



**KINETIC AND STATISTICAL CRITERIA FOR THE SELECTION OF CONDITIONS OF EXTRACTION OF VOLATILE COMPOUNDS OF PIQUIN PEPPER (*Capsicum annuum* L. var. *glabriusculum*)**

**CRITERIOS CINÉTICOS Y ESTADÍSTICOS PARA LA SELECCIÓN DE LAS CONDICIONES DE EXTRACCIÓN DE COMPUESTOS VOLÁTILES EN CHILE PIQUÍN (*Capsicum annuum* L. var. *glabriusculum*)**

C.F. Vázquez-Cárdenas<sup>1</sup>, J.I. Valiente-Banuet<sup>2,\*</sup>, P. Caballero-Mata<sup>3</sup>, H. Mújica-Paz<sup>1</sup>,  
J. Rodríguez-Rodríguez<sup>3</sup>, J. Welti-Chanes<sup>1\*</sup>

<sup>1</sup>Escuela de Ingeniería y Ciencias. Centro de Biotecnología FEMSA. Tecnológico de Monterrey. Av. Eugenio Garza Sada 2501. Monterrey, NL., C.P. 64849, México.

<sup>2</sup>Escuela de Biotecnología y Ciencias de la Salud. Centro de Biotecnología FEMSA. Tecnológico de Monterrey. Av. Eugenio Garza Sada 2501. Monterrey, NL., C.P. 64849, México.

<sup>3</sup>Escuela de Ingeniería y Tecnologías de Información. Centro de Calidad Ambiental. Tecnológico de Monterrey. Av. Eugenio Garza Sada 2501. Monterrey, NL., C.P. 64849, México.

Received March 4, 2015; Accepted April 11, 2015

**Abstract**

This study was aimed to analyze the effect of temperature (40, 60, and 80 °C) and time (10, 40, and 80 min) on extraction of major volatile compounds (VC) from piquin peppers (*Capsicum annuum* L. var. *glabriusculum*) using HS-SPME. VC extraction was significantly affected ( $P < 0.05$ ) by temperature and time, which was described by a kinetic fractional conversion equation and the Arrhenius model. However, principal component analysis (PCA) performed on major VC determined a differential response of VC to extraction conditions. Two distinctive groups of compounds were identified using kinetic and statistical criteria: compounds associated (GR1) and poorly associated (GR2) with the aromatic profile of peppers. Extraction of GR1 compounds was favored at 40 °C and 80 min; whereas for GR2, extraction was favored at 80 °C and 80 min. The best extraction condition of VC associated with aroma of piquin pepper was determined (40 °C and 80 min) considering the individual effect of temperature that favored extraction of VC within GR1. The results showed that the use PCA can be a useful tool for a better selection of extraction conditions of the aromatic fraction of interest.

**Keywords:** aromatic fraction, bird pepper, fractional conversion model, HS-SPME and GC-MS, principal component analysis (PCA), volatile profile.

**Resumen**

El objetivo de este estudio fue analizar el efecto de temperatura (40, 60 y 80 °C) y tiempo (10, 40 y 80 min) sobre la extracción de los componentes volátiles mayoritarios (CV) asociados al aroma del chile piquín (*Capsicum annuum* L. var. *glabriusculum*) mediante HS-SPME. La extracción de CV fue afectada significativamente ( $P < 0.05$ ) por la temperatura y el tiempo en un efecto descrito por un modelo cinético de conversión fraccional y el modelo de Arrhenius. Al aplicar análisis de componentes principales (ACP) a los CV se determinó una respuesta diferente a las condiciones de extracción. El uso de criterios cinéticos y estadísticos permitió identificar dos grupos de CV asociados (GR1) y pobremente asociados (GR2) al perfil aromático de los chiles. La mayor extracción de GR1 se obtuvo a 40 °C y 80 min; mientras que la extracción de GR2 fue favorecida a mayor temperatura y tiempo. La condición de extracción seleccionada fue de 40 °C y 80 min que favorece la extracción de los CV asociados al aroma de los chiles. Los resultados indican que el ACP es una herramienta útil para la selección de las mejores condiciones de extracción de la fracción aromática de interés.

**Palabras clave:** fracción aromática, chiltepín, piquín, modelo de conversión fraccional, HS-SPME y GC-MS, análisis de componentes principales (ACP), perfil volátil.

\*Corresponding authors. E-mails: : valiente@itesm.mx; jwelti@itesm.mx

## 1 Introduction

*Capsicum* fruits (peppers) are consumed throughout the world due to their attributes of pungency, aroma, and color (Cisneros-Pineda *et al.*, 2007, Valdez-Fragoso *et al.*, 2013). Mexico is considered to hold the greatest biodiversity of *Capsicum* genus plants and is a center of geographical origin of cultivated peppers. Piquin pepper (*C. annuum* L. var. *glabriusculum*) is a predecessor of domesticated cultivars of *Capsicum* and, as a product, is highly appreciated due to its unique organoleptic characteristics (Kraf *et al.*, 2013; Montoya-Ballesteros *et al.*, 2010; Rochín-Wong *et al.*, 2013; Rodríguez-del Bosque *et al.*, 2005). Since piquin pepper fruits have not undergone a full domestication process, and its corresponding reduction in genetic variability, they provide a complex matrix of volatile compounds responsible for pungency and aroma.

Capsaicinoids are the compounds responsible for pungency and have been associated with sensory and functional properties of interest in the food and pharmaceuticals industries (Moreno *et al.*, 2012). Capsaicinoids also have recognized functional properties in pain and anticancer treatments, among others (Chrubasik *et al.*, 2010; Cisneros-Pineda *et al.*, 2007; Maity *et al.*, 2010; Perva-Uzunalić *et al.*, 2004).

