8.26 ± 0.37
	Pectin (%)	4.35 ± 0.98	3.60 ± 1.26	2.25 ± 0.76
	Carbohydrates	+++	++++	++++
Phytochemistry screening (Extract Pulp)	Reducing carbohydrates	++	+++	++++
	Carbohydrates nonreducing	++++	++	++
	Polyphenols	++++	+++	+++
	Tannines	+++	+	+
	Flavonoids	+++	+	++
	Alkaloids	N.D.	N.D.	N.D.
	Anthocyanins	N.D.	N.D.	N.D.
	Saponins	+	+	N.D.
	Phenylpropanoids	++	+	++
	Iridoids	N.D.	N.D.	N.D.
	Lactones	+++	+	++
	Cardiac glycosides	N.D.	N.D.	N.D.
	Terpenes / steroids	+++	++	++
	Anthraquinone	N.D.	N.D.	N.D.

* mg kg^{-1}

** %

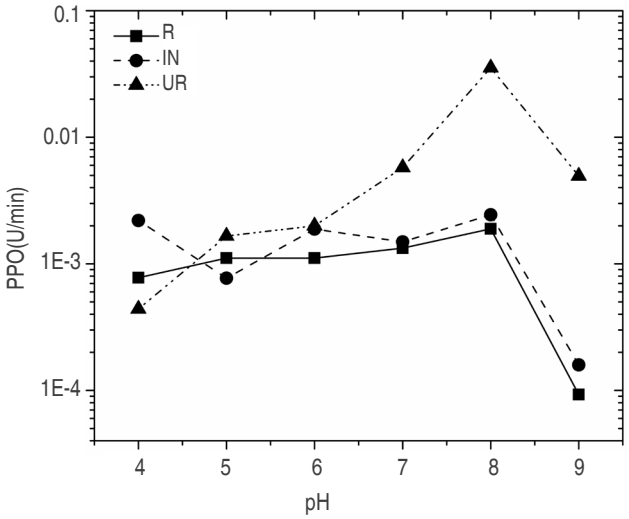


Figure 2. Effect of pH vs. activity of the PPO enzyme in three stages of carambolo ripening.

It was found that optimum pH value of carambolo PPO was higher than that reported in the literature using catechol as a substrate (Dogan *et al*., 2013). Variations in the optimum pH in diverse vegetables can be explained because of the different substrates used in the activity evaluation and the location of the enzyme in the plant or in the cell (Assis *et al*., 2006). The temperature influence on the activity of the PPO of *A. carambola* for three ripening stage is shown in Figure 3. The maximum enzyme activity for all stages was revealed at 40 °C. However, there was no significant difference (P>0.05) between the evaluated temperature (10 to 60 °C), but between stages (P<0.05).

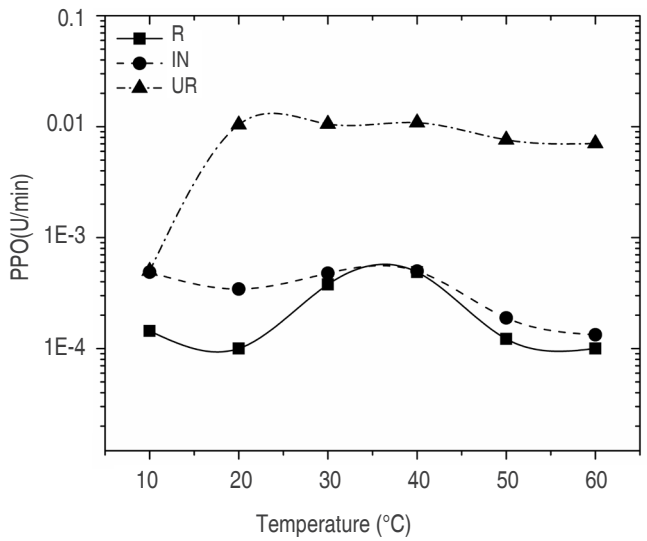


Figure 3. Effect of temperature vs. the PPO enzyme activity in all three carambolo stages of ripening.

