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Changes in the physicochemical, rheological, biological, and sensorial properties of habanero chili pastes affected by ripening stage, natural preservative and thermal processing

Cambios en las propiedades fisicoquímicas, reológicas, biológicas y sensoriales de pastas de chile habanero afectadas por el estado de madurez, conservador natural y procesamiento térmico

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Abstract
Physicochemical and rheological properties, total phenolic content (TPC), capsaicinoids identification and quantification, antioxidant activity (AA) and sensorial analysis were determined on two ripening stages of habanero (*Capsicum chinense* Jacq.) pepper pastes added with lime essential oil (LEO) as natural preservative and subjected to thermal process. Rheological parameters indicated that orange ripening stage and thermal processing presented the higher consistency coefficient, flow index, and viscoelastic (G' and G") behavior values of the evaluated habanero chili pastes. The addition of LEO favored the increment of TPC and AA, as well as color preservation. Thermal processing potentiated total capsaicinoid content (TCC), TPC, and AA, but affected the color characteristics. The combination of LEO and thermal processing significantly affect the sensorial attributes in two of the three sensorial tests performed. These results suggested that apart from sensorial perception, the natural preservative LEO and the thermal processing improved physicochemical and rheological properties as well as biological activity of habanero pastes of the two ripening stage increasing habanero pepper pastes quality and marketing possibilities.
Keywords: *Capsicum chinense* Jacq., viscoelasticity, lime essential oil, capsaicin, antioxidant activity.

Resumen
Las propiedades fisicoquímicas y reológicas, contenido total de fenoles (TPC), identificación y cuantificación de capsaicinoides, actividad antioxidante (AA) y análisis sensoriales fueron determinados en pastas de chile habanero (*Capsicum chinense* Jacq.) de dos estados de madurez, adicionados con aceite esencial de limón (LEO) como conservador natural y sometidos a un proceso térmico. Los parámetros reológicos indicaron que el estado de madurez naranja y procesamiento térmico presentaron los valores más altos de coeficiente de consistencia, índice de flujo y comportamiento viscoelástico (G' y G") de las pastas de chile habanero evaluadas. La adición del LEO favoreció el incremento de TPC y AA, así como la conservación del color. El procesamiento térmico potenció el TPC, contenido total de capsaicinoides (TCC) y AA, pero afectó las características del color. La combinación de LEO y procesamiento térmico afectó significativamente los atributos sensoriales en dos de los tres análisis realizados. Estos resultados sugieren que, sin considerar la percepción sensorial, el uso de conservador natural (LEO) y el procesamiento térmico mejoraron las propiedades fisicoquímicas y reológicas, así como la actividad biológica de las pastas en los dos estados de madurez de chile habanero incrementando su calidad y sus posibilidades de mercado.
Palabras clave: *Capsicum chinense* Jacq., viscoelasticidad, aceite esencial de limón, capsaicina, actividad antioxidante.

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1 Introduction

Habanero chili pepper (*Capsicum chinense* Jacq.) stands out among many species of peppers grown in Mexico mainly because of its flavor, taste and high pungency, characteristics supported by the Protected Designation of Origin obtained in 2010 (Fabela-Morón *et al*., 2020). This pepper is of great interest due to its nutritional and biological properties that are influenced by climatic conditions, ripening time, genotype, among other factors (Antonio *et al*., 2018). The maturity stage of habanero peppers highly influences the compositional quality of fruits, since during ripening, several biochemical, physicochemical and structural modifications happened (Menichini *et al*., 2009).

Habanero fruits can be marketed fresh and processed, either as dry pepper, sauce or paste, the last one defined as a product of thick consistency obtained from the grinding of the fruit that allows pepper preservation in a simple and low cost way, which guarantees its supply, representing an advantage for its commercialization (Ornelas-Paz *et al*., 2013; Rochín-Wong *et al*., 2013). The quality parameters such as physicochemical, rheological and sensorial properties of habanero pastes are of great interest for commercial purposes (Bozkurt & Erkmen, 2004). Moisture and pH of pastes are important parameters related to microbial growth and product stability, neutral values of pH in habanero pepper pastes can favor the development of bacteria that affects its composition and flavor (Gamonpilas *et al*., 2011; Qadri *et al*., 2015). Additionally, color is an important physicochemical parameter to consider during pepper processing as it is largely used as coloring and flavoring agents in several products (Pino *et al*., 2007). To decrease spoilage by microorganisms during pastes storage, the use of organic acids and thermal treatments have been positive tested (Calo *et al*., 2015; Ornelas-Paz *et al*., 2013). Nevertheless, low concentations of organic acids have shown negative effects on color stability and antioxidan activity of procesed products (Patras, 2019), and the heat has demonstrated the alteration of the phytochemicals presented on them (Ornelas-Paz *et al*., 2013). As an alternative, natural origin compounds such as essential oils (EOs) have been recently studied as food natural preservatives due to their antimicrobial effects and high antioxidant activity (Dima & Dima, 2015; Tavares *et al*., 2012). The importance of EOs is attributed to their availability, low toxicity and better biodegradability (Dwivedy *et al*., 2016). Among the wide variety of EOs, those obtained from citrus fruits have gained great acceptance being recognized as GRAS products (Fisher & Phillips, 2008). Lime essential oil (LEO) has successfully been proven in various food products extending their shelf life by protecting against several microorganisms (Pathan *et al*., 2012). The biological activity of LEO has been linked to the presence of volatile compounds, including monoterpenes (Espina *et al*., 2011). However, the biological activity can also be attributed to the presence of phenolic compounds such as flavones, flavanones, hydroxycinnamic acids and vitamin C (Gironés-Vilaplana *et al*., 2014). Moreover, essential oils have demonstrated to favor phytochemicals solubilization increasing aromas and flavors (Amruthraj *et al*., 2014). In this sense, phytochemical compounds present in food products and habanero peppers such as phenolic compounds and capsaicinoids have demonstrated the ability to reduce the risk of suffering from some degenerative diseases (Olguín-Rojas *et al*., 2019; Ji *et al*., 2020; Vallejo-Castillo *et al*., 2020).

Capsaicinoids are formed by the union of a vanillylamine compound with a fatty acid and synthesized in the placenta (Antonio *et al*., 2018; Montoya-Ballesteros *et al*., 2010). Capsaicin and dihydrocapsaicin are responsible for up to 90% of the pungency in peppers and their content depend on factors such as: genotype, growing conditions, ripening stage, harvest, among others (Antonio *et al*., 2018; Montoya-Ballesteros *et al*., 2010).

Rheological tests such as rotational and oscillatory analyses are the main tests of individual ingredients and food matrixes, closely linked to the development of home-based foods and their industrial production (Fischer *et al*., 2009). The small amplitude oscillatory shear analysis is a dynamic response of a food material that allows to measure the changes in the microstructure and entanglement of complex foods during heating/cooling or shearing in-situ without damaging the structure of the test specimens (Ahmed, 2018). These rheological test are a powerful tool to obtain information about the microscopic structure of viscoelastic materials (Dzul-Cauich *et al*., 2013). The modification of the physicochemical characteristics of chili pepper paste by ripening stage or heat processing induces alterations in its functional properties, such as rheological parameters and sensorial attributes, and it has not been reported before in habanero pastes added with LEO.

