GRASAS Y ACEITES 69 (1)
January–March 2018, e240
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0884171

Evaluation of the oxidative stability of Chipotle chili (*Capsicum annuum* L.) oleoresins in avocado oil

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Submitted: 03 August 2017; Accepted: 30 November 2017

SUMMARY: *Capsicum annuum* L. (Chipotle chili) is a natural source of bioactive metabolites with antioxidant properties. The objective of this research was to obtain and characterize the oxidative stability under storage of Chipotle chili oleoresins extracted with cold-pressed avocado oil. The most efficient conditions obtained to extract carotenoids and phenolic compounds were at 1:3 ratio (chipotle chili: avocado oil; w:v) at room temperature in darkness during 48 h. At the end of the harshest conditions (45 °C, 30 days), the extracts were stable to lipid oxidation with a final Totox value of 27.34, a carotenoid preservation of 85.6%, antioxidant activity retention of 80.66% and a color change (ΔE) of 1.783. The kinetic constants obtained were higher for peroxide formation than for carotenoid degradation. The oleoresins obtained could be considered an economic and sustainable alternative to extract carotenoids with good oxidation stability that could be used in foodstuffs.

KEYWORDS: Capsicum annum *L.; Carotenoids; Lipid stability; Oleoresin*

RESUMEN: ***Evaluación de la estabilidad oxidativa de oleorresinas de chile Chipotle (*Capsicum annuum *L.) en aceite de aguacate.*** *Capsicum annuum* L. (chile Chipotle) es una fuente natural de metabolitos bioactivos. El objetivo de esta investigación consistió en evaluar la estabilidad oxidativa durante el almacenamiento de aceites de aguacate extraído en frio en presencia de oleorresinas de Chile Chipotle. Las condiciones más eficientes obtenidas para la extracción de carotenoides se dieron a una concentración 1:3 (p/v: chipotle chile/aceite de aguacate) durante 48 h en oscuridad a temperatura ambiente. Al final de las condiciones de almacenamiento más severas (45 °C, 30 días) los extractos fueron estables a la oxidación lipídica con un valor de Totox de 27.34, una conservación de carotenoides del 85.6%, una retención de la actividad antioxidante del 80.66% y un cambio de color (ΔE) de 1.783. Las constantes cinéticas obtenidas fueron mayores para la formación de peróxidos que en la degradación de carotenoides. En conclusión, las oleorresinas obtenidas bajo las condiciones anteriores pueden considerarse como una alternativa económica y sustentable para la extracción de carotenoides con una buena estabilidad oxidativa.

PALABRAS CLAVE: Capsicum annum *L.; Carotenoides; Estabilidad oxidativa; Oleorresinas*

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Citation/Cómo citar este artículo: Cerecedo-Cruz L, Azuara-Nieto E, Hernández-Álvarez AJ, González-González CR, Melgar-Lalanne G. 2108. Evaluation of the oxidative stability of Chipotle chili (*Capsicum annuum* L.) oleoresins in avocado oil. *Grasas Aceites* **69** (1), e240. https://doi.org/10.3989/gya.0884171

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1. INTRODUCTION

Adding additives is a common practice in food processing to avoid spoilage, food alterations and to improve some sensorial characteristics in an economical and competitive way. However, nowadays, consumers are concerned about the safety of additives (Bearth *et al.,* 2014). Therefore, the food industry is migrating to develop safer additives, usually extracted from natural sources capable of providing preservative functions in addition to sensorial characteristics (Melgar-Lalanne *et al.,* 2016).

Chipotle chili (*Capsicum annuum* L.) is a medium pungency smoke-dyed Jalapeño chili widely appreciated for its unique flavor in the preparation of marinated sauces, purees, canned products, snacks and as an ingredient in traditional Mexican cuisine (Gómez-Moriel *et al.,* 2012). Although their sensorial characteristics remain as the main reason for their industrial and domestic use, the high quantity and quality of the antioxidants present in this fruit make them an outstanding natural antioxidant. This high antioxidant capacity is provided by bioactive metabolites such as phenolic compounds, capsaicinoids and carotenoids where the hydrophilic fraction of these compounds is the main contributor to the total antioxidant activity of the fruit (Kittisakulam *et al.,* 2016). However, when extracted in non-polar solvents, only a small portion of the hydrophilic phenolic compounds could be extracted while the lipophilic fraction is extracted in its majority (Guadarrama-Lezama *et al.,* 2012; Cavazza *et al.,* 2015). These non-polar extracts are usually obtained with organic solvents which require them to be removed before their addition to foods (Guadarrama-Lezama *et al.,* 2012). Moreover, the high process temperatures (> 60 °C) during industrial processing may produce structural changes in some valuable compounds leading to a possible loss in functionality (Melgar-Lalanne *et al.,* 2016).

Carotenoids, which are important both as colorants and antioxidants, are hydrophobic extracted compounds that belong to the family of terpenoids with a wide range of organic solvents such as hexane, acetone and methanol with different extraction rates (Melgar-Lalanne *et al.,* 2016). Recently, the use of vegetable oils as non-polar solvents has been explored with a relatively good extraction rate to extract lipophilic metabolites in different matrixes (Goula *et al.,* 2017; Guadarrama-Lezama *et al.,* 2012). Their low polarity (between 5–15 mN/m) (Kovacs *et al.,* 2016) and high bio-accessibility of carotenoids in vegetable oils (Victoria-Campos *et al.,* 2013; Unlu *et al.,* 2005) makes them an economic and sustainable option. Although few studies have used vegetable oils to extract *Capsicum* bioactive metabolites (Guadarrama-Lezama *et al.,* 2012; Amruthraj *et al.,* 2014), carotenoids have been successfully extracted in edible oils from other sources such as shrimp waste and pomegranates (Sachindra and Mahendrakar, 2005; Goula *et al.,* 2017). Moreover, the presence of carotenoids was responsible for preventing oxygen degradation in olive oil during storage (Cavazza *et al.,* 2015; Salazar *et al.,* 2012).