Volatile compounds (VC) are primarily responsible for the characteristic aroma of peppers (*Capsicum* spp). Type and concentration of VCs are affected by cultivar and stage of maturity (Forero *et al.*, 2009; Mazida *et al.*, 2005; Pino *et al.*, 2006). The VC profile of hot peppers is considered very complex because of the number and type of compounds involved. Seven families of VC have been identified including esters, alcohols, aldehydes, alkanes, sesquiterpenes, monoterpenes, and ketones (Bogusz Junior *et al.*, 2011). Previous reports have identified 63 VC for *C. annuum* cv. Mazurka (bell pepper), 36 for *C. annuum* (wild pepper) and *C. chinense* (habanero), 140 for *C. annuum* L. var. *glabriusculum* (Colombian wild pepper), 150 for *C. baccatum* (ají sudamericano), and *C. pubescens* (rocoto) (Forero *et al.*, 2009; Kollmannsberger *et al.*, 2011; Luning *et al.*, 1994; Moreno *et al.*, 2012).

Volatile profiles of peppers change according to the fruit maturation stage, the maximum concentration of VC is obtained in unripe stages and gradually decreases during maturation (Bogusz Junior *et al.*, 2012; Mazida *et al.*, 2005; Pino *et al.*, 2006). This reduction has been observed in varieties as Colombian piquin (*C. annuum* L. var. *glabriusculum*) and

Mizurka (*C. annuum*) (Forero *et al.*, 2009; Luning *et al.*, 1994).

The characterization of volatile profiles is usually incomplete due mainly to limitations of the extraction procedures and methods used as hydrodistillation, dynamic headspace, and distillation with organic solvents (Forero *et al.*, 2009; Luning *et al.*, 1994; Gahungu *et al.*, 2011; Pino *et al.*, 2011). Limitations of these methods include excessive extraction time, residues associated to the use of organic solvents, and degradation caused by high temperatures. By contrast, the method of headspace-solid phase microextraction (HS-SPME) is considered a good option to extract volatile compounds of food products. HS-SPME does not use organic solvents and extraction times are shorter (40-50 min). This method involves exposing an adsorbent fiber in the headspace of a vial where volatile compounds are retained for further characterization by chromatography (Mazida *et al.*, 2005; Kollmannsberger *et al.*, 2011; Bogusz Junior *et al.*, 2012).

Volatile characterization in peppers using HS-SPME depends on the adequate selection of an adsorption fiber that allows the extraction of a larger number of VC. The fiber that has consistently yielded better results for pepper fruits is composed of three coatings: divinylbenzene, carboxen, and polydimethylsiloxane (DVB/CAR/PDMS). This fiber composition allows the extraction of a greater number and types of VC with different polarity, and this fiber has been previously used in other peppers including malagueta (*C. frutescens*), dedo-to-moça (*C. baccatum* var. *pendulum*), murupi (*C. chinense*), ají (*C. baccatum*), and rocoto peppers (*C. pubescens*) (Bogusz Junior *et al.*, 2011; Bogusz Junior *et al.*, 2012; Kollmannsberger *et al.*, 2011; Mazida *et al.*, 2005).

The selection of extraction conditions is important because high extraction temperatures increase the variability of the measurements due to possible fiber desorption of some of the VC (Sousa *et al.*, 2006). Some authors have selected a temperature of 40 °C to prevent possible degradation of VC at higher temperatures (Moreno *et al.*, 2012; Rodríguez-Burruezo *et al.*, 2010). However, the combined effect of time and temperature on the extraction efficiency of VC using HS-SPME has not yet been entirely elucidated.

Diverse studies on the characterization of volatiles report total area of all peaks in a chromatogram as the primary response variable to optimize the extraction and characterization of the aromatic fraction. Total

area is reported under the tacit assumption that extraction of all the volatile compounds is affected by temperature and time in the same form (Moreno *et al.*, 2012; Bogusz Junior *et al.*, 2012; Sousa *et al.*, 2006). However VC profiles in peppers are complex, and total peak area may not be used as a single response variable for the analysis of individual compounds because temperature and time could be affecting differentially the extraction of volatile compounds.

The objective of this study was to evaluate the effect of different conditions of temperature and time on the extraction of volatile compounds associated with aroma of green piquin pepper (*Capsicum annuum* L var. *glabriusculum*) using HS-SPME, and to apply kinetic and statistical criteria to determine extractive conditions that would define an adequate VC profile of this complex *Capsicum* matrix.

## 2 Materials and methods

### 2.1 Materials

Piquin pepper plants (*Capsicum annuum* L. var. *glabriusculum*, family *Solanaceae*) were grown under standard cultivated conditions in Marin, Nuevo León, Mexico. Four kg of unripe (green) piquin peppers were harvested, cleaned from impurities, washed with tap water, and dried with paper towels. Samples of 15 g were used for physicochemical analyses, and the remaining portion was frozen at -80 °C for later use.

### 2.2 Physicochemical analysis

All physicochemical measurements of piquin pepper were carried out in triplicate. Fruit size (length and maximum equatorial diameter) of 40 peppers was measured using an electronic caliper (VWR, Nuevo León, Mexico). Volume was determined by the displacement of chia seeds (*Salvia hispanica* L) with a measured density of 0.6842 g mL<sup>-1</sup> (Mohsenin, 1980). Moisture content (g of water / 100 g fresh weight) was determined using weight differences of samples of 1 g of ground peppers dried at 100 °C for 24 h (AOAC, 1998). Total soluble solids (TSS) and titratable acidity (TA) were determined in a solution obtained by homogenization of 5 g of piquin fruits in 95 mL of distilled water for 2 min, followed by filtration. Titratable acidity (TA) (g of citric acid/ 100 g wet weight) was determined by titration of 10 mL of filtered solution with 0.1 N NaOH solution using phenolphthalein as indicator (Rao *et al.*, 2011). TSS

(°Bx) were determined using a refractometer at 25 °C (Atago PR-101α, Minato-ku, Tokyo, Japan).