On the other hand, sensory evaluation has been reported as a useful tool to evaluate product quality in new raw materials incorporations and conditions of processed food and peppers (De los Rios-Deras *et al*., 2015; Postemsky *et al*., 2017). have evaluated thought the aroma and overall acceptance the effect of salt incorporation on processed jalapeño pepper. Since physicochemical, rheological, and sensorial analysis are of great interest as quality parameters of habanero paste thermally treated and few studies have been reported (Fischer *et al*., 2009). The objective of this study was to evaluate the effect of ripening stage, LEO, and thermal processing on the rheological, physicochemical, biological, and sensorial properties of habanero (*Capsicum chinense* Jacq.) chili pastes from the Yucatan Peninsula, Mexico.

2 Materials and methods

2.1 Materials

Habanero chili pepper fruit (*Capsicum chinense* Jacq.) with the accepted commercial characteristics for processing in two different ripening stages (green and orange) were purchased from a commercial supplier located in Conkal, Yucatan, Mexico. Mexican lime essential oil (LEO) obtained from Mexican lime was purchased from the AELTSA company (Tecoman, Colima, Mexico). The characteristics of refractive index of 1.482 - 1.486, citral content of 4.2-8%, appearance yellow to brownish-green liquid, and fresh citrus odour were commercial parameters indicated by the supplies. Acetic acid, Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), capsaicin (CS) and dihydrocapsaicin (DHC) standards, HPLC grade water, HPLC grade acetonitrile, HPLC grade methanol, and ethanol were purchased from Sigma Aldrich (Toluca, Mexico).

2.2 Elaboration of habanero pepper pastes

Habanero pepper pastes were obtained by the grinding of chili peppers (green and orange in separated containers) with water in a ratio 1:1 (w/v) using an immersion blender to mix the mixture for 15 min. Five treatments including fresh fruit were performed for each ripening stage. Conditions of each treatment were as follows: Fresh fruit (F), acetic acid addition of 3% (v/v) as control (C) (Gironés-Vilaplana *et al*., 2014), LEO of 0.8% (v/v), previously evaluated at our laboratory (data not shown). C and LEO treatments were subjected to a thermal processing (TT) conducted in an autoclave (Felisa FE-399, Mexico) with a temperature of 121 °C, the pressure of 15 psi, and time of 15 min, to obtain the C-TT and LEO+TT treatments.

2.3 Physicochemical analysis

2.3.1 Moisture, water activity, and pH determinations

Moisture content (%) and water activity were determined according to the AOAC official method (1990). pH was measured with a pH meter (Sper Scientific, Scottsdale USA). Determinations were performed in the fresh fruit before paste preparation, in the pastes after addition of acetic acid and LEO, and finally after thermal processing. All measurements were performed in triplicate.

2.3.2 Surface color measurement

The color parameters of each habanero chili pepper paste were measured using a MiniScan EZ4500L colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA). The CIELAB values L* (0=black; 100=white), a* (negative-green; positive-red), and b* (negative-blue; positive-yellow) were determined. Determinations were performed in the fresh fruit before paste preparation, in the pastes after addition of acetic acid and LEO, and finally after thermal processing. All measurements were performed in triplicate. Color squares were produced by converting L*, a*, and b* values to R, G, and B using http://colormine.org/convert/rgb-to-lab and Microsoft PowerPoint software (Yang *et al*., 2020) The Hue angle, Chroma, and Saturation were also calculated as follows:

$$Hue\ angle = \tan^{-1}\left(\frac{b*}{a*}\right) \tag{1}$$

$$Chroma = \left(a*^2 + b*^2\right)^{0.5} \tag{2}$$

$$Saturation = \frac{Chroma}{L*}\pi r^2 \tag{3}$$

Additionally, a designation of the name of the color was performed using the Royal Horticultural Society Color Charts Edition V (http://rhscf.orgfree.com/).

2.4 Rheological measurements

Rheological measurements were carried out using a Discovery Hybrid Rheometer DHR-2 (TA Instruments, New Castle, DE, USA) with a plate-plate geometry (diameter = 40 mm). Steady-state rheological behavior of habanero pepper pastes was studied at 25 °C in the shear rate range from 10^{-2} to 10^2 s^{-1} at a gap of 1 mm. The rheological parameters were calculated with the power-law model using TRIOS software (TA Instruments, New Castle, DE, USA).

$$\eta_{app} = \kappa \dot{\gamma}^n \tag{4}$$

where η_{app}= apparent viscosity; $\dot{\gamma}$= shear rate; κ= consistency coefficient, and n= flow index.

The linear viscoelastic region (LVR) was determined by amplitude sweep tests at a frequency of 1 Hz and an increasing strain from 0.01 to 100% (data not shown). Frequency sweep tests were determined by applying a 1% strain within the LVR over a frequency range between 0.1 to 100 rad/s at 25 °C. The mechanical spectra were plotted in terms of the storage modulus (G') and loss modulus (G") as a function of frequency. The frequency dependence of G' and G" values was fitted to the power-law model follows:

$$G' = af^b \tag{5}$$

$$G'' = cf^d \tag{6}$$

where f is the frequency, while a, b, c, and d coefficients are rheological parameters. The parameter a and c are indications of the elastic (solid) or viscous (liquid) behavior whereas the b and d parameters describe the nonlinearity relationship of the G' and G" versus frequency (Fang *et al*., 2020).

2.5 Total Phenolic Content (TPC)

Total phenolic compounds were extracted and measured following the Ana *et al*. (2018) methodology using Folin-Ciocalteau reagent. Briefly, 250 μL of Folin-Ciocalteau (1N) was mixed with 20 μL of each extract, 1250 μL of Na_2CO_3 (7.5%) was added to each sample after 8 min and then the solution was diluted with 480 μL of distilled water. The absorbance of the reactions mixtures was measured at 760 nm using an UV-vis spectrophotometer (Thermo Fisher Scientific, Biomate 3S, Madison, WI, USA). TPC analysis was performed in triplicate, and mean values were expressed as mg Gallic Acid Equivalents (GAE)/g of dry weight (dw) according to the slope generated from a calibration curve (R^2 = 0.995). Determinations were performed in the fresh fruit before paste preparation, in the pastes after addition of acetic acid and LEO, and finally after thermal processing. All measurements were performed in triplicate.