All juices showed a possible inhibition from 40 °C and low activity at 10 °C, similar to the reported by Zhang and Shao (2015). In general, PPO exposure to temperatures between 70 to 90 °C destroys its catalytic activity, but the time required for the inactivation depends on the vegetable product. Chutintrasri and Noomhorm (2006) found that the pineapple PPO reduces its activity by 60% after heat exposure between 40 to 60 °C (30 min).

Polyphenoloxidase is an oxide reductase copper-dependent, also known as phenolase or catechol oxidase. It participates as a catalyst in the reaction between catechol and oxygen, resulting in the formation of quinones, which are able to react with cell components leading to formation and precipitation of a dark polymer similar to melanin, a phenomenon known as enzymatic browning (Mejía-Doria *et al*., 2014). Thus, PPO properties are also of interest, just as the information obtained in these studies can be of use to drawn important conclusions for food chemistry.

Quantification of protein concentration (enzyme)

Protein content in carambolo decreased with ripening: 390.2 mg g^{-1}, 75.9 mg g^{-1} and 39.4 mg g^{-1}, for the UR, IN and R, respectively; which is lower than earlier reported for the same fruit (Edem *et al*., 2008). Differences between our data and the literature could be explained by soil nutrients deficiency, for example, with low organic matter.

Peroxidase enzyme activity (POD)

The activity of this enzyme during the carambolo ripening was similar to that shown by the PPO, it means a decreasing tendency from the unripe fruit (0.0076 UPOD h^{-1}), intermediate (0.0045 UPOD h^{-1}) and ripe fruit (0.0028 UPOD h^{-1}). This implies that when peroxidase shows low activity, the increase of hydrogen peroxide is allowed and promotes contact between phenols and polyphenoloxidase, leading to browning process (Mizobutsi *et al*., 2010).

The peroxidase is an enzyme that controls the physiological growth, differentiation and development of the plants. POD activity in the carambolo could also be a result of the participation of the enzyme in the last step of polymerization of cinnamyl alcohols to form lignin, showing the process of lignification in the darkening of tissues subjected to mechanical damage. It could also be noticed that in senescence, H_2O_2 levels increase and lysis of vacuoles containing phenols occurs (Cheftel *et al*., 1989), which would provide suitable conditions for PPO activity to increase significantly, and allow the observed enzymatic browning in *A. carambola* fruits.

Rheological behavior of carambolo

The study showed that there was no variation of shear thixotropic time. The variation of shear strength concerning the strain rate was adjusted to different rheological models (power law, Herschel-Bulkley equation, Bingham equation and Newton equation).

Figure 4 shows the rheograms corresponding to the ripe state of carambolo juices (10 °C), for different soluble solid contents. For the other states of ripening and different temperatures, there are analog rheograms obtained to those illustrated in Figure 4 (data not shown). Depending on the sample, the best adjustments were obtained with the Bingham and Newton equations. Results of these adjustments for the three states of ripening studied are shown in Tables 2, 3 and 4. Is well known, that for a determined

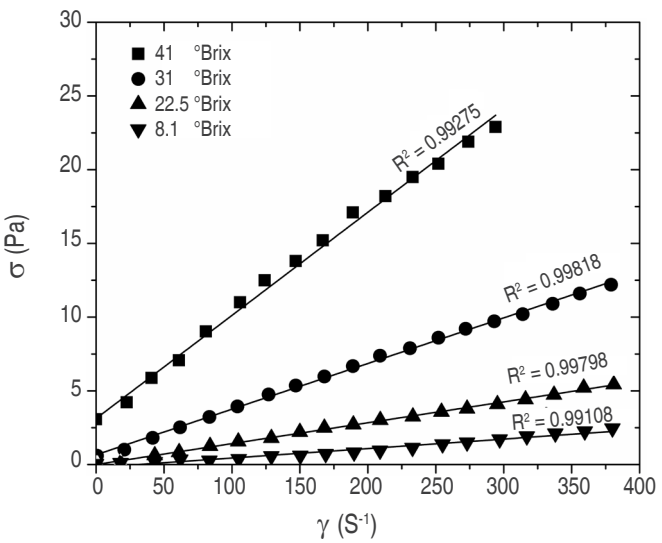


Figure 4. Rheogram of mature stage carambolo juice at 10 °C with different soluble solids.