### 2.3 Extraction of VC by HS-SPME

Samples of 1.6 ± 0.13 g of whole piquin peppers were ground in a mortar, and 1 g was placed in a 15 mL vial with top open and septa PTFE/silicone (Supelco, Bellefonte, PA., USA). The fiber used for the extraction was a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA., USA). A pad holder needle was introduced into the vial through the septum. The vial was placed in a temperature-controlled bath (Fisher Scientific, USA) for five minutes to reach the temperature to be evaluated (Table 1). Subsequently the fiber contained in the holder was exposed to the vial headspace during the time and temperature indicated in the experimental design (Table 1). At the end of adsorption time, the fiber was introduced into the GC injector at 250 °C for 1 min to desorb the volatiles. The fiber was reconditioned in the GC injector every three extraction processes at 250 °C for 10 min in split mode 1:100 to prevent accumulation of residues not fully desorbed (Bogusz Junior *et al.*, 2011).

### 2.4 Gas chromatography-mass spectrometry (GC-MS)

Volatile compounds analyses were carried out using a GC-MS Agilent 5975C (USA) with a capillary column HP-5 MS (5% phenyl/95% dimethylpolysiloxane) (30 x 0.25 mm x 0.25 μm) (J & W Scientific, USA). The temperature ramp used was as follows: injector split mode 1:15 for 1 min at 250 °C, helium at 1 mL min<sup>-1</sup>, an oven at 45 °C for 1 min, increasing to 240 °C at 5 °C min<sup>-1</sup> and a holding time of 4 min, temperature of interface, 240 °C; electron ionization at 70 eV, quadrupole mass analyzer and mass range 20-350 m/z. Compounds were identified using the Wiley7n database.

### 2.5 Kinetic and thermodynamic analysis

Kinetic of VC extraction was described using a fractional conversion model (FCM), a variation of a first-order kinetics model (Eq 1) (Serment-Moreno *et al.*, 2014).

$$\frac{dA_t}{dt} = -k(A_\infty - A_t) \quad (1)$$

where,  $A_t$  is the concentration of extracted volatiles (total peak area) at time  $t$  (min), and  $k$  is the extraction rate constant ( $\text{min}^{-1}$ ).  $A_\infty$  is the concentration of extracted volatiles at 80 min, for each evaluated temperature, assuming that at longer extraction times, the change in the concentration of volatiles extracted is very small. The rate constants were estimated by linear regression analysis (Eq 2).

$$\ln\left(\frac{A_\infty - A_t}{A_\infty}\right) = -kt \quad (2)$$

The effect of temperature on extraction of VC was described using the linearized Arrhenius relationship (Eq 3).

$$\ln(k) = \ln(A) - \frac{Ea}{RT} \quad (3)$$

where,  $k$  is the rate constant ( $\text{min}^{-1}$ ) at temperature  $T$ ,  $A$  is the frequency factor,  $Ea$  is the activation energy ( $\text{J mol}^{-1}$ ),  $R$  is the gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $T$  is temperature (K).

## 2.6 Principal component analysis (PCA)

Methodology of principal component analysis (PCA) was used to group the volatiles as a function of extraction time and temperature combinations. The volatile compounds that were taken into consideration for this analysis represented at least 10% of the total area of the major peak at any extraction condition. This analysis was carried out using IBM SPSS Statistics software version 18.

## 2.7 Experimental design

The experimental design used for the selection of the extraction conditions of VC using HS-SPME was a full factorial design with the total peak area as the response variable and two factors: temperature ( $^{\circ}\text{C}$ ) and extraction time (min) at three levels. Extraction factors and levels used are presented in Table 1. All determinations were carried out in triplicate and the results are reported as average values. Minitab software (Minitab Inc., 2003-14 © 1972) was used for the statistical analysis.

# 3 Results and discussion

## 3.1 Physicochemical analysis of piquin pepper

Physicochemical characteristics of piquin pepper fruits are presented in Table 2.

Table 1. Experimental levels used for a factorial design ( $3^2$ ) for the selection of extraction conditions of volatile compounds of piquin pepper

| Variables                            | Levels |    |    |
|--------------------------------------|--------|----|----|
|                                      | 1      | 2  | 3  |
| Temperature (T, $^{\circ}\text{C}$ ) | 40     | 60 | 80 |
| Extraction time (t, min)             | 10     | 40 | 80 |

Table 2. Physicochemical analysis of green piquin pepper fruits

| Characteristic   | Average $\pm$ SD |
|--|------------------|
| Fruit length (mm)  | 7.43 $\pm$ 1.41  |
| Equatorial diameter (mm)                                     | 6.68 $\pm$ 0.58  |
| Volume ( $\text{cm}^3$ )                                     | 2.02 $\pm$ 0.11  |
| Density ( $\text{g/mL}$ )                                    | 0.95 $\pm$ 0.02  |
| Water content (g water/100 g fresh pepper) <sup>a</sup>      | 82.14 $\pm$ 0.31 |
| Total soluble solids ( $^{\circ}\text{Bx}$ )                 | 8.39 $\pm$ 0.83  |
| Titrateable acidity (g $\text{ca}^b$ / 100 g $\text{ww}^a$ ) | 0.49 $\pm$ 0.02  |

<sup>a</sup> wet basis

<sup>b</sup> Citric acid

Unripe piquin pepper fruits are small and ovoid shape with average fruit length of  $7.43 \pm 1.41$  mm, equatorial diameter of  $6.68 \pm 0.58$  mm, and volume of  $2.02 \pm 0.11 \text{ cm}^3$ . Average moisture content was  $82.14 \pm 0.31 \%$ , which was relatively low in comparison to other cultivars of green pepper (range of 92.7 to 94.7% in *C. annuum*) (Guil-Guerrero *et al.*, 2006). Lower moisture content in piquin pepper may be associated with higher number of seeds and smaller proportion of pericarp tissue. A high density of  $0.95 \text{ g/cm}^3$  indicates that piquin fruits are compact with a proportionally higher content of dry matter. For this reason, the ratio fresh weight/dry weight of  $5.59 \pm 0.09$  (g/g) for piquin pepper was lower than previously reported values of 15.19 to 15.35 g/g for unripe padrón pepper fruits (*C. annuum* L.) (Estrada *et al.*, 2000).