2.6 Capsaicinoids profile and content

2.6.1 Total capsaicinoids extraction

The capsaicinoids were extracted according to the methodology reported by Collins *et al*. (1995). Extractions were performed in the fresh fruit before paste preparation, in the pastes after addition of acetic acid and LEO, and finally after thermal processing. Each sample (green and orange) was mixed with methanol in a 1:10 (w/v) ratio. The solution was placed in a water bath (Buchi B-491, Switzerland) at 80 °C for 4 h. Samples were removed from the water bath and cooled to room temperature. After that were centrifuged at 4000 rpm during 10 min. Finally, the supernatant of each sample was filtered through 0.45 μm filter paper into a HPLC sample vial using a 5 mL disposable syringe (Millipore, Bedford, MA, USA). The amber vials were stored at 4 °C until further analysis.

2.6.2 Total capsaicinoids identification and quantification

The identification and quantification of capsaicinoids were performed by chromatographic analyses in a UPLC Acquity H Class (Waters, Milford, CA, USA) chromatographer, equipped with a sample manager (UPSMFTN), a quaternary pump (UPQSM), and a photodiode array detector (UPPDALTC). Data acquisition was completed with the Empower 3 software program (Waters). The capsaicinoids separation was performed with a Gemini C18 column (5 μm, 110A, 150 X 4.6 mm i.d.) (Phenomenex, Torrance, CA, USA). The Official Analytical Method 21.3 of the American Spice Trade Association (ASTA) was followed with slightly modifications: 0.5 mL/min isocratic system flow, and 10 μL injection volume. CS and DHC quantification were obtained using calibration curves for the respective analytical standards in a range from 5 to 300 μg/mL. All measurements were carried performed in triplicate.

2.7 *Antioxidant Activity determination*

The antioxidant activity was performed based on the scavenging of DPPH radical inhibition described by Ana *et al*. (2018). The DPPH radical scavenging effect of the sample was expressed as inhibition percentage of DPPH radical measured at 515 nm using an UV-vis spectrophotometer (Thermo Fisher Scientific, Biomate 3S, Madison, WI, USA). Briefly, 100 μL of sample extract was added to 2900 μL of 0.01 mM DPPH in methanol, then stored in darkness for 30 min at 24 °C. Obtained absorbances were related to a previous calibration curve using Trolox as an antioxidant molecule to obtain the inhibition percentage.

2.8 *Sensory evaluation*

The sensory evaluation of the habanero chili pastes in two different ripening stages was based on color, odor, texture, and flavor determination. Three sessions were carried out, where 41 untrained panelists participated. The test was carried out only in samples with thermal treatment (C+TT, LEO+TT) to guarantee the food safety of habanero pastes. The sensory analysis was performed in a temperature room (25 °C) and controlled lighting. Habanero pastes were put in white, opaque containers and coded using three-digit numbers chosen randomly. Tomato sauce as a vehicle was used for flavor evaluation to minimize the pungency degree in each sample and to avoid saturation during the test. Register formats for sensory analysis were adapted from the proposed by Poste LM, Mackie (2011).

2.8.1 *Triangle test*

A triangle test was carried out to evaluate significant differences in the color, odor, and texture attributes among habanero pepper pastes. The panelists were asked about smell and taste sensorial attributes in one different sample (added with LEO) and two identical samples (control) of habanero pepper paste to indicate the odd sample. Results were compared with tables of a minimum number of correct responses required for significance as described in a ISO normativity (ISO 4120, 2004).

2.8.2 *Quantitative response scale test*

In the quantitative response scale test, the panelists were asked about the smell, and taste of the habanero pepper paste, and they evaluated its juiciness, color, odor, and texture (ISO 4121, 2003). Each panelist evaluated these characteristics on a standard 5-point hedonic scale from 1 to 5, where 1 corresponded to extremely unsatisfactory and 5 corresponded to extremely satisfactory.

2.8.3 *Preference test*

Preference test was used to rank the different treatments, the panelists were asked to assign an order to the samples according to their preference in flavor, indicating their greater preference with the number 1 and their lowest preference with the number 2. Results were analyzed as the ratio of number of preferred answers and the total answers (Ornelas-Paz *et al*., 2013).

2.9 *Statistical analysis*

All experiments were conducted in triplicate and the results were reported as the mean ± standard deviation. Data were subjected to one-way analyses of variance (ANOVA) and LSD means comparation analysis. Significance was established at p
leq 0.05. Data analysis was carried out using the Statgraphics Centurion XVI software (Statistical Graphics Corp., Manugistics, Inc., Cambridge, MA, USA).

3 Results and discussion

3.1 *Physicochemical characterization*

The results of the physicochemical analysis indicated values of water activity of the fresh fruit and pastes from 0.46 to 0.89 and moisture content from 86.8 to 88.4%. The statistical analysis showed that LEO and thermal processing did not affect significantly ($p<0.05$) water activity and moisture content on both, green and orange habanero pepper pastes (data not shown). The values of pH of the green and orange habanero fruits were 5.0 and 5.3 respectively, control treatments added with acetic acid presented values of 3.7 in both ripening stages, the addition of LEO reduced pH values to 4.7 also for both ripening stages. After the thermal processing the pH values of control samples remained constant, nevertheless a reduction of around 0.3 units was observed in LEO treatments reaching values around 4.4. The neutral pH in pepper pastes could favor the development of bacteria and they affect their composition and flavor, in this sense acid pH values (4 - 5) are recommended to preserve pastes (Gamonpilas *et al*., 2011; Qadri *et al*., 2015; Sun *et al*., 2007). A final reduction of around 1 pH unit could favor the preservation of physicochemical parameters due to the reduction of possible spoilage of the product.

3.2 *Surface color*

Surface color determination indicated that orange and green pastes presented colors between orange-brown and medium brown and green-brown and dark green, respectively. The green color is attributed to the chlorophyll content and as in other fruits by the time they synthetize other pigments such as carotenoids (Montoya-Ballesteros *et al*., 2010). The addition of acetic acid in control samples and LEO, as well as the thermal processing, significantly decreased ($p<0.05$) the lightness (L*) values on green pastes compared to fresh fruits. Is notable that even though LEO addition presented a reduction on lightness, it was lower than the presented by the combination of acetic acid of the control sample and thermal treatment (C+TT) (around 10 units) (Fig. 1). However, the orange pastes only presented significantly ($p<0.05$) lightness (L*) reduction in sample added with acetic acid and subjected to thermal processing with a decrease of 3 units compared to the fresh product. In both cases, several factors may decrease lightness in the treatments, nevertheless the main factors can be explained by the degradation of pigments, phenolic compounds oxidation, and the formation of dark color compounds due to the nonenzymatic browning reactions such as the Maillard reaction favor by heat treatment and acidic media (Patrón-Vázquez *et al*., 2019) the last one also possible for the presence of proteins and sugars from the habanero fruit previously reported (Ahmed *et al*., 2018). On the other hand, the redness (a*) values in green pastes showed a significantly increased ($p<0.05$) compare to fresh fruit sample, when the addition of acetic acid, LEO, and thermal processing was performed; this variation was not observed for orange pastes (Fig. 1). The results of b* values (yellowness) and Chroma values indicated that thermal treatment and higher acidification affected negatively green pastes, while orange pastes were positively affected. In this sense, the reduction of b* values in green pastes are also related to the formation of dark color decreasing yellowness, in orange pastes this variation is not perceptible. For chroma values in both pastes a difference from both fruits to a gray color of the same lightness or brightness that corresponds to saturation of the perceived color is observed.