Cold-pressed avocado oil is extracted similarly to extra virgin olive oil and appreciated for culinary purposes, for its potential health benefits and for being environmentally friendly (Wong *et al.,* 2011). It contains mainly oleic acid (69–74%) as well as a small minority of desirable compounds like vitamins, phytosterols, chlorophylls and carotenes that play a positive role in reducing coronary heart disease risk, cataracts and diabetes (Sun-Waterhouse *et al.,* 2011a). Avocado oil was able to increase the *in vivo* absorption of carotenoids in *in vivo* trials (Unlu *et al.,* 2005). From a technological point of view, cold-pressed avocado oil (also cold virgin avocado oil) is a high quality oil, rich in oleic acid, extracted without the presence of organic solvents and without the addition of artificial antioxidants which improves its shelf life in a eco-friendly way. These characteristics allow cold-pressed avocado oil (similarly to extra-virgin olive oil) to be a suitable model to study the lipid oxidation of oils and fats when a substance with proven antioxidant properties is added (Wong *et al.,* 2011).

In this scenario, the use of avocado oil as solvent may offer an attractive advantage compared to conventional methods for carotenoid extraction due to its low polarity, high accessibility, oxidative stability and safety. Furthermore, being that it is a cold-pressed oil there are no traces of organic solvents in the oleoresin composition (Güneser *et al.,* 2017).

The objective of this research was to explore the oxidative stability under storage of Chipotle chili (*C. annuum* L.) oleoresins extracted with commercial cold-pressed avocado oil.

2. MATERIALS AND METHODS

2.1. Materials

Chipotle chili fruits (*Capsicum annuum* L.) and cold-pressed avocado oil (Ahuacatlán, Mexico) were bought in a supermarket in Xalapa, Veracruz (Mexico). Only whole and free of damage Chipotle fruits were considered for this research. All chemicals used were purchased from Sigma-Adrich (USA), Merk (Germany), or Dibico (Mexico) and were of analytical grade.

2.2. Chipotle chili oleoresins preparation

Due to the high relative humidity in Xalapa, Ver. Mex. (around 78% annual, Conagua http://200.4.8.21/observatorios/historica/veracruz.pdf, *reviewed on Nov, 17, 2017*), and although

Chipotle chili is a sun dried chili, the fruits were previously conditioned at 45 °C in order to reduce the water content until constant weight (48–72 h) and facilitate grinding. The initial moisture content of commercial chipotle chili was 33.97 ± 0.14 gH_20/100g sample; and after the conditioning at 45 °C the water content was 20.81 ±0.51 gH_20/100g sample. Then, the chilies were ground in a commercial mill (Hamilton Beach, EUA), and subsequently sieved using a 1 mm mesh. Milled chili was mixed with avocado oil in two different ratios: 1: 2 and 1:3 (w/v: Chipotle chili/avocado oil) and conserved in amber glass bottles in the dark for 48 h at room temperature (~25 °C). The oleoresins were centrifuged (3,500 g, 15 min, 4 °C) to facilitate solid separation, decanted, filtered and stored in amber glass bottles protected from light at 4°C until further use.

The total carotenoids and total phenolic compounds extraction yield (%) were calculated as equation (1):

$$Extraction\ Yield\ (\%) = \frac{[A_{oil} - A_{avocado}]}{[A_{chili}]} \times 100 \quad \text{Eq. (1)}$$

Where: A_{oil} is the total carotenoid or total phenolic compound concentration in the oleoresin (µg/mL); $A_{avocado}$ is the total carotenoid or total phenolic compound concentration in the avocado oil and A_{chili} is the total carotenoid or total phenolic compound concentration of chili powder (µg/mL).

2.3. Total carotenoid content determination

The total carotenoid content was determined (Hornero-Méndez and Mínguez-Mosquera, 2001). Briefly, 100 µL of Chipotle oleoresin (~120 µg) were diluted with 25 mL of acetone and mixed in the dark to avoid oxidation. Then, 1 mL of each sample was read with a spectrophotometer (Velab VE-5600UV, Mexico City, Mexico) at 472 and 508 nm with acetone as blank. Total carotenoids were expressed following the Lambert-Beer law with the following equations (2)-(4).

$$CR = \frac{2144.0 \times A_{508} - 403.3 \times A_{472}}{270.9} \quad \text{Eq. (2)}$$

$$CY = \frac{1724.3 \times A_{472} - 2450.1 \times A_{508}}{270.9} \quad \text{Eq (3)}$$

$$CT = CR + CY \quad \text{Eq (4)}$$

Where: CR is the red carotenoid fraction; CY is the yellow carotenoid fraction and CT is the total carotenoids; A_{508} and A_{472} is the read absorbance at these wavelengths expressed in nm.

2.4. Determination of total phenolic compounds

Total phenolic compounds were determined by the Folin test as described by Guadarrama-Lezama *et al.,* 2012 using the Folin-Ciocalteau reagent. The Folin-Ciocalteu reagent was diluted in deionized water 1:10 (w/w) prior to use. Each sample was diluted 1:5 (w/w) with ethanol, and a 0.1 mL aliquot was mixed with 0.75 mL of the previously prepared Folin-Ciocalteau reagent. The solution was incubated at room temperature for 5 min and 0.75 mL of sodium bicarbonate (60 g/L) were added and thoroughly mixed. The mixture was incubated at room temperature for 90 min and filtered by using a 0.45 µm syringe filter (Whatman, International Ltd., Maidstone, Kent, UK). Absorbance was measured at 750 nm. Gallic acid was used as standard. Results were expressed as gallic acid equivalents (µg) per mL of oleoresin.

2.5. Antioxidant activity

2.5.1. Ferric reduction antioxidant power (FRAP)

FRAP was determined as described by Benzie and Strain (1996) with slight modifications. FRAP reagent was prepared as follows: 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ-HCl (2,4,6-Tripyridyl-s-Triazine:HCl 40 mM), and 20 mM ferric chloride 10:1:1 (v:v:v) were heated at 37 °C for 30 min. Then, 100 µL of samples were diluted with 1 mL of acetone. Absorbance was measured as 593 nm and TEAC (trolox equivalent antioxidant capacity) was expressed in milimoles (mM) of TEAC/g. A standard curve of trolox (6-hydroxy-2,5,7,8-tetramethylchloroman-2-carboxulic acid) was used to estimate the antioxidant capacity of the samples.