Measured TSS were  $8.39 \text{ }^{\circ}\text{Bx}$ , which is a higher value than those reported for unripe bell pepper cv. Domino ( $5\text{-}6 \text{ }^{\circ}\text{Bx}$ ) (Estrada *et al.*, 2000). TA was  $0.49 \text{ g/100 g ww}$  for piquin pepper; this value is higher than  $0.13 \text{ g/100 g ww}$  reported for immature sweet pepper (*C. annuum* L.) cv. Somontano (Tadesse *et al.*, 2002).

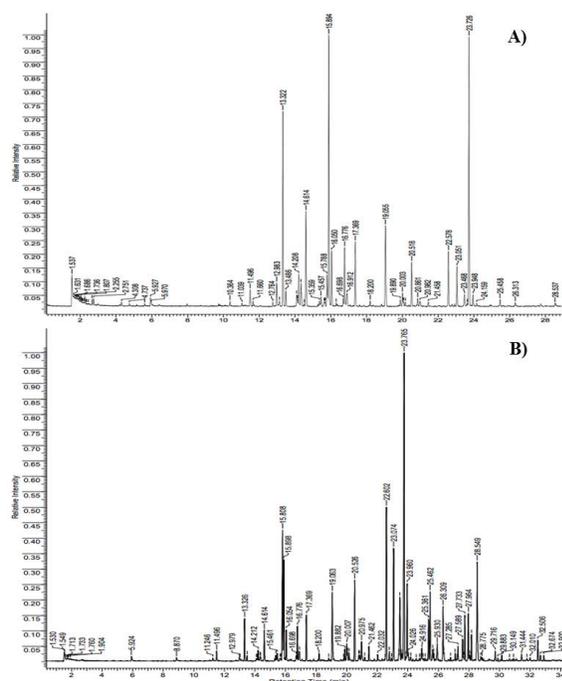


Fig. 1. Chromatograms of the extraction of volatiles of green piquin peppers for different temperature and time of extraction conditions: (A) 40 °C and 10 min and (B) 80 °C and 80 min.

### 3.2 Effect of temperature and time on total extraction of VC

Total content and number of VC extracted by HS-SPME, as well as total peak area were affected by extraction conditions (temperature and extraction time). A total of 60 peaks were obtained with a total peak area of  $1.99\text{E}+08$  when extraction conditions were set at 40 °C and 10 min. In comparison a total of 136 peaks with  $9.80\text{E}+08$  total peak area were obtained when extraction conditions were set at 80 °C and 80 min. Chromatograms of volatile extraction obtained at both conditions are presented in Figure 1.

For purposes of this study, a group of all major volatile compounds of the profile of green piquin pepper profile was selected. The group consisted of all VC that represented at least 10% of the area of the major peak (Figure 1 and Table 3). Esters of hydrocarbon chains were more abundant at shorter retention times (13-20 min), whereas a group of mainly alkanes, sesquiterpenes, and alcohols were obtained at longer retention times (23-28 min). The effects of temperature and extraction time were observed in the peaks of major VC, in which an

increase of temperature (from 40 °C to 80 °C) decreased the concentration of extracted compounds (P1-P12) with shorter retention times (13-20 min) except methyl salicylate (retention time of 15.801 min). By contrast, in the same conditions the extraction of compounds (P12-P26) was increased. A comprehensive list of all compounds selected for this study is presented in Table 3.

All of the identified VC shown in Table 3 have been previously reported for other varieties of peppers as *C. annum*, *C. chinense*, and *C. frutescens*. In particular, some esters have been identified as major compounds of aroma in varieties of peppers. For example, hexenyl 2-methyl propanoate was determined with the highest concentration in *C. chinense*. Other compounds as cis-3-hexenyl 2-methyl butanoate and cis-3-hexenyl 3-methyl butyrate have been identified in varieties of *C. annum*, *C. chinense*, and *C. frutescens*. Moreover the cis-3-hexenyl 2-methyl butanoate compound was identified in Brazilian peppers (*Capsicum* spp) as a major component within the group of esters (Bogusz Junior *et al.*, 2012; Rodríguez-Burruezo *et al.*, 2010). In our findings, an important characteristic of the profile of volatile compounds in green piquin pepper is the presence of caryophyllene as a major component. This feature differs from some Brazilian varieties of *C. annum*, *C. chinense*, and *C. frutescens*. Within these varieties, caryophyllene was not part of the profile or was present at low concentrations (Bogusz Junior *et al.*, 2012; Rodríguez-Burruezo *et al.*, 2010).

Extraction of volatiles of green piquin peppers was significantly affected by temperature (T), extraction time (t), and their interaction (T\*t) at a confidence level of 95% (Table 4), with a coefficient of determination ( $R^2$ ) of 0.96. A higher total peak area was obtained as temperature and extraction time increased (Figure 2). Extraction conditions of 80 °C and 80 min generated the highest total content of VC with a value of  $9.8\text{E}+08$  for the total peak area. Bogusz Junior *et al.* (2011) using comparable methods and conditions described in the current study for the extraction of volatiles in Malagueta pepper (*C. frutescens*), reported a  $7.16\text{E}+07$  total peak area at the optimal extraction condition of 40 °C and 80 min, and a peak area of  $6.61\text{E}+08$  was obtained at the same conditions for piquin pepper. Furthermore, this would indicate that piquin pepper has 9.23 times higher content of volatile compounds when compared to Malagueta pepper.