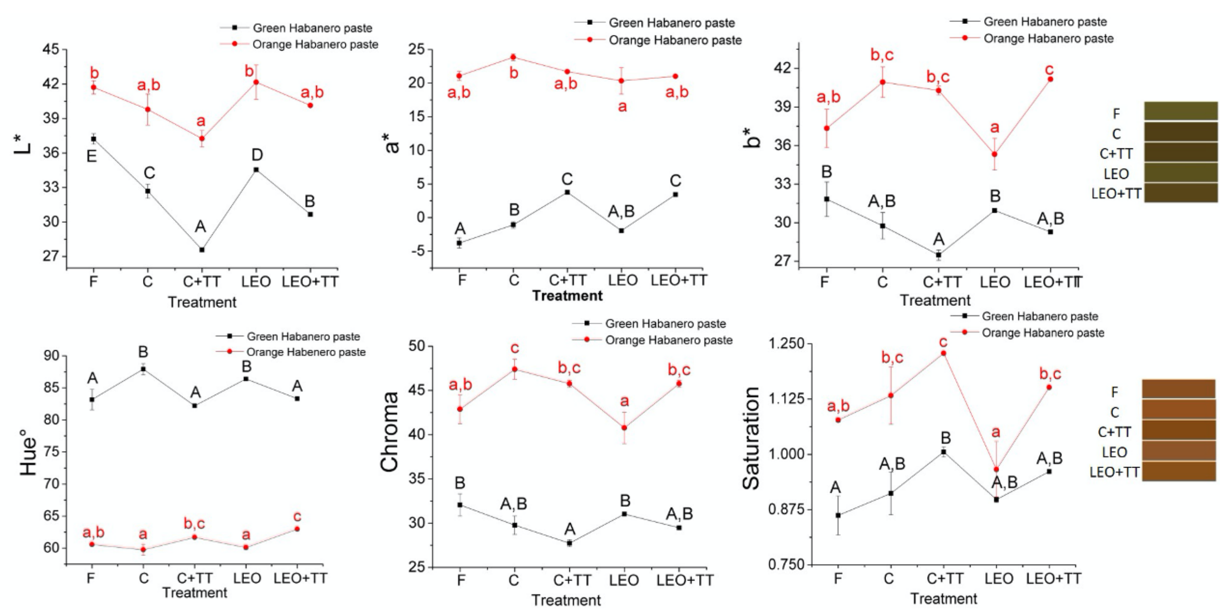

Fig. 1. Surface color: L*= lightness, a*= redness, b*= yellowness, Hueº, Chroma, and saturation of green and orange habanero pepper paste. The same letter in the same graphic (lowercase letters: orange habanero pastes and capital letters: green habanero pastes) indicates that mean values are not statistically different ($p < 0.05$). Mean ± SD; n = 3. F= Fresh fruit; C= Control paste with acetic acid addition; C+TT=Control paste with Thermal Treatment; LEO= Paste with LEO addition; LEO+TT= Paste with LEO and Thermal treatment.

A significantly ($p<0.05$) increase in Hue angle was observed for orange pastes when LEO addition and thermal processing were performed (Fig. 1). Likewise, the Hue angle values increased for green pastes when acid acetic and LEO were added without thermal processing. Saturation values related to the color purity indicated that both, orange and green pastes were not significantly different among treatments (Fig. 1). (Montoya-Ballesteros *et al*., 2014), also reported that color is strongly affected when acidification and thermal processing was applied, additionally they found that higher acidification in samples of artisanal chiltepin pastes clearly affected the final color.

3.3 Rheological properties

3.3.1 Steady shear measurements

Rheological behavior is a reliable tool for understanding molecular structure changes during the preparation and processing of the products (Makroo *et al*., 2019). The flow curves obtained for the orange and green habanero pastes are shown in Fig. 2 a, b. The ripening stage of habanero pastes affected considerably the apparent viscosity. All the green habanero pastes presented the lowest viscosity values (3.10 - 0.30 Pa s at 0.01 s^{-1} and 0.03 - 0.02 Pa s at 100 s^{-1}) compared to the orange habanero pastes (436.01 - 80.42 Pa s at 0.01 s^{-1} and 0.53 - 0.30 Pa s at 100 s^{-1}). For the interval of shear rate studied, a deep descent of the apparent viscosity decreased when the shear rate increased, and the flow became non-Newtonian behavior (pseudoplastic fluid). The flow behavior showed high correlation values (R^2 = 0.95-0.99) with power-law model. The ripening stage of habanero fruits caused a significant effect on the viscosity (consistency coefficient) of pastes. The κ values were 0.153, 0.146, 0.303, 0.104, and 0.186 Pa s^n corresponding to F, C, C+TT, LEO and LEO+TT, respectively in green habanero pastes, while that the κ values of orange habanero pastes were 5.012, 5.583, 14.103, 6.750, and 10.881 Pa s^n for F, C, C+TT, LEO and LEO+TT, respectively. The degree of pseudo-plasticity could be measured by the flow behavior index (n), which decreases when pseudoplastic increases (flow index < 1 = shear thinning behavior and flow index > 1 shear thickening behavior). The corresponding n values were 0.334, 0.415, 0.518, 0.465,0.419 and 0.621, 0.617, 0.732, 0.633, 0.683 for F, C, C+TT, LEO and LEO+TT for green and orange habanero pastes, respectively. The sample of orange habanero paste added with acetic acid and subjected to thermal processing (C+TT) presented the highest κ (14.103 Pa s^n) and n (0.732) values, indicating the most consistency (highest apparent viscosity) and more pronounced shear thinning behavior. Such differences were not only by the variations among the ripening state of habanero chili pepper but also of the thermal processing and used preservative in the paste.