2.5.2. Scavenging of $ABTS^{\cdot+}$ radical

The scavenging activity of $ABTS^+$ radical was determined as described by Sousa *et al.,* (2015) based on the capacity of a sample to inhibit the $ABTS^+$ radical produced by reacting 7 mM ABTS stock solution with 2.45 mM PotasiumPersulfate ($K_2S_2O_7$) and allowing it to stand in the dark at room temperature for 12–16 h before use. The stock solution of $ABTS^+$ was diluted in ethanol until an absorbance of 0.70 ± 0.02 at 734 nm at 25 °C. Once the radical was formed, 2.9 mL of the $ABTS^+$ radical solution was mixed with 100 µL of each sample and the absorbance measured at 734 nm. A calibration curve was prepared with Trolox diluted in ethanol at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM and read at 734 nm. Inhibition (%) was calculated as Equation (5):

$$\%inhibition = \frac{A_{st0} - A_{stf}}{A_{st0} - \left(\frac{A_{sd0} - A_{sdf}}{A_{sd0}} \right)} \quad \text{Eq (5)}$$

Where A_{sto} and A_{stf} are the initial and final absorbance; A_{dto} and $A_{dtf}$are the initial and final absorbance of the dissolvent. The results were expressed as μM trolox / g.

2.6. Color measurement

Color measurements of chili oleoresins were carried out using a colorimeter (Color Flex XC1115 Hunter Lab, USA). The instrument was calibrated with a tile (L^*= 97.02, a^* = 0.08, b^* = 1.75) before the measurements and expressed in the CIELab color model. At least 10 measurements of each sample were taken and average values were determined for further calculation. Color changes can be measured as the modulus of the distance vector between the initial color values and the actual color coordinated. This concept is named "total color difference" (Pathare *et al.,* 2013) and is calculated in the CIELab color model as Equation (6).

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad \text{Eq (6)}$$

Where L^* value is the measurement of lightness, ranging from 0 (black) to 100 (white); a^* value ranges from −100 (green) to +100 (red); b^* value ranges from −100 (blue) to +100 (yellow); ΔL*, Δa*, Δb* are the absolute differences between the initial and final values. ΔE is the total difference between the reference and sample color. Differences in color were classified as very distinct ($\Delta E > 3$), distinct ($1.5 < \Delta E < 3$) and small difference ($\Delta E < 1.5$) as according to Pathare *et al.,* 2013.

In addition, a more appropriate measurement of color was obtained from the calculation Chroma (Eq.7) and hue angle (Eq.8). Hue angle is the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue and yellow. So the higher the hue angle is, the less yellow. Chroma is an index somewhat analogous to color saturation or intensity of a color. Hence, the higher Chroma value, the higher the color intensity. Both parameters are polar coordinates and easy to visualize, giving a more reliable appreciation of color.

$$Chroma = \sqrt{\left(a^2 + b^2\right)} \quad \text{Eq. (7)}$$

$$Hue\ angle = \arctan\left(\frac{b}{a}\right) \quad \text{Eq. (8)}$$

2.7. Degradation under storage conditions

Chipotle chili oleoresin in avocado oil at 1:2 (CHOAO 1:2) (w/v) and Chipotle chili oleoresin in avocado oil 1:3 (CHOAO 1:3) (w/v) and avocado oil (AO) (as control) were exposed to three different temperatures under darkness (8, 25 and 45 °C) and stored for 28 days. Darkness was carried out to guarantee that the oxidation was only due to the temperature and not to light (photo-oxidation); temperatures were determined in function of refrigeration conditions (8 °C), room temperature (25 °C) and accelerated oxidation conditions along with extreme environmental temperatures in tropical zones (45 °C); 28 days of storage was considered since it was a sufficient lapse of time to observe the degradation of the components analyzed. A chemical analysis were done each third day. Degradation was calculated as a percentage the difference between the metabolite (total carotenoids or total phenolic compounds) at a determined time and at initial time.

2.8. Peroxide, *p*-anisidine and Totox values

Peroxide value (PV), *p*-anisidine value (AV) and Totox values were determined. PV was evaluated following the AOCS official method (Cd 8–53) and was expressed as peroxide miliequivalents of active oxygen per kilogram of oil ($meqO_2$ / kg). An aliquot (0.5 g) of oil was used for each assay. A blank sample as reagent control was used and carried through all the steps. AV was determined with the AOCS official method (Cd 8–53) and expressed as mg /kg, an aliquot (0.5 g) of oil was used for each assay and the sample's absorbance was measured at 350 nm (AOCS, 2000). The Totox value (a total oxidation value) gives an overall and empirical measurement of the relevant precursors, the non-volatile carbonyls, present in the oils (Wei *et al.,* 2009) and was calculated with Equation. (9).

$$Totox\ value = 2PV + AV \quad \text{Eq. (9)}$$

2.9. Kinetics considerations for total carotenoid degradation and peroxide formation

The total carotenoid degradation and peroxide formation due to storage were assumed to follow first order kinetics (Equation. (10) as Vikram *et al.,* 2005.

$$\pm\frac{dC}{dt} = kC \quad \text{Eq. (10)}$$

Where C is the total carotenoids or peroxide index present in the oleoresin at time *t*; *t* is the storage time; and *k* is the kinetic constant for carotenoid

degradation or peroxide formation, C_0 is the carotenoid concentration or peroxide index at initial time, so the linear relationship for Equation (11) is:

$$lnC = lnC_0 \pm kt \qquad \text{Eq. (11)}$$

For equations (10) and (11) the plus sign (+) is applied to peroxide formation and the minus sign (-) for carotenoid degradation.