Table 2. Rheological flow behavior of ripening stage carambolo juice, depending on temperature and soluble solids

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
41.6	10	3.06	69.60	0.992
	20	2.33	47.30	0.994
	30	1.57	36.30	0.996
	40	1.13	28.80	0.998
	50	0.89	25.10	0.999
31.5	10	0.87	29.30	0.998
	20	0.54	21.70	0.999
	30	0.36	16.50	0.999
	40	0.25	13.10	0.999
	50	0.18	10.90	1.000
22.5	10	0.22	13.90	0.999
	20	0.14	10.60	0.999
	30	0.08	8.30	0.999
	40	0.06	6.80	0.999
	50	0.04	5.70	0.997
8.1	10	0.13	5.30	0.997
	20	0.09	4.40	0.994
	30	0.08	3.70	0.997
	40	0.06	3.30	0.990
	50	0.05	2.90	0.992

Table 3. Rheological flow behavior of intermediate maturation stage carambolo juice, depending on temperature and soluble solids.

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
40.1	10	0.07	15.10	0.999
	20	0.03	11.30	1.000
	30	0.01	8.40	0.999
	40	0.00	6.90	0.997
	50	0.00	6.10	0.985
31.6	10	0.02	10.90	0.999
	20	0.01	8.10	0.999
	30	0.00	6.30	0.999
	40	0.01	5.00	0.999
	50	0.00	4.60	0.981
21.3	10	0.00	6.50	0.999
	20	0.00	4.90	0.999
	30	0.00	3.80	0.999
	40	0.00	3.20	0.994
	50	0.00	2.70	0.994
7.2	10	0.00	2.80	0.999
	20	0.00	2.20	0.999
	30	0.00	1.80	0.999
	40	0.00	1.50	0.999
	50	0.00	1.40	0.998

state of ripening and soluble solids content, the viscosity of the samples decreases as the temperature increases (Tables 2, 3 and 4). In the same way, for a given state of ripening and a certain temperature, the viscosity decreases as well as soluble solids content. It is evident that the threshold stress responses are very small and only in the ripe state samples have non-zero values.

Moreover, threshold effort values less than unity can be neglected (Syang-Peng and Mei-Sia, 2013). It can be said that the only samples with some plastic behavior are juices from ripe fruit with a soluble solids content of 41.6 °Brix, although their values are close to unity. Therefore, it can be considered that the samples tested have an almost Newtonian flow behavior.

Regarding the influence of the ripe stage, it is observed that for a given concentration of soluble solids and the same temperature, the samples have a higher viscosity as the ripening state increases.

Tabla 4. Rheological flow behavior of unripe stage carambolo juice, depending on temperature and soluble solids.

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
42.5	10	0.03	13.40	1.000
	20	0.00	10.00	0.999
	30	0.00	7.40	0.999
	40	0.00	6.20	0.989
	50	0.00	5.60	0.975
33.0	10	0.00	10.80	1.000
	20	0.00	7.90	0.999
	30	0.00	6.60	0.982
	40	0.00	5.20	0.984
	50	0.00	4.20	0.984
22.0	10	0.00	6.70	0.997
	20	0.00	5.20	0.987
	30	0.00	4.20	0.979
	40	0.00	3.60	0.969
	50	0.00	3.10	0.968
5.2	10	0.00	2.70	0.973
	20	0.00	2.20	0.965
	30	0.00	1.70	0.979
	40	0.00	1.30	0.997
	50	0.00	1.10	0.997

Effect of temperature

In Tables 2, 3 and 4 it is observed that the viscosity of any of the studied samples depends on temperature, so it is important to quantify the effect that temperature has on the viscosity of those samples. The variation of viscosity with temperature can be described by an Arrhenius type equation (Keshani *et al*., 2012; Saghir *et al*., 2013).

$$\eta = K_0 \exp\left(\frac{E_a}{RT}\right) \qquad (5)$$

Where η is the viscosity, K_0 is a constant, E_a is the activation energy of flow, R is the gas constant and T is the absolute temperature in Kelvin.

The values of viscosity shown in Tables 2, 3 and 4 are set to the linearized form of Equation 5 by linear regression. These settings allow obtaining the values of K_0 and E_a for each concentration and ripeness (Table 5). Both the settings as estimates of the parameters have been significant at 95% probability. Figure 5 shows data for juice made from ripe fruit, showing that the data is properly adjusted to the

linearized form of the equation 5. For the other ripening states similar figures were obtained (data not shown).