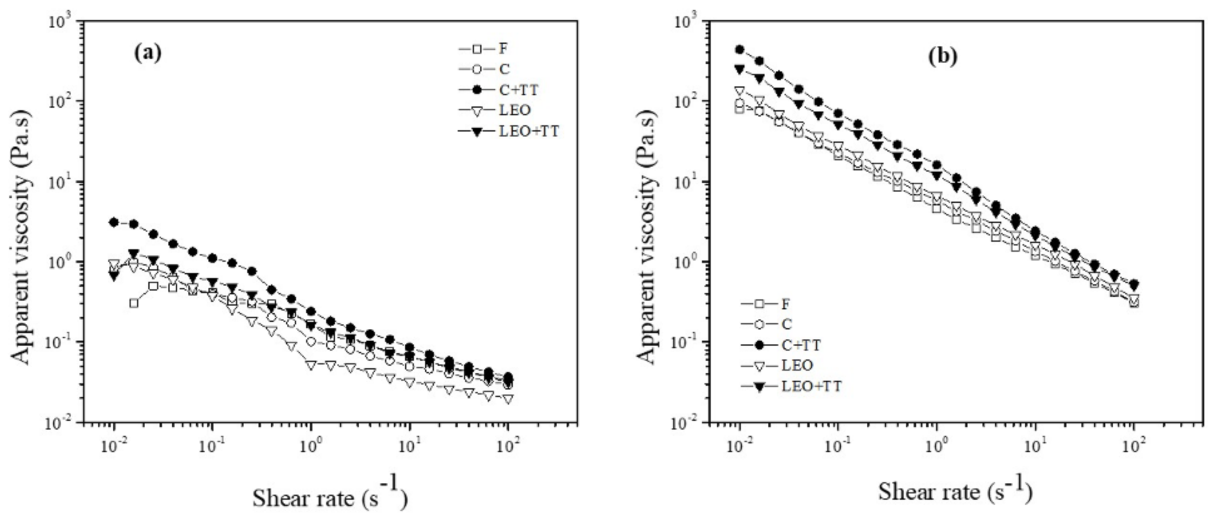

Fig. 2. Apparent viscosity versus shear rate of habanero pepper pastes with or without thermal treatment, acid acetic, and essential oil. (a) Green habanero pepper pastes and (b) orange habanero pepper pastes. F= Fresh fruit paste; C= Control paste with acetic acid addition; C+TT=Control paste with Thermal Treatment; LEO= Paste with LEO addition; LEO+TT= Paste with LEO and Thermal treatment.

The combined effect of the thermal processing and acid acetic in the orange ripening stage of habanero paste presented the highest apparent viscosity values in all shear rate range. (Huang *et al*., 2018) reported that when a small amount of acid was added to the fruit pulp dispersion, the hydrogen bonding between the molecules was strengthened, and lead to the increase of the molecular interactions and viscosity. The shear thinning characteristic is beneficial to organoleptic qualities, e.g. flavor release and mouth-feel, in food products and promotes mixability, pumpability and flowability characteristics which are important factors for the design of flow systems, product development and for scale-up and mechanization of the process (Gamonpilas *et al*., 2011). Similar results have been reported for other food products as pimento purée and litchi pulp (Huang *et al*., 2018). Ahmed *et al*. (2002) reported flow index (0.961 - 0.887) values in green chili puree during thermal processing, which it indicates a shear-thinning fluid, while Martínez-Padilla & Rivera-Vargas, (2006) showed the rheological (n and κ) parameters for Mexican commercial heterogeneous sauces. Cepeda & Collado, (2014) reported that the pseudo-plasticity could be attributed to the alterations in the molecular structure due to shear stress that produces an orientation of the particles with the flow direction (Meléndez-Martínez *et al*., 2010). Cho *et al*. (2017) attributed the shear-thinning movement to the particle aggregation from particle interaction in the fermented red pepper paste. Carrillo-Navas *et al*. (2014) reported that in the shear thinning region, as the shear rate produces a stronger flow pattern, the particles are aligned along the flow direction.

3.3.2 *Viscoelastic behavior*

The viscoelastic properties are important to understand and predict the physical-chemical stability of food dispersions (Augusto *et al*., 2011). The G' and G" modulus for an angular frequency range between 0.1 and 100 rad/s in habanero pastes with different ripening stage and thermal processing with natural preservative are shown in Fig. 3. In general, the variation of G' and G" dynamic modulus with angular frequency to determinate the viscoelastic behavior displayed an increase with respect to angular frequency (Fig. 3a, b, A, B). Carrillo-Navas *et al*. (2014) suggested that this behavior may be due to avoid microstructure complete fracture, the network becomes increasingly elastic for shear-frequency disturbances, as well as it is internally reorganized for responding to shear disturbances.

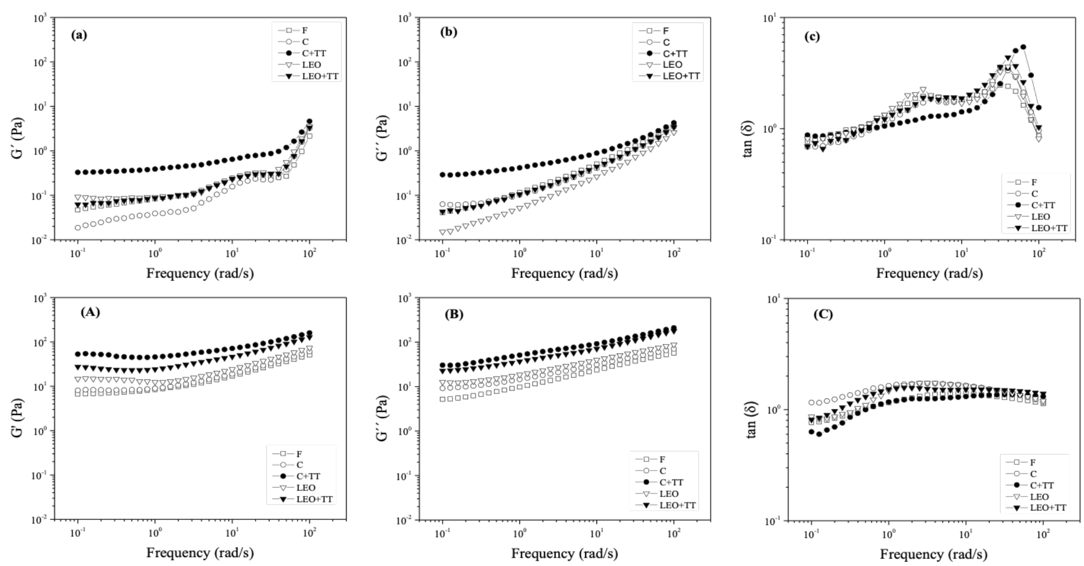

Fig. 3. The dynamic oscillatory behavior of habanero pepper pastes with or without thermal treatment, acetic acid, and essential oil. (a) Storage modulus (G'), (b) loss modulus (G"), (c) tan (δ) for green habanero pepper pastes and (A) storage modulus (G'), (B) loss modulus (G"), (C) tan (δ) for orange habanero pepper pastes. F= Fresh fruit; C= Control paste with acetic acid addition; C+TT=Control paste with Thermal Treatment; LEO= Paste with LEO addition; LEO+TT= Paste with LEO and Thermal treatment.