The kinetic constant (k) was assumed to vary with the absolute temperature (T), according to the Arrhenius-type equation:

$$k = K_0 \exp\left(\frac{-E_a}{RT}\right) \qquad \text{Eq. (12)}$$

Where K_0 is the frequency factor (weeks^{-1}), Ea is the activation energy (kJ/mol), R is the gas constant (8.314J/molK) and T is the absolute temperature (K).

2.10. Statistical analysis

Experimental data for the different parameters were fitted and processed with Excel 7 (Microsoft, Redmond, WA, USA) and Sigma Plot 11.0 software (SPSS Inc, Chicago, Il, USA). All results were expressed as mean values of triplicates. Statistical comparisons between two groups were made using the Student's test. With several groups, one-way analysis of variance was used. When significant F values were obtained, group differences were evaluated by the Tukey test. The significant level considered was $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction of total carotenoids and total phenolic compounds and color parameters

The data of the initial total carotenoids, phenolic amount and color parameters of chipotle chili powder, avocado oil, CHOAO 1:2 and CHOAO 1:3 are shown in table 1. The best extraction of total carotenoids and total phenolic compounds as well as the main antioxidant activity was obtained with CHOAO 1:3. In this oleoresin, the total carotenoid extraction rate was 40.08% (w/v) and the total phenolic extraction rate 2.26% (Table 1), however, CHOAO 1:3 was more diluted than CHOAO 1.2. CHOAO 1:2 had significantly ($p \leq 0.05$) higher antioxidant activity than CHOAO 1:3 (Figure 1c). This could be attributed to the higher total content of carotenoids as well as phenolic compounds presents in CHOAO 1:2 when expressed in oil ml (408.86 µg carotenoids/ mL oil in CHAOA 1:2 *vs.* 309.84 µg carotenoids/ mL oil in CHOAO 1:3). The tested sample of avocado oil showed a similar composition

TABLE 1 Total initial carotenoids, total phenolic compounds extracted from Chipotle Chili powder (CH) and color parameters of Chiptole oleoresins in avocado oil 1:2 (CHOAO 1:2) and 1:3 (CHOAO 1:3); Chipotle chilli powder and avocado oil (AO). Carotenoid extraction rate (%) and total phenolic extraction rate (%) of CHOAO 1:2 and CHOAO 1:3

Sample	Total Carotenoids (µg /g CH)	Total Phenolics (µg gallic acid /g CH)	Extraction rate (%)		Color parameters				
			Total Carotenoids	Total Phenolics	L*	a*	b*	Chroma	Hue
CHOAO 1:2	817.720 ± 9.30 ^{c}	423.822 ± 2.97 ^{c}	40.08	1.96	0.860 ± 0.04	3.160 ± 0.04	0.570 ± 0.05	10.322 ± 075	3.210 ± 0.05
CHOAO 1:3	929.529 ± 1.68 ^{b}	487.419 ± 2.33 ^{b}	43.98	2.26	1.620 ± 0.05	4.740 ± 0.05	0.920 ± 0.06	10.970 ± 0.64	4.830 ± 0.06
AO**	40.079 ± 1.36 ^{a}	8.737 ± 0.75 ^{a}			20.556 ± 1.09	-2.843 ± 1.08	7.946 ± 0.20	8.443 ± 0.14	69.800 ± 2.09
CH	1840.0 ± 3.0 ^{d}	21089.35 ± 184.77 ^{d}			37.766 ±0.40	18.230 ± 0.10	32.920 ± 0.245	37.08 ± 0.20	60.530 ±0.27

*Values represent the mean of three measurements (± SD). Values in the same column with different letters are significantly different (Tukey test, $P < 0.05$). ** AO: Avocado oil expressed as µg / mL

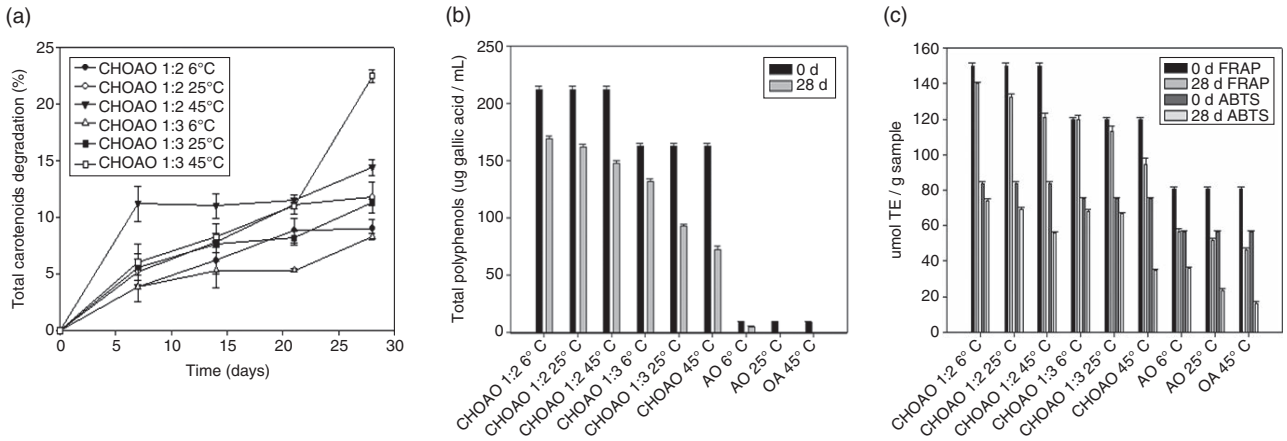

FIGURE 1. Effect of temperature and storage time on degradation of a) Total carotenoids expressed as difference between final and initial carotenoids; b) Total phenolic compounds; c) Antioxidant activity expressed as µmol TE/ g sample of Chipotle oleoresin in avocado oil 1:2 (CHOAO 1:2), Chipotle oleoresin in avocado oil 1:3 (CHOAO 1:3) and avocado oil (AO).

to the one previously reported (Qin and Zhong, 2016). However, it was poor in carotenoids and phenolic compounds when compared with the oleoresin obtained.