Table 3. Major compounds present in green piquin pepper extracted by HS-SPME at three different extraction conditions

| Label | Retention Time (RT) | Compound                            | Extraction Conditions                               |   |   |
|-------|---------------------|-------------------------------------|---|---|---|
|       |                     |                                     | 40 °C and 10 min ( $\times 10^6$ , Total Peak Area) | 40 °C and 80 min ( $\times 10^6$ , Total Peak Area) | 80 °C and 80 min ( $\times 10^6$ , Total Peak Area) |
| P1    | 13.329              | Hexyl 2-methyl propanoate           | 19.49   | 25.39   | 14.93   |
| P2    | 13.487              | E-4, 8-dimethyl-1, 3, 7-nonatriene  | 2.22  | 4.87  | 2.89  |
| P3    | 14.209              | cis-3-hexenyl 2-methyl propanoate   | 3.8   | 8.44  | 4.7   |
| P4    | 14.62               | 1-Hexyl 2-methyl propanoate         | 12.33   | 27.39   | 14.34   |
| P5    | 15.801              | Methyl salicylate                   | 5.11  | 31.39   | 54.1  |
| P6    | 15.898              | 2-methylpropyl 2-methylbutirate     | 28.17   | 64.91   | 40.91   |
| P7    | 16.06               | Hexyl isovalerate                   | 6.17  | 16.75   | 10.81   |
| P8    | 16.777              | cis-3-Hexenyl 2-methylbutanoate     | 6.66  | 24.88   | 14.35   |
| P9    | 16.914              | cis-3-hexenyl valerate              | 2.67  | 12.03   | 6.86  |
| P10   | 17.374              | Hexyl valerate                      | 6.84  | 23.07   | 17.69   |
| P11   | 19.06               | Unknown                             | 10.37   | 40.15   | 33.22   |
| P12   | 20.01               | Hexyl hexanoate                     | 2.47  | 10.56   | 10.32   |
| P13   | 20.521              | 2-methyl tridecane                  | 6.36  | 24.56   | 32.32   |
| P14   | 20.976              | Unknown                             | 0.97  | 4.56  | 9.61  |
| P15   | 22.03               | $\alpha$ -ionone                    | 0   | 0.97  | 3.03  |
| P16   | 22.587              | 2-butyl-1,1,3-trimethyl cyclohexane | 6.84  | 27.51   | 58.59   |
| P17   | 23.069              | 2-methyl tetradecane                | 5.06  | 20.45   | 48.6  |
| P18   | 23.477              | Irisone ( $\beta$ -ionone)          | 2.06  | 11.26   | 32.02   |
| P19   | 23.758              | Caryophyllene                       | 33.8  | 133.87  | 181.3   |
| P20   | 23.951              | Pentadecane                         | 2.57  | 13.97   | 32.54   |
| P21   | 25.358              | Nerolidol                           | 0   | 1.45  | 13.57   |
| P22   | 25.45               | Nonadecane                          | 1.34  | 7.3   | 27.28   |
| P23   | 26.308              | Hexadecane                          | 0.97  | 4.71  | 21.71   |
| P24   | 27.729              | 2-methyl hexadecane                 | 0.49  | 2.22  | 18.05   |
| P25   | 27.961              | 2-Tridecen-1-ol                     | 0   | 0.57  | 18.42   |
| P26   | 28.536              | Heptadecane                         | 1.06  | 5.93  | 48.25   |

Table 4. Analysis of variance by the minimum squares method for the parameters of temperature, extraction time, and interaction for the extraction of volatile compounds in piquin pepper by HS-SPME

| Sources of variation     | Sum of the squares | Degrees of liberty | Mean of squares | $F_{calculated}$ | $P_{value}$ |
|--------------------------|--------------------|--------------------|-----------------|------------------|-------------|
| Temperature (T, °C)      | 5.36E+17           | 5.36E+17           | 2.68E+17        | 218.82           | 0.0001      |
| Extraction time (t, min) | 9.79E+17           | 9.79E+17           | 4.89E+17        | 399.26           | 0.0001      |
| Interaction (T*t)        | 1.43E+16           | 1.43E+16           | 3.58E+15        | 2.92             | 0.05        |
| Error                    | 2.20E+16           | 2.20E+16           | 1.22E+15        |                  |             |
| Total                    | 1.55E+18           |                    |                 |                  |             |
| R <sup>2</sup>           | 98.58              |                    |                 |                  |             |

<sup>a</sup> Response variable: total peak area

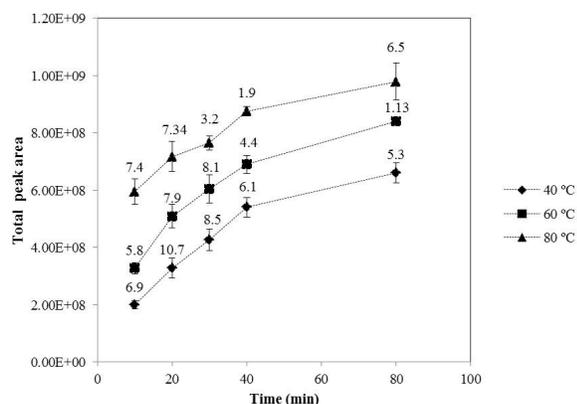


Fig. 2. Effect of time and temperature on the extraction of volatile compounds of green piquin pepper by HS-SPME. Values on the points of the curves represent the coefficients of variation (CV) for each condition.

The high variability of the total VC content in pepper (*C. chinense*), and lack of fit in the designs used at elevated temperatures (74 °C) was attributed to possible desorption of volatiles from the fiber by the effect of temperature (Bogusz Junior *et al.*, 2011). The high level of variability could be attributed to the analysis being performed in the splitless mode. In our work, when the splitless mode was used the chromatograms overlapped and the peaks squared (data not shown), which suggested that the signals were outside the detection limit. Splitless mode most likely increased variability in the repetitions of the experiments and caused lack of fit, therefore, this work was performed in split mode 1:15 which allowed us to obtain coefficients of variation (CV) below 10%, and even in the most extreme conditions of 80 °C and 80 min the CV was 6.5% (Figure 2).