The viscoelastic properties were influenced by ripening stage, the thermal processing and acidity in the orange habanero pastes (Fig. 3 A, B). C+TT in orange habanero paste had the highest G' and G" values in all habanero pastes. These factors could present an important effect in the food viscoelasticity to increase the water-soluble pectin of the food matrix after the thermal process at low pH. The water-soluble pectins could act as mass connecting elastic within the food network in the orange habanero paste, as well as could be a consequence of the movement of calcium ions from fruit to water, changes in membrane permeability, and changes in the three-dimensional structure of fruit tissue (Ramos-Aguilar *et al*., 2015). Also, both the temperature and pH could inactivate the polyphenol oxidase enzyme, leading to the inhibition of polyphenolics degradation, therefore a more stable food matrix (Alam *et al*., 2018). de Oliveira Carvalho & Orlanda, 2017 reported the effect of pH and temperature on enzyme activity in buriti (*Mauritia flexuosa* Linnaeus f.) pulp, indicating that enzymatic activity is more effectively inhibited in an acid pH (below 4.0) and thermal processing (77 °C during 4 min). The G" data showed high correlation values (R^2 = 0.95-0.99) with power-law model, while G' correlation data ranged from 0.75 to 0.94. The *a* rheological parameter values were 0.09, 0.04, 0.42, 0.11, 0.10 Pa and 9.63, 10.84, 54.94, 16.35, 30.20 Pa for F, C, C+TT, LEO and LEO+TT for green and orange habanero pastes, respectively. The *c* rheological parameter values were 0.12, 0.12, 0.46, 0.05, and 0.11 Pa corresponding to F, C, C+TT, LEO and LEO+TT in green habanero pastes, while that the c values of orange habanero pastes were 10.30, 15.60, 51.10, 20.02, and 38.24 Pa for F, C, C+TT, LEO and LEO+TT, respectively. The *b* and *d* values ranged from 0.15 to 0.85 in all habanero chili pastes, which indicated the frequency dependence of the G' and G" modulus, indicating semi-solid viscoelastic behaviors. When the parameters are 0, moduli are independent of frequency, indicating that the sample exhibits an elastic behavior and the values between 0 and 1 are semi-solid viscoelastic behaviors (Fang *et al*., 2020). According to G' and G" versus frequency data fitted with power-law model. C+TT in orange habanero paste showed the highest *a* and *c* rheological parameter values for G' and G" modulus such as the lowest *b* parameter value (0.15) indicating the highest semi-solid behavior in this habanero paste (G' > G"). Gamonpilas *et al*. (2011) showed that chili sauces had G' higher than G", hence a dominant elastic behavior as compared to the viscous behavior, typically in suspensions with network-like structure. The weak gel-like behavior is typically observed in suspensions with network-like structures, which has been reported for fruit pulps from *Colocasia esculenta* L. Schott (Yu *et al*., 2016). On the other hand, Barbieri *et al*. (2018) reported the thermal stability of the gabirola (*Campomanesia xanthocarpa* Berg) pulp at a wide range of temperatures, reflecting a predominance of elastic behavior, like a gel and low internal structure changes. Similar behavior has been reported for other food products as tomato juice (Yu *et al*., 2016). The tan γ curves of green and orange habanero chili pastes are presented in Fig. 3c and Fig. 3C, respectively. The tan (γ) values for green habanero pastes were > 1, exhibiting viscoelastic behavior as predominantly viscous (Fig. 3c), while that the orange habanero pastes presented a viscoelastic behavior (tan $\gamma \approx 1$) over the tested frequency (Fig. 3C). The a and c rheological parameter data fitted were used to obtain the tan γ values. LEO and C+TT values of tan γ in green and orange habanero chili pastes were 0.50 and 0.93, respectively, which indicating a viscoelastic behavior as predominantly elastic (tan γ < 1). The tan γ values in rest of treatments of both habanero paste ranged from 1.06 to 2.81, exhibiting viscoelastic behavior as predominantly viscous (tan γ > 1). (Gamonpilas *et al*., 2011) reported that network structure in chilli sauce was the weakest owing to the most frequency-sensitive G' and the highest magnitude of tan γ.

3.4 TPC and antioxidant activity

The TPC and antioxidant activity are presented in Fig. 4. TPC values of all green and orange habanero pastes ranged from 0.43 to 1.51 mg GAE/g dw and 0.48 to 1.33 mg GAE/g dw, respectively. The slightly difference on TCP between green and orange control pastes could be explained by the ripening stage, physiological age, environmental conditions, and possible stress during plants growing (Loizzo *et al*., 2015). The addition of acetic acid in control samples produced a significantly ($p<0.05$) increment in TPC compared with the green fresh fruit, that could be attributed to the fact that the polyphenolic compounds present greater stability when found in the acidic conditions (pH 3) (Hurtado & Pérez, 2014), or to the cleavage effect of acids on the glycosidic bonds in phenolic compounds leading to the separation of glucosides and aglycones, nevertheless this effect was not observed for orange paste.

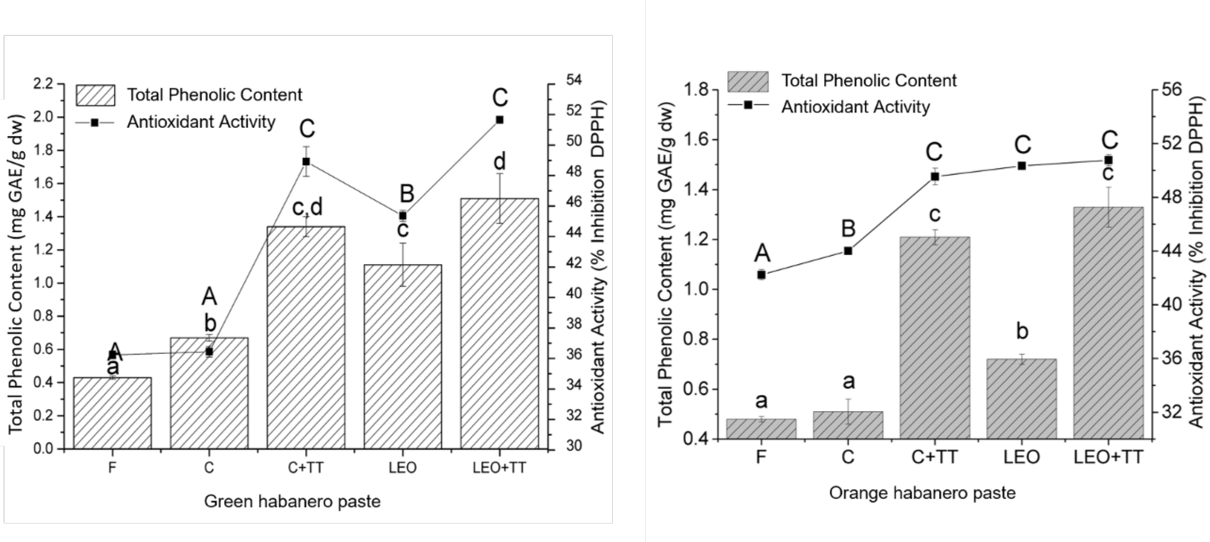

Fig. 4. Correlation of Total Phenol Content (TPC) and Antioxidant activity of A) green and B) orange habanero pepper pastes. Mean ± SD; n = 3. The same letter in the same graphic (lowercase letters for bar and capital letters for line) indicates that mean values are statistically different ($p < 0.05$). F= Fresh fruit paste; C= Control paste with acetic acid addition; C+TT=Control paste with Thermal Treatment; LEO= Paste with LEO addition; LEO+TT= Paste with LEO and Thermal treatment.