The carotenoids extracted in the present research with avocado oil were higher (929.529 ± 1.68 µg/ g Chipotle powder; CHOAO 1:3) than previous reports using maceration with other vegetable oils. So, Guadarrama-Lezama *et al.,* 2012 obtained up to 231.1 µg Carotenoids /mL oil in *Capsicum annuum* L. *grossum* when extracted with corn oil at higher temperatures (between 60 and 80 °C) and similar to those obtained with the use of supercritical CO_2 and avocado oil as solvents in chili obtained up to 280 to 460 µg of capsanthin /g dry matter a major carotenoid in chili (Barros *et al.,* 2016). Differences regarding other vegetable oils are probably due to the diverse nature of the fatty acids present amongst them (Sachindra *et al.,* 2005).

The hydrophilic nature of phenolic compounds could be responsible for their poor extraction in the present research (Asnin and Park, 2015). Hence, their presence cannot be considered as an important factor in the prevention of the lipid oxidation of oleoresins. Nonetheless, the phenolic compounds measured were slightly higher than those reported in corn oil by Guadarrama-Lezama *et al.,* 2012 with other pungent chili and the degradation rate was lower in both oleoresins than in the avocado oil. This fact shows that the carotenoids may have a key role in oleoresin protection against oxidation.

Therefore, the color components from the chili powder, mainly red components, could be extracted from oil. Both oleoresins showed a very dark red color (almost black) as can be appreciated in Table 1, with the most concentrated one being the darkest, as previously reported (Akbas *et al.,* 2017).

3.2. Lipid oxidation during storage

Peroxides are primary oxidation products that can further undergo degradation to form low molecular weight aldehydes and ketones, hence giving an idea about the present oxidation state of oils and fats and are the main oxidation products formed during auto-oxidation. Radicals are prone to produce peroxidation and follow the typical chain reactions. Meanwhile, the anisidine determination determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) in fats and oils and gives evidence about the shelf life and the secondary oxidation products, mainly aldehydes and ketones. Finally, the Totox value is used to estimate the oxidative deterioration of food lipids since it includes both parameters and is an indicator of the past history of the oil (as reflected in AV) and the present state (as evidenced in PV). Subsequently, PV can decrease over time when secondary products are formed and AV and Totox values should be considered for a better interpretation of the oxidation phenomena (Shahidi and Wanasundara, 2002).

The PV and AV of CHOAO 1:2, CHOAO 1:3 and AO during storage at different temperatures (6, 25 and 45 °C) are shown in Table 2 and Totox kinetics are presented in Figure 2. In avocado oil, the results of PV and AV were lower than those previously reported in avocado oils from different varieties (Prescha *et al.,* 2014; Espinosa-Alonso *et al.,* 2017). No references about lipid oxidation in high concentrated Capsicum oleoresins were found. However, in infused edible oils (up to 3% of Capsicum powder or Capsicum oleoresins), the presence of the Capsicum compounds, mainly the carotenoid fraction, provided a protective effect against lipid oxidation in infused oils due to their non-polar nature (Rege and Momin, 2017, Cavazza *et al.,* 2015; Salazar *et al*., 2012).

TABLE 2. Peroxide value (meq O_2 / kg) and *p*-Anisidine value (mmol / kg) of Chipotle oleoresin in avocado oil 1:2 (CHOAO 1:2), Chipotle oleoresin in avocado oil 1:3 (CHOAO 1:3) and avocado oil (AO)

Time (days)	T (°C)	Peroxide value (PV)			*p*-anisidine value (AV)		
		CHOAO 1:2	CHOAO 1:3	AO	CHOAO 1:2	CHOAO 1:3	AO
0	25	2.827 ± 0.257b,A	2.514 ± 0.149b,A	1.847 ± 0.094a,A	1.0445 ± 0.1760^{b,A}	1.0588 ± 0.1784^{b,A}	0.2057 ± 0.0617^{a,A}
7	6	2.892 ± 0.136a,B	2.904 ± 0.136a,A	2.787 ± 0.087a,A	1.9631 ±0.2619^{b,B}	1.8576 ± 0.2349^{b,A}	0.2862 ± 0.0593^{a,A}
	25	2.492 ± 0.125a,A	2.926 ± 0.339a,A	3.161 ± 0.079a,A	1.1519 ±0.4391^{b,A}	2.1604 ± 0.3585^{c,A}	0.3232 ± 0.1141^{a,A}
	45	5.055 ± 0.148b,C	3.174 ± 0.067a,A	5.113 ± 0.123b,B	1.5372 ± 0.0721^{b,AB}	2.0157 ± 0.1979^{b,A}	0.5469 ± 0.0895^{a,A}
14	6	3.305 ± 0.117a,A	3.124 ± 0.091a,A	2.789 ± 0.089a,A	1.1519 ± 0.4391^{b,A}	1.5725 ± 0.3856^{b,A}	0.3198 ± 0.0581^{a,A}
	25	3.824 ± 0.074a,A	3.672 ± 0.164a,A	3.718 ± 0.059a,B	1.4085 ± 0.1532^{b,AB}	1.4004 ± 0.0197^{b,A}	0.6214 ± 0.2068^{a,A}
	45	7.919 ± 0.122ab,B	7.479 ± 0.014a,B	8.454 ± 0.062b,C	1.9649 ± 0.1640^{b,B}	1.3274 ±0.0411^{ab,A}	0.9198 ± 0.1222^{a,A}
21	6	3.345 ± 0.104a,A	3.258 ± 569a,A	3.788 ± 0.052a,A	1.1442 ± 0.0994^{b,A}	1.5906 ± 0.2263^{b,A}	0.3327 ± 0.0824^{a,A}
	25	3.943 ± 0.159a,B	4.705 ± 0.232b,B	3.788 ± 0.135a,B	1.0180 ± 0.0200^{ab,A}	1.5443 ± 0.3416^{b,A}	0.7634 ± 0.1192^{a,AB}
	45	12.220 ± 0.275a,C	13.446 ± 0.101b,C	12.169 ± 0.121a,C	1.3801 ± 0.2148^{a,A}	1.3801 ±0.2148^{a,A}	1.1577 ± 0.1198^{a,B}
28	6	3.397 ± 0.195ab,A	3.565 ± 0.267b,A	2.828 ± 0.056a,A	1.4507 ± 0.3356^{c,A}	2.3229 ± 0.1717^{b,B}	0.4799 ± 0.1456^{a,A}
	25	4.227 ± 0.091a,B	4.702 ± 0.059a,B	4.209 ± 0.061a,B	1.0083 ± 0.3363^{a,A}	1.6104 ± 0.2816^{a,A}	1.0026 ± 0.0657^{a,AB}
	45	13.009 ± 0.178a,C	14.882 ± 0.191b,C	18.355 ± 0.245c,C	1.1166 ± 0.2399^{a,A}	1.8902 ± 0.1403^{a,AB}	1.3036 ± 0.0951^{ab,B}

*Values represent the mean of three measurements (± SD). Rows with equal lowercase letters and columns with equal capital letters indicate that there is no significant difference ($p > 0.05$).