Table 5 shows the parameters of the Arrhenius equation obtained in the fittings. In the case of the ripening state it is observed that the values of the activation energy (*Ea*) and the parameter K_0 tend to increase with the soluble solids content. This trend is similar to that shown by other types of juices (Guerrero and Alzamora, 1997, 1998; Ibarz *et al*., 2009), although the values of the activation energy of flow is lower, which is attributed to the presence of pulp in the carambola juice studied.

Table 5. Parameters of the Arrhenius equation for different carambolo juice soluble solids content and ripening stages.

State	C (°Brix)	K_0 (mPa·s)	E_a (kJ mol^{-1})	R^2
Mature	41.60	17.3x10^{-3}	19.39	0.981
	31.50	9.3x10^{-3}	18.92	0.997
	22.50	10.1x10^{-3}	16.97	0.998
	8.10	4.13x10^{-3}	11.39	0.995
Intermediate	40.10	8.05x10^{-3}	16.88	0.980
	31.60	8.17x10^{-3}	17.60	0.984
	21.30	5.33x10^{-3}	16.65	0.993
	7.20	8.64x10^{-3}	13.52	0.980
Green	42.50	9.32x10^{-3}	17.01	0.975
	33.00	6.10x10^{-3}	17.55	0.996
	22.00	13.38x10^{-3}	14.56	0.993
	5.20	11.52x10^{-3}	17.65	0.995

It is observed that the activation energy of flow varies from 19.39 to 11.39 kJ/mol, for juices of 41.6 °Brix and 8.1 °Brix, respectively, indicating that for more concentrated juices one variation of temperature will affect more the viscosity variation. To the intermediate ripening state is observed a similar trend, although the range of variation of the activation energy of flow values is lower. In the case of juice obtained from unripe carambola, there is not obtained a definite trend of the variation in activation energy with the soluble solids content, with values close to 17 kJ/mol.

This trend is similar to that shown by other types of juices (Diamante and Umemoto, 2015), although the values of the activation energy of flow are lower, which is attributed to the presence of pulp in the carambola juice studied.

Figure 5 shows data for juice made from ripe fruit; it is properly adjusted to the linearized form of the equation 5. For the other ripening states, similar figures were obtained (data not shown).

It is observed that the activation energy of flow varies from 19.39 to 11.39 kJ mol^{-1}, for juices of 41.6 °Brix and 8.1 °Brix, respectively. For the intermediate ripening state a similar trend is observed, although the range of variation of the activation energy of flow values is lower. In the case of juice obtained from unripe carambola, a definite trend of the variation of activation energy with the soluble solids content, with values close to 17 kJ mol^{-1} was not obtained.

Effect of soluble solids

The data shown in Tables 2, 3 and 4 are set to an exponential equation, allowing to predict the viscosity of the samples in function of the soluble solids content:

$$\eta = \eta_0 \exp(bC) \qquad (6)$$

where η is the viscosity, η_0 is the viscosity when the soluble solids content is 0 °Brix, *b* is a constant and *C* is the concentration expressed in °Brix.

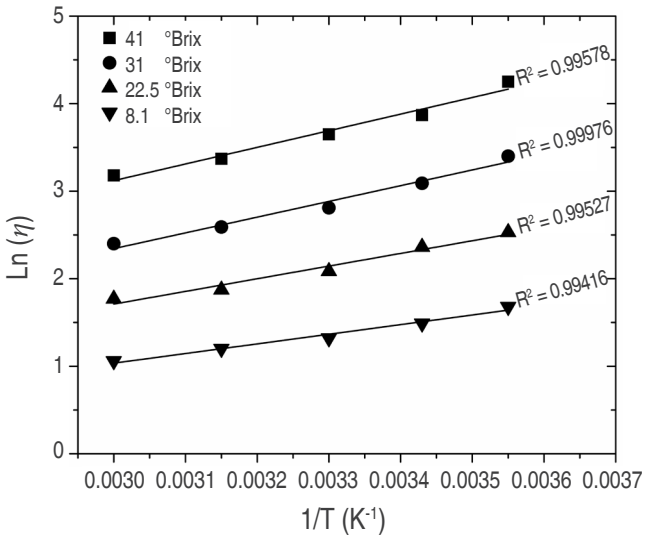


Figure 5. Effect of temperature on mature state carambolo juice viscosity in function of soluble solids.