The LEO addition resulted in a higher significantly increased ($p<0.05$) of TPC respect to the fresh and control treatments in both habanero pastes. This could be attributed to the contribution of phenol content of the LEO (25.49 mg GAE/g dw), which was directly related to the presence of flavonoid compounds (Cheong *et al*., 2012). Additionally, thermal processing improved significantly ($p<0.05$) the TPC in all cases, obtaining the highest values of TPC for samples added with LEO in pastes of both ripening stages. According to Martins *et al*. (2017), the dehydration of a food matrix may improve phenolic compound extraction by the disruption of the cellular matrix and the increase of compounds bioavailability. Additionally, the cleavage and subsequent oxidation of capsaicinoids in the thermal processing leads to the formation of vanillin, which could be used to produce other substituted phenols associated with TCC (Perucka & Materska, 2003; Pino *et al*., 2011). On the other hand, the antioxidant activity values in all green and orange habanero pastes ranged from 36.23 to 51.65% and 42.24 to 50.76% inhibition of DPPH, respectively. The antioxidant activity observed in habanero pepper could be attributed to the presence of ascorbic acid, capsaicinoids, and phenolic compounds (Perucka & Materska, 2003; Pino *et al*., 2011), such as gallic, ferulic, o-coumaric, p-coumaric, sinapinic, and caffeic acids (Troconis-Torres *et al*., 2012). The antioxidant activity significantly increased ($p < 0.05$) in green and orange habanero pastes after the addition of LEO and thermal processing. According to (Zhang *et al*., 2015), thermal processing could induce the non-enzymatic browning reactions that produced compounds exhibiting antioxidant activity. Additionally, it could be inferred that the antioxidant activity of habanero pepper pastes was linked to the TPC, as reported in previous studies (Covarrubias-Cárdenas *et al*., 2018). Although a lower value of TPC was presented in orange habanero pastes compare to green habanero pastes, the higher antioxidant activity with LEO addition in orange habanero pastes could be related to the presence of specific phenolic compounds present in the orange pastes that could favor antioxidant activity (Covarrubias-Cárdenas *et al*., 2018).

3.5 *Capsaicinoids profile and total content*

The profile of capsaicinoids is presented in Fig. 5, as it can be observed both ripening stage pastes presented a similar profile with the highest chromatographic pick (1) at elution time of 2.65min corresponding to the capsaicin compound and the lowest one (2) at elution time of 3.41min corresponding to the dihydrocapsaicin compound, the none defined picks presented around the elution time at 1 min corresponded to the mixture of phenolic compounds present in the sample.

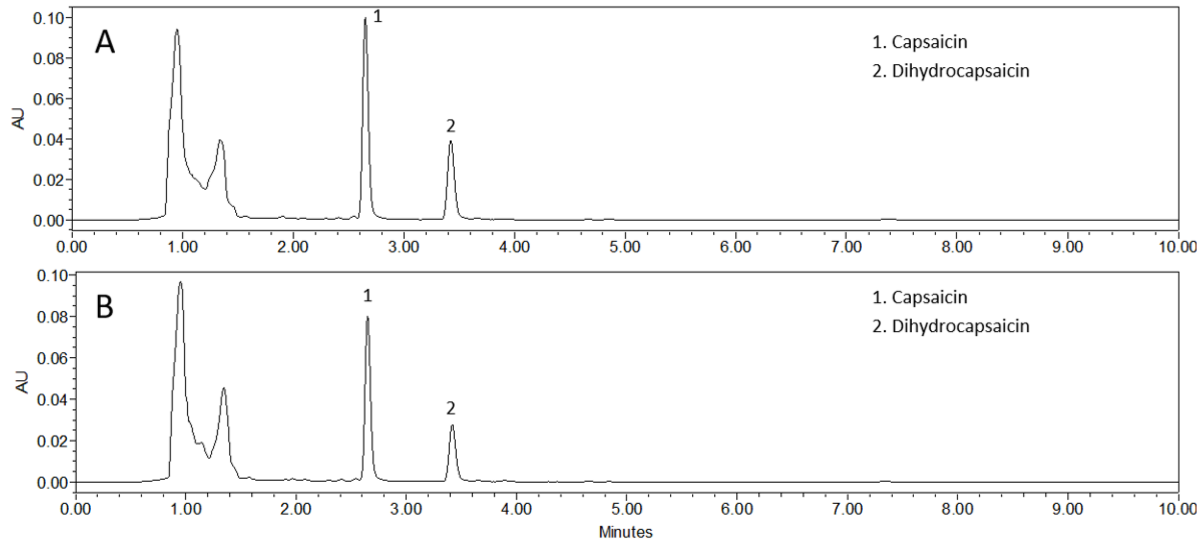

Fig. 5. Capsaicinoid profile of A) green habanero control paste and B) orange habanero control paste obtained by UPLC.

Table 1. Total capsaicinoids content (TCC) on green and orange habanero pepper pastes.

Code	Green habanero paste			Orange habanero paste		
	CS (mg/g dw)	DHC (mg/g dw)	TCC (Scoville Units)	CS (mg/g dw)	DHC (mg/g dw)	TCC (Scoville units)
F	10.27 ± 0.27[a]	2.50 ± 0.08[a]	204,452 ± 4,702[a]	9.43 ± 0.04[a]	2.76 ± 0.01[a]	195,105 ± 807[a]
C	13.13 ± 0.26[b]	3.20 ± 0.06[b]	261,539 ± 5,199[b]	12.45 ± 0.08[e]	3.64 ± 0.02[e]	257,706 ± 1,540[e]
C+TT	14.42 ± 0.09[d]	3.52 ± 0.02[d]	287,225 ± 1,692[d]	12.02 ± 0.02[d]	3.51 ± 0.01[d]	248,628 ± 420[d]
LEO	13.94 ± 0.21[c]	3.40 ± 0.05[c]	277,677 ± 4,204[c]	11.06 ± 0.04[b]	3.23 ± 0.01[b]	228,792 ± 924[b]
LEO+TT	13.18 ± 0.05[b]	3.22 ± 0.01[b]	262,480 ± 879[b]	11.22 ± 0.05[c]	3.28 ± 0.02[c]	232,154 ± 1,111[c]

CS: Capsaicin; DHC: Dihydrocapsaicin; Mean ± SD; n = 3. The same letter in the same column indicates that mean values are not statistically different ($p > 0.05$). F= Fresh fruit; C= Control paste with acetic acid addition; C+TT=Control paste with Thermal Treatment; LEO= Paste with LEO addition; LEO+TT= Paste with LEO and Thermal treatment.