The results of the AV of avocado oil and both oleoresins are shown in Table 2. The AV value increased during all the storage times analyzed in the AO. However, the behaviors in both oleoresins were different. In CHOAO 1:2 AV increased during the first 7 days and after this period the value was reduced. Meanwhile, in CHOAO 1:3, it decreased during the first week and after that, increased during the second and third weeks. A similar behavior was observed in sardine oil when different essential oils were added as antioxidant. This behavior may be due to the presence of carotenoids and polyphenols which can suffer structural changes during the oxidation process which affect the formation of aldehydes and ketones (Vaisali *et al.,* 2016).

In order to appropriately determine this phenomenon in edible oils, in addition to the detection of primary and secondary oxidation products, a combination of methods which measures the scavenging electron radical capacity such as ABTS and FRAP (Moon and Shibamoto, 2009; Shahidi and Zhong, 2015) as well as the degradation of antioxidant compounds (carotenoids and phenolic compounds) present was applied (Figure 1). Antioxidants can neutralize the radical cation $ABTS^{\bullet+}$ by direct reduction via electron donation or by radical quenching via hydrogen atom donation. The balance of these two mechanisms is determined by antioxidant structure and the pH of the medium (Prior *et al.,* 2005). Hence, although the TEAC assay is usually classified as an electron transfer based method, the hydrogen atom mechanism also applies. While FRAP antioxidants are also known as reductants, their electron donating capability can not only allow the scavenging of free radicals and other oxygen derived reactive oxygen species, but also reduce higher valent elements to their lower their valence state. The redox potential or reducing power of antioxidants is an important indicator of their antioxidant efficacy. The FRAP assay is a typical electron transfer based method that measures the reduction of ferric ion (Fe^{3+})–ligand complex to the intensely blue colored ferrous (Fe^{2+}) complex by antioxidants in acidic media. Both tests showed the protective effect of the antioxidant compounds present in the Chipotle chili to avoid the rancidity of the oleoresins when compared with the avocado oil.

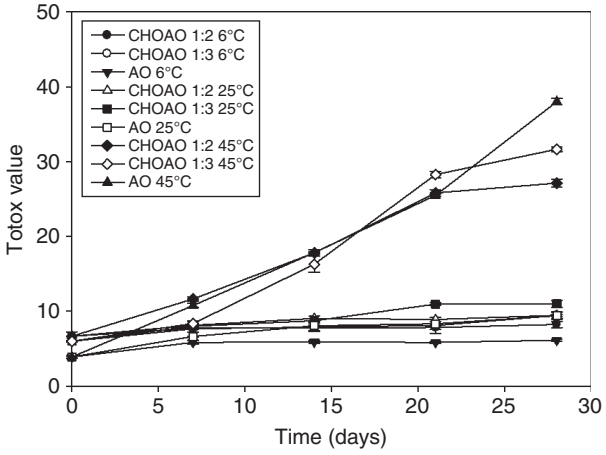

FIGURE 2. Totox value of Chipotle oleoresins in avocado oil 1:2 (CHOAO 1:2), 1:3 (CHOAO 1:3) and avocado oil (AO) at different temperatures during storage time.

During the storage time, $ABTS^{\bullet+}$ and FRAP capacities showed similar trends; avocado oil and both oleoresins were capable of scavenging both radicals in a concentration/temperature dependent manner (Fig. 1). For all samples tested at 0 days (beginning of storage) both radical capacities were stable and showed no difference between them, with no effect from the temperature applied. Nonetheless, CHOAO 1:2 showed the best scavenging electron radical capacities with an antioxidant activity loss at the end of the storage time at 45 °C of 36.671 ± 1.316 % measured by $ABTS^{\bullet+}$ and 18.214 ±1.952% measured by FRAP assay. This might be due to the higher content of carotenoids and other antioxidants which reduces oxidative degradation. Furthermore, CHOAO 1:3 showed no differences ($p \leq 0.05$) between 6 °C and 25 °C, for both capacities, suggesting a possible alternative antioxidant (AOX) resistance of the compounds contained in the oleoresin (storing temperatures which do not exceed 20–25 °C better preserve carotenoid

TABLE 3. Kinetic parameters of total carotenoid degradation and peroxide formation of Chipotle oleoresin in avocado oil 1:2 (CHOAO 1:2) and Chipotle oleoresin in avocado oil 1:3 (CHOAO 1:3) under storage at different temperatures.

		Carotenoid degradation				*Peroxide formation*			
Sample	**T (°C)**	***k* x 10^2 (weeks^{-1})**	**R^2**	**Ea (kJ/mol)**	**K_0 (weeks^{-1})**	***k* x 10^2 (weeks^{-1})**	**R^2**	**Ea (kJ/mol)**	**K_0 (weeks^{-1})**
CHOAO 1:2	6	2.42	0.966	8.707	1.040	5.13	0.927	38.337	659343.55
	25	3.14	0.971			9.26	0.941		
	45	3.83	0.961			39.35	0.973		
CHOAO 1:3	6	1.89	0.949	20.550	123.53	8.05	0.947	34.598	228433.40
	25	2.68	0.954			17.89	0.975		
	45	5.63	0.943			50.24	0.971		

stability, therefore safeguarding the nutritional value) (Hidalgo *et al.,* 2008). At 45 °C the antioxidant capacities showed a drastic decrease, indicating a faster degradation of the AOX compounds present in all oleoresins (accelerated oxidation temperature). The stability of carotenoids in the oleoresins, *per se,* is a function of storage conditions. Higher temperatures exert a strong influence on the kinetics of degradation, accelerating the rate of pigment decomposition, which is related as well with reaction rates (Table 3).