### 3.3 Kinetic analysis of the extraction for total content of VC

Extraction process of VC from piquin pepper, and its relation with time and temperature, could be integrated by three phenomena: 1. Release of VC from the pepper matrix, 2. Adsorption and possible desorption from the fiber, and 3. VC degradation. The effect of time and temperature on extraction of VC of green piquin pepper by HS-SPME was described in this study using a kinetic fractional conversion model (Eq. 1). The rate constants evaluated for the extraction processes at temperatures of 40, 60, and 80 °C were 0.0327, 0.0371, and 0.0409 min<sup>-1</sup>, respectively, and the equations obtained from the linear regressions (Eq.

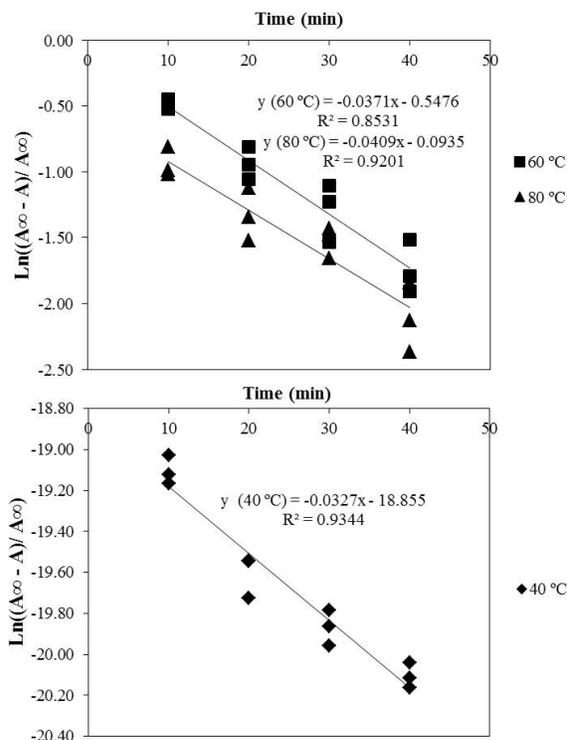


Fig. 3. First order kinetics of effect of time and temperature on the extraction of volatile compounds of green piquin pepper by HS-SPME.

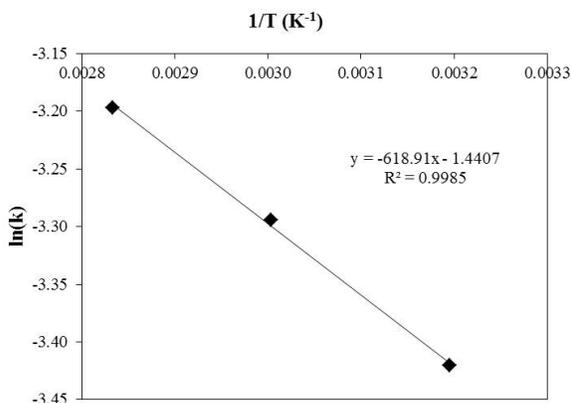


Fig. 4. Arrhenius plot of effect of temperature on the extraction of volatile compounds of unripe (green) piquin pepper by HS-SPME.

2) are shown in Figure 3.

Additionally, the dependence of the extraction rate constants with temperature was adequately described by the Arrhenius equation ( $R^2 = 0.994$ ) (Figure 4). For the extraction process at temperatures at 40, 60, and 80 °C, the value of the activation energy ( $E_a$ ) was 5.145 kJ mol<sup>-1</sup>. As shown Arrhenius equation and

FCM can be used to predict total contents of VC under temperature and time of extraction. However, these analyses do not identify the individual contribution of extraction kinetics phenomena (release of VC from the matrix, adsorption and possible desorption from the fiber, and VC degradation) on the extraction process.

### 3.4 PCA application to evaluate the effect of temperature and extraction time on the profile of VC

The extraction of the VC of the named “full aromatic profile” is directly related to temperature and time. However, the complexity of the profile might indicate that not all volatile compounds present the same response to changes in temperature and time. Therefore, utilization of total area of all peaks in a chromatogram should not be used as a sole criterion to select the “best” extraction conditions. In this study, a PCA was used to determine the effects of the evaluated extraction conditions (time and temperature) to increase or decrease the individual concentration of each component on the volatile fraction (due to the phenomena of release, adsorption, desorption, and degradation). PCA was performed to the major volatile compounds (Table 3) present in green piquin pepper extracted by HS-SPME, to classify the compounds by their response to temperature and time of extraction.

Results of the PCA application for the volatiles of green piquin peppers feature four components (statistical criteria) that explain 86.18% of the total variance with a mean sampling adequacy Kaiser-Meyer-Olkin (KMO) value of 0.72. The PCA also showed two major groups of volatile compounds for green piquin pepper (Figure 5). The first group (GR1), composed of P1 to P12 peaks, corresponded to compounds with shorter retention times, mainly esters; whereas the second group (GR2) with the P13 to P26 peaks was mostly composed of sesquiterpenes, alcohols, and alkanes. GR1 included the VC mostly associated with aroma of pepper, whereas GR2 provided a greater content of VC. However, the compounds within GR2 have not been widely recognized as primarily responsible for the aroma of *Capsicum* fruits.