The CS and DHC contents were expressed in mg/g and the TCC was reported as Scoville Units (SHU) of green and orange habanero pastes (Table 1). In general, green habanero pastes presented higher SHU values (from 204,452 to 287,225) than orange habanero pastes (from 184,615 to 257,706). In both cases, the CS content was higher than DHS content, nevertheless, the green pastes presented around 80% of CS of the TCC and orange pastes around 77%. The TCC of habanero pepper pastes reported herein was like the reported by Giuffrida *et al*. (2013) for different varieties of habanero pepper, such as habanero golden (155,703 ± 26,262 SHU), habanero orange (238,155 ± 13,850 SHU), and habanero white (166,567 ± 37,860 SHU). All thermal processing increased significantly ($p<0.05$) the TCC in both pastes compared to the fresh fruit, these results may be attributed to the deactivating of the polyphenol oxidase enzyme in an acidic medium leading to an increase in capsaicinoids (Schweiggert *et al*., 2005) and the hydrophobic nature of the essential oil which improved capsaicinoids solubility raising extraction yield (Chinn *et al*., 2017). Thermal processing in some cases tented to lower TCC of the habanero pepper pastes, similar results were reported by Srisajjalertwaja *et al*. (2012), who observed a loss on TCC after subjecting samples of Thai green chili (*Capsicum annum*) to thermal treatment (180-210 °C for 20 min). Cheok *et al*. (2017) observed similar results, reporting a significant reduction ($p<0.05$) of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin contents (62.18%, 64.72%, and 62.95%, respectively) after the thermal processing, which could be attributed to the degradation of the capsaicin molecules (8-methyl-N-vanillyl-6-nonenamide) through cleavage of the alkyl group attached to the amide.

3.6 Sensory analysis

3.6.1 Triangle test

A triangle test was conducted to assess the perception of differences in the color, odor, and texture attributes between the control habanero pepper pastes and added with LEO. The results for green and orange habanero pepper pastes showed that 37 out of 41 persons correctly identified the samples, suggesting significant differences ($p < 0.05$) in the perception of the evaluated pastes. The habanero pepper pastes with the addition of LEO significantly affected the sensory quality of the product. According to the sensory test, the odor was the most significant difference in the habanero pepper pastes with LEO, which was attributed to the presence of volatile compounds such as cis-2,4-dodecadienal, trans-2,4-dodecadienal hexanol, cis-3-hexenol, and trans-nerolidol, which was capable of imparting fruity notes, that could mask the aroma of the habanero chili pepper (Cheong *et al*., 2012).

3.6.2 Quantitative response scale test

Results from the quantitative response scale test are presented in Table 2. LEO did not significantly affect ($p<0.05$) the color and texture on green and orange habanero pastes, nevertheless the odor was affected in both pastes. According to the quantitative response scale test, the results inferred that the addition of the LEO only affected the odor attribute of the sample. Volatile compounds characteristic in the habanero pepper such as 2-isobutyl-3-methoxypyrazine, 2,6-nonadienal, and decadienal for green pastes, and aliphatic esters such as isobutanoic, 2-methylbutyric, isopentanoic, and hexanoic acids in the orange pastes (Pino *et al*., 2011) could be masked by the presence of the volatile compounds from the LEO.

3.6.3 Preference test

A preference test was conducted to assess if one of the two samples presented better acceptable sensory characteristics expressed as flavor preference of the green and orange habanero pepper pastes, and the results are presented in the Table 3. Contrary to the quantitative response scale test, in the preference test, control sample was preferred as it received the highest number of preferred answers reported as preferred answers/total answers, with statistically significant difference ($p < 0.05$) for both, green and orange habanero pepper pastes. Similar results were reported by Bozkurt & Erkmen, (2005), who evaluated the effect of the addition of *Saccharomyces* and *Streptomyces* on hot pepper pastes and panelist clearly detected a difference. Mexis *et al*. (2012) who used LEO as a food additive also reported a negative effect on the sensorial quality of fresh chicken meats added with LEO observing that an increase in concentration of the LEO (0.1 and 0.2 mL / 100 g) decreased general acceptability because of the panelist's perception of a fruity flavor. However, the rosemary or clove essential oils have improved acceptance to the final products which could be represented an alternative for future studies in habanero chili pastes (Contini *et al*., 2014; Viuda-Martos *et al*., 2010).

Table 2. Sensory evaluation by quantitative response scale test of green and orange habanero pepper pastes.

Sensory parameters	Treatment	Green habanero pepper paste	Orange habanero pepper paste
Color	C+TT	3.86 ± 0.76^a	3.20 ± 1.10^a
	LEO+TT	3.55 ± 0.69^a	3.59 ± 0.86^a
Odor	C+TT	3.84 ± 0.95^b	3.27 ± 0.84^b
	LEO+TT	2.73 ± 0.99^a	2.34 ± 0.95^a
Texture	C+TT	3.61 ± 0.71^a	3.52 ± 0.92^a
	LEO+TT	3.41 ± 0.75^a	3.64 ± 0.91^a

Values with different letters are significantly different ($p < 0.05$) within each stage of maturity (green or orange) and for each sensory parameter. Mean ± SD; n = 41. C+TT=Control paste with Thermal Treatment; LEO+TT= Paste with LEO and Thermal treatment.

Table 3. Sensory evaluation by preference test of green and orange habanero pepper pastes.

Treatment	Green habanero pepper paste	Orange habanero pepper paste
C+TT	20/41[a]	16/41[a]
LEO+TT	8/41[b]	9/41[b]

Results are expressed as the number of preferred answers/total answers. Mean ± SD; n = 41. The same letter in the same column indicates that mean values are not statistically different (p > 0.05). C+TT= Control paste with thermal treatment; LEO+TT=Paste with Lemon Essential Oil with thermal treatment.

Conclusions

Physicochemical, rheological, biological, and sensorial properties of habanero pepper pastes (*Capsicum chinense* Jacq.) were significantly affected by ripening stage, thermal processing and LEO. Physicochemical properties indicated that thermal processing significantly affected the color parameters, but the addition of LEO favored color preservation maintaining the original color of the pastes and decreasing the pH value. TPC, TCC and antioxidant activity values were also favored by LEO addition compare to the fresh fruit, furthermore, TPC and antioxidant activity were potentiated with thermal process indicating that pastes added with LEO and subjected to thermal processing were the best conditions. The rheological behavior indicated that the orange paste presented the best flow index and consistency coefficient parameters as well as viscoelastic (G' and G") properties standing out the behavior of the orange paste added with acetic acid and subjected to the thermal processing. The sensory evaluation indicated that the combination of LEO and thermal processing significantly affected odor attributes maybe because of the presence of volatile compounds from LEO, nevertheless color and texture did not present significant differences. Further analysis of shelf life test could be interesting to evaluate to continue increasing the value of these products. These results highlight the importance of the physicochemical, rheological, biological, and sensorial evaluations as well as the natural additives and thermal processing to increase habanero pepper marketing possibilities, in this case, to develop a processed product that will satisfy the needs and demands of the consumers and thus promote the habanero pepper consumption.

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