Itis well established that carotenoids are susceptible to oxidation when exposed to light, oxygen and enzymes; this oxidation could be important in chili and paprika powder storage under room temperatures both in light or darkness conditions (Namitha and Negi, 2010) and their presence as a result of their addition in cold-pressed oils can protect them from lipid oxidation (Alavi and Golmakani, 2017).

In the present research, at the end of the storage, different grades of degradation in the total carotenoid content loss (%) in both oleoresins were found (Figure 1), being notably higher under the most thermo-degrading conditions (45 °C). A similar behavior was observed in total phenolic compound degradation. The degradation of both antioxidant components may influence the total lipid oxidation of the oleoresin expressed in the Totox values (Figure 2).

At the end of the storage time, the Totox values were significantly higher at 45 °C (which was observed at early storage time from 7 days). This fact suggests the importance of the assessment of tertiary oxidation compounds in the tested oil. Moreover, only for 21 days of storage at 45 °C could the oleoresin not be acceptable for its consumption at the highest temperature tested (Totox > 30) (Sun-Waterhouse *et al.,* 2011b). Nonetheless, the oil degradation was not perceptible when preserved at room conditions, which suggests that it can be stored for more than a month without further organoleptic and/or nutritional modifications and without the addition of artificial antioxidants.

However, differences in the carotenoid degradation between both oleoresins were not statistically significant (P=0.570). The total carotenoid degradation is a big quality problem in paprika and chili powder because it is strongly related with color loss; the addition of other antioxidant molecules (such as tocopherols or BHT) is recommended (Koncsek *et al.,* 2016). Moreover, in avocado oil the natural presence of tocopherols acts as a natural antioxidant (Berasategui *et al.,* 2012). Meanwhile, in chili oleoresins microencapsulation has been used to reduce carotenoid loss during storage (Guadararrama-Lezama *et al.,* 2014), although the carotenoid loss presented here is similar to those encapsulated chili extracts (Dominguez-Cañedo *et al.,* 2015).

The carotenoid loss in both Capsicum oleoresins assayed depended on the initial carotenoid concentration and the temperature applied during storage. The values obtained indicated that the addition of Chipotle chili clearly increases the antioxidant activity *per se,* reducing the peroxide formation during storage in avocado oil.

Phenolic compounds are bioactive metabolites that might mitigate the oxidative damage and the risk of chronic diseases due to their ability to reduce free radical formation and to scavenge free radicals (González-Gallego *et al.,* 2010). However, due to its polar nature just a small amount was extracted in avocado oil in spite of being of great importance and present in the avocado pulp (Di Stefano *et al.,* 2017). Phenolic degradation was total in AO at 25 and 45 °C at the end of storage time, similarly to previously reported studies (Sun-Waterhouse *et al.,* 2011). The presence of capsicum oleoresin showed a protective effect, resulting in a reduction in the phenolic loss dependent on the capsicum concentration probably for the carotenoid protective effect in the oxidation process.

3.3. Color changes during storage

Total color difference and chroma are considered the most sensitive parameters for the measurement of color degradation in some foods in response to storage under different temperature conditions (Pathare *et al.,* 2013). The total color differences and Chroma during storage at different temperatures of both oleoresins and avocado oil are shown in Figure 3. The avocado oil exhibited significant differences ($p \leq 0.05$) in color from both oleoresins. These changes may be due to lipidoxidation during storage as the cold-pressed avocado oil used did not have antioxidants as additives in its composition. While the avocado oil showed a slightly greenish color both Chipotle extracts were reddish (see Table 1). Moreover, ground Chipotle chili showed red and blue color components (see Table1). The color of Capsicum oleoresins are clearly related with their carotenoid fraction, composed of more than 50 molecules identified and their loss during storage is an important quality issue although studies about the color stability in capsicum oleoresins are limited (Amruthraj *et al.,* 2014). No Pearson correlation could be found in the present research between total carotenoid degradation and total color changes (P < 90%). Nonetheless, color changes were slightly appreciated at the end of storage time in both oleoresins only when stored at 45 °C (AE> 1) and could not be perceptible at room temperature, which relates to the quality and stability of both oleoresins under appropriate storage conditions. The color differences suggest the presence of color-protective factors, such as the antioxidants coming from the Chipotle chili carotenoid fraction (Figure 3) which