Table 6 shows the parameter values of the settings and the correlation coefficients for each temperature tested for the three ripening stages. In the case of juice from ripe fruits and intermediate, the trend of the parameters η_0 and *b* is to decrease when temperature increases. Similar trends have been observed in other works (Ibarz *et al*., 2009).

Table 6. Exponential equation parameters of the effect of soluble solids in the carambolo juice viscosity at different temperatures and maturation states.

State	T (°C)	η_0 (mPa·s)	b (°Brix^{-1})	R^2
Mature	10	2.68	0.077	0.996
	20	2.34	0.071	0.996
	30	1.99	0.068	0.993
	40	1.80	0.065	0.988
	50	1.55	0.064	0.981
Intermediate	10	2.03	0.052	0.992
	20	1.60	0.050	0.994
	30	1.33	0.048	0.993
	40	1.12	0.047	0.994
	50	1.02	0.046	0.995
Green	10	2.32	0.044	0.977
	20	1.91	0.041	0.980
	30	1.52	0.041	0.954
	40	1.18	0.043	0.948
	50	0.98	0.043	0.959

The parameter *b* is a measure of the influence that the soluble solids content exerted on the viscosity, and since its value decreases with increasing temperature, it indicates that higher temperatures influence less on the change in viscosity when soluble solids content varies, (considered statistically insignificant). In the case of juice from unripe fruits, the parameter *b* hardly varies with the temperature change, indicating that the changes in soluble solids content similarly influenced, independently of the working temperature considered.

Combined effect temperature-soluble solids content

From the engineering point of view, it is useful to have a single equation that describes the combined effect of temperature and soluble solids content on the viscosity of the carambola juice. Different authors have used diverse equations (Assis *et al*., 2006; Bozdogan, 2015; Guerrero and Alzamora, 1997, 1998; Ibarz *et al*., 2009). Since the influence on the viscosity of the temperature and the soluble solids is described by exponential equations (Eq. 5 and 6), in this work it has been used the following equation:

$$\eta = a\exp\left(\frac{E_a}{RT} + bC\right) \qquad (7)$$

in which a is a pre-exponential factor, E_a is the activation energy of flow, b is a constant, R is the gas constant, T is the absolute temperature, and C is the concentration expressed in °Brix.

In both cases, both the fittings and the estimates of the parameters prove significant at a probability level of 95%. Table 7 shows the parameters values obtained in the adjustment analyses. It is observed that the values of the activation energy of flow are similar for the three ripening states, so that the temperature effect on the carambola juice viscosity is analogous and independent of the ripening state. For the parameter *b*, it is noted that its value increases with the ripening state increasing, indicating a greater effect of concentration on the viscosity as fruit ripening increases.

Table 7. Combined effect temperature-soluble solid content on viscosity carambola juice with different state of maturation.

Estado	*a* (mPa·s)	E_a (kJ/mol)	*b* ($^oBrix^{-1}$)	R^2
Mature	$2.526x10^{-3}$	16.83	0.069	0.988
Intermediate	$2.213x10^{-3}$	16.17	0.048	0.990
Green	$1.967x10^{-3}$	16.69	0.042	0.970

CONCLUSIONS

The study describes the characterization, not reported before, of the fruits and juices of *Averrhoa carambola* in three ripening stages. The maximum activity of carambolo polyphenoloxidase, for all stages, allowed to classify as basic type; the maximum temperature of the enzyme activity was observed at 40 °C, with a potential inhibition from 50 °C and low activity at 10 °C. The characterization of the polyphenoloxidase enzyme of carambolo fruits can be performed at temperatures below 20 °C and pH less than 7, which would slightly control enzymatic browning.

The rheological study of carambolo juice allowed to establish that the viscosity variation with temperature is adjusted to the Arrhenius equation, whereas the effect of soluble solids content on the viscosity of the samples was adequately described by an exponential equation. This appears to be the first work to study the rheological properties and polyphenol oxidase enzyme of *A. carambola* fruit in our country; the information provided here could be helpful to the successful development of new food functional products.

All authors have no conflict of interest to declare.

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