A factorial analysis was performed for the two VC groups to determine possible differences in the effect of extraction temperature and time between the groups (Table 5). Results of this analysis indicated that while extraction time and the interaction of temperature and time had significant effects ( $P < 0.05$ ) on total content

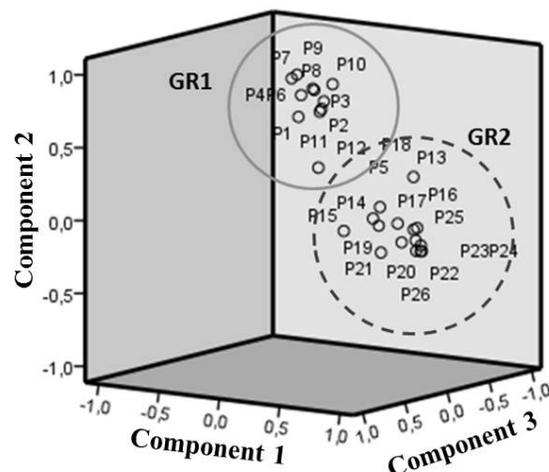


Fig. 5. Principal components analysis for the extraction of volatile compounds in piquin pepper by HS-SPME, at temperatures of 40, 60 and 80 °C.

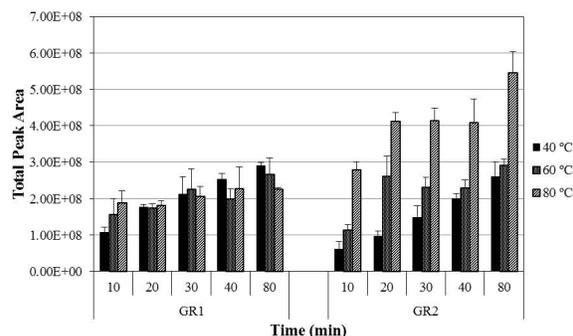


Fig 6. Effect of time and temperature on the extraction of volatile compounds (GR1 and GR2) piquin pepper by HS-SPME.

Table 5. Factorial analysis of the effect of temperature and extraction time of VCs in green piquin pepper by group of volatile compounds (for GR1 and GR2 groups)

| Group of volatile compounds | Factors             | P-value             | R <sup>2</sup> |
|-----------------------------|---------------------|---------------------|----------------|
| GR1                         | Temperature (T, °C) | 0.84                | 79.46          |
|                             | Time (t, min)       | 0.0001 <sup>b</sup> |                |
|                             | Interaction (T*t)   | 0.01 <sup>b</sup>   |                |
| GR2                         | Temperature (T, °C) | 0.0001 <sup>b</sup> | 95.78          |
|                             | Time (t, min)       | 0.0001 <sup>b</sup> |                |
|                             | Interaction (T*t)   | 0.19                |                |

<sup>a</sup>Significant difference with a  $P < 0.05$ .

extracted of components from the GR1, temperature did not have a significant effect ( $P < 0.05$ ). By contrast, temperature and extraction time had a significant effect ( $P < 0.05$ ) on total extraction of compounds of

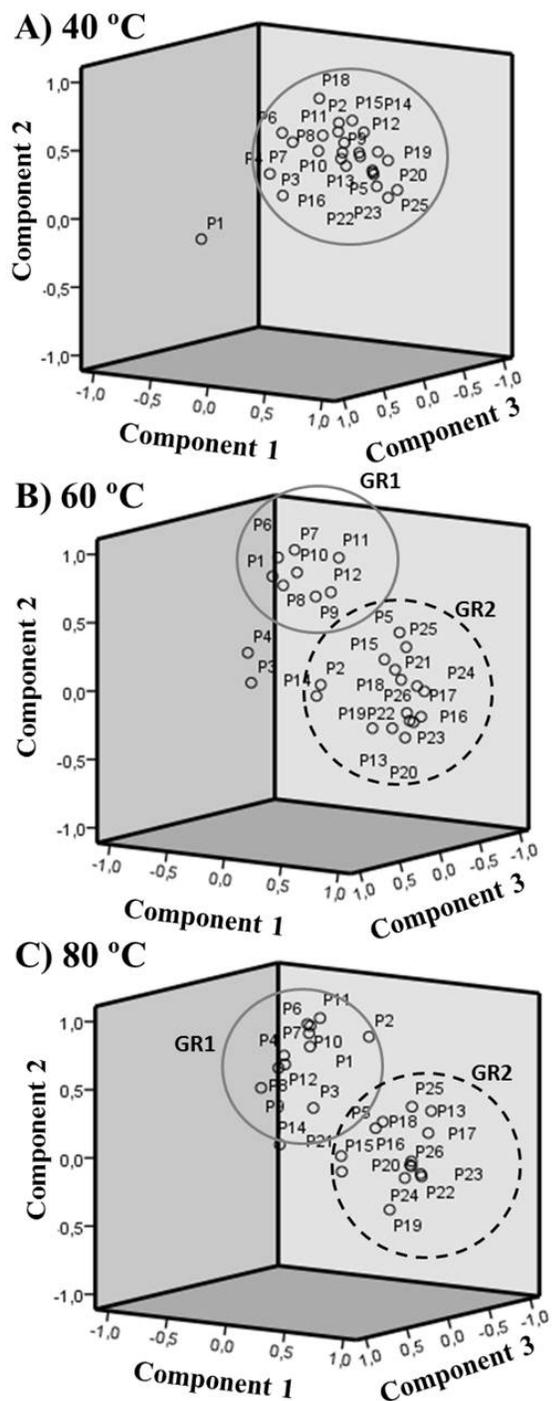


Fig. 7. Principal components analysis to the extraction of volatile compounds in piquin pepper by HS-SPME, at temperature of: A) 40 °C, B) 60 °C y C) 80 °C.

GR2, but the interaction of temperature and time did not. The effect of time and temperature on total content extracted of volatiles from GR1 showed a

reduction in the VC extraction as temperature and time increased (reduction of 7.9% from 40 °C to 60 °C and 22.4% from 40 °C to 80 °C, at 80 min) (Figure 6). For the compounds of GR2, extraction was favored with increasing temperatures and time obtaining an increment of 12.4% from 40 °C to 60 °C and 110.2% from 40 °C to 80 °C, at 80 min (Figure 7).

For GR1, a reduction of VC could be related to a possible desorption from the fiber at high temperatures, therefore the highest levels of extraction are reported at lower temperatures (40 °C). Moreno *et al.*, (2012), Rodríguez-Burruezo *et al.*, (2010), and Bogusz Junior *et al.*, (2012) chose temperatures of approximately 40 °C assuming it would prevent degradations caused by high temperatures and to obtain a more accurate characteristic composition profile. Furthermore, the main compounds responsible for the aroma in green pepper were: 2-isobutyl-3-methoxypyrazine, hexanal, trans-2-hexenal, linalool, 2,3-butadienon, and 3-carene (Mazida *et al.*, 2005). This group of compounds is characterized by short retention times, which would suggest that they belong to the GR1.

Therefore, the selection of the best extraction conditions of VC associated with aroma of piquin pepper were chosen considering the individual effects of temperature and extraction time by group of compounds. For the VC grouped within GR1 extraction was favored at 40 °C and 80 min, whereas for GR2 the best conditions were at 80 °C and 80 min. As GR1 compounds represent the group of VC that are more clearly associated to the aromatic profile of peppers, the extraction conditions for the characterization of the aromatic profile of piquin pepper should be 40 °C and 80 min.

## Conclusions

Characterization of volatile compounds of Piquín pepper fruits is significantly affected (and in different forms) by temperature and extraction time. Temperature effects on VC extraction were fitted at a kinetic fractional conversion model and adequately described by the Arrhenius equation ( $R^2 = 0.9942$ ) with an activation energy ( $E_a$ ) of  $5.145 \text{ kJ mol}^{-1}$ . The best extraction conditions of VC on compounds associated with aroma were selected considering the individual effects of temperature and extraction time. Principal component analysis indicates two different groups of volatile compounds as they relate to temperature and extraction time. For the first group

(mainly esters) extraction was favored at 40 °C and 80 min, whereas for GR2 (composed of sesquiterpenes, alcohols, and alkanes) these conditions were set at 80 °C and 80 min. The best extraction condition was 40 °C and 80 min, considering the effect of temperature and extraction time on individual volatile compounds; addition to the contribution of the group of volatile compounds on the aromatic profile pepper. Our study showed that a more complete analysis of the effect of temperature and time on the extraction of individual VC was necessary. This criterion was most appropriate for the best selection of extraction conditions of VC in piquin pepper, by HS-SPME method.

### Acknowledgements

This research was funded by the Mexican Consejo Nacional de Ciencia y Tecnología (CONACyT) scholarship to Carlos Fernando Vázquez Cárdenas; and the Cátedra of Tecnologías Emergentes para la Conservación de Alimentos (0020CAT200), Cátedra Empresarial de Tecnologías de Agricultura Intensiva (0020CAB002), Cátedra de Biotecnología Agrícola y Ambiental (0020PRY078), and the Cátedra de Nutrigenómica (0020CDB081) of the Instituto Tecnológico y de Estudios Superiores de Monterrey; and personal donation of José A. Fernández Carbajal.

### References

- AOAC. (1998). Method 934.06. *Official Methods of Analysis. Association of Official Analytical Chemists.*
- Bogusz Junior, S., Melo, A. M., Zini, C. A., Godoy, H. T. (2011). Optimization of the extraction conditions of the volatile compounds from chili peppers by headspace solid phase micro-extraction. *Journal of Chromatography A* 1218, 3345-3350.
- Bogusz Junior, S. B., Melo, A. M., Zini, C. A., Godoy, H. T. (2012). Analysis of the volatile compounds of Brazilian chilli peppers (*Capsicum* spp.) at two stages of maturity by solid phase micro-extraction and gas. *Food Research International* 48, 98-107.
- Chrubasik, S., Weiser, T., Beime, B. (2010). Effectiveness and safety of topical capsaicin cream in the treatment of chronic soft tissue pain. *Phytotherapy Research* 24, 1877-1885.
- Cisneros-Pineda, O., Torres-Tapia, L. W., Gutiérrez-Pacheco, L. C., Contreras-Martín, F., González-Estrada, T., Peraza-Sánchez, S. R. (2007). Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. *Food Chemistry* 104, 1755-1760.
- Estrada, B., Bernal, M. A., Díaz, J., Pomar, F., Merino, F. (2000). Fruit development in *Capsicum annuum*: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *Journal of Agricultural and Food Chemistry* 48, 6234-6239.
- Forero, M.D., Quijano, C. E., Pino, J. A. (2009). Volatile compounds of chile pepper (*Capsicum annuum* L. var. *glabriusculum*) at two ripening stages. *Flavour and Fragrance Journal* 24, 25-30.
- Gahungu, A., Ruganintwali, E., Karangwa, E., Zhang, X., Mukunzi, D. (2011). Volatile compounds and capsaicinoid content of fresh hot peppers (*Capsicum chinense*) Scotch Bonnet variety at Red stage. *Advance Journal of Food Science and Technology* 3, 211-218.
- Guil-Guerrero, J. L., Martínez-Guirado, C., Reboloso-Fuentes, M., Carrique-Pérez, A. (2006). Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuum*) varieties. *European Food Research and Technology* 224, 1-9.
- Kollmannsberger, H., Rodríguez-Burruezo, A., Nitz, S., Nuez, F. (2011). Volatile and capsaicinoid composition of ají (*Capsicum baccatum*) and rocoto (*Capsicum pubescens*), two Andean species of chile peppers. *Journal of the Science of Food and Agriculture* 91, 1598-1611.
- Kraft, H., Luna-Ruíz, J. de J., Gepts, P. (2013). A new collection of wild populations of *Capsicum* in Mexico and southern United States