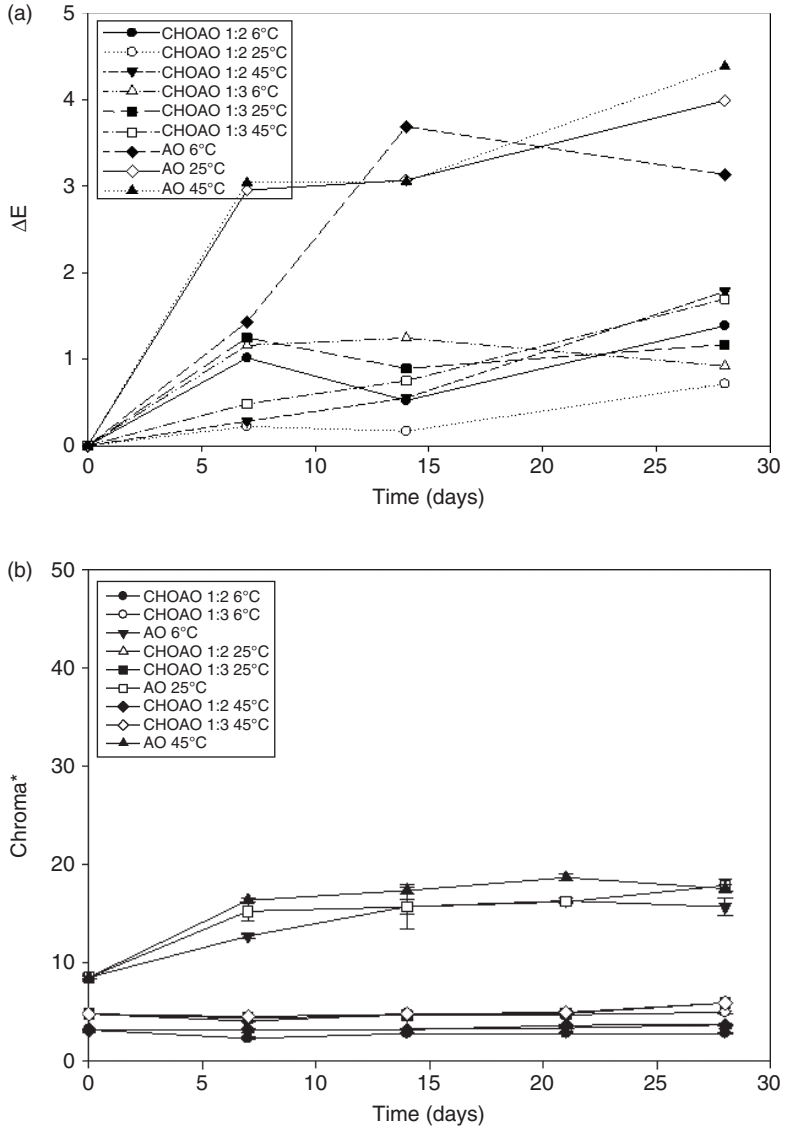

FIGURE 3. Effect of temperature and storage time on a) color changes(ΔE) and b) Chroma changes in Chipotle oleoresin in avocado oil 1:2 (CHOAO 1:2), Chipotle oleoresin in avocado oil 1:3 (CHOAO 1:3) and avocado oil (AO) during storage.

are partially degraded during the storage time. As storage advanced, the oleoresin samples became less saturated and slightly reddish. Great differences in perceivable color (ΔE > 3) could be observed in avocado oil at the end of the storage time in all temperatures tested, but was significantly higher at 45 °C. However, for both oleoresins the changes observed at the end of storage (28 days) were not detectable at first sight (1 < ΔE > 3) which indicates that oleoresin may be used as food colorant in foods with short and medium storage times (a month or less). Moreover, in the case of CHOAO 1:2 stored at 25 °C no color changes were detected (ΔE < 1). Longer storage studies should be performed to appreciate noticeable color changes and determine their shelf-life as a colorant.

3.4. Kinetics

Kinetic parameters for carotenoid degradation and peroxide formation are shown in Table 3. The carotenoid degradation and peroxide formation data corresponded to a first-order kinetic model, with 0.927 being the smallest value for the linear regression coefficients (R^2) and these are related to oxidative stability. As Table 3 shows, the higher the temperature, the higher the kinetic constant (k) and, therefore the faster the carotenoid degradation and peroxide formation. As previously mentioned, the total peroxides during storage increased with time, mainly at 45 °C and were higher in avocado oil than in both oleoresins (Table 2). For total carotenoid degradation, the activation energy (Ea) in CHOAO

1:2 was lower than in CHOAO 1:3, but for the peroxide formation the Ea variation may be considered minimal (see Table 3).

Comparing the results with those previously reported is somewhat difficult since different conditions are involved. However, Ea values were lower than previously reported for carotene degradation (Hidalgo *et al.,* 2008). The degradation is mediated at lower temperatures by the Ea, but as the temperature rises, the degradation reaction is controlled by the particle collisions found (K_0). In the case of peroxide formation, Piedrahita *et al.,* 2015 observed that when natural antioxidants were added to choibá oil, the Ea was higher than in the control without natural antioxidants, indicating that this addition reduced the oxidation rate and increased the lifetime of the product.

The kinetic constants are higher for peroxide formation than for carotenoid degradation (Table 3). The high increase in the number of collisions between molecules more than the slight change in the activation energy is the main factor. The frequency factor K_0 is an entropic factor which indicates the number of collisions that the reactant molecules suffer. Hence, in a reaction controlled by the enthalpy, the changes in the activation energy are proportionally bigger than the changes in the frequency factor. Additionally, in reactions controlled by the entropy, changes in the frequency factor are bigger than changes in the activation energy; in this case the reaction rate might be benefited from a change that may cause a bigger number of collisions of the reactant molecules. Hence, an entropic control implies that the frequency factor is the kinetic parameter that influences the most, instead of the activation energy as in the enthalpic control (Aguilar *et al*., 2016).

4. CONCLUSIONS

The maceration of Chipotle chili (*C. annuum* L.) in cold-pressed avocado oil could be considered as an economic and green solvent alternative to extract carotenoids with good oxidation stability under normal conditions. The most efficient conditions obtained were a 1:3 (w/v) ratio between chipotle chili and avocado oil, although the most concentrated oleoresin resulted was CHOAO 1:2. The extract obtained was stable against lipid oxidation during storage at room temperature as well as under refrigeration, although lipid oxidation was of importance at the end of the harshest conditions tested (45 °C). Moreover, Chipotle oleoresins showed a relatively high antioxidant activity, which is preserved at 6 and 25 °C. An appropriate storage under room temperature (≤ 25 °C) could be essential to conserve the oleoresin obtained for long time periods with a minimal loss in their functional and bioactive properties.

Thus, both oleoresins may be considered as natural additives. However, studies regarding their application in food products should be carried out to validate this fact, mainly because in foodstuffs the oleoresins will be diluted and interact with other food components. Furthermore, the influence of cooking temperatures in the antioxidant properties of the oleoresins should be completed to have a more comprehensive understanding of the protective effect of these bioactive compounds present before, during and after processing for different food products.

ACKNOWLEDGMENTS

This work was supported by the Consejo Nacional de Ciencia y Tecnología (Conacyt) through a Masters grant to Liesel Cerecedo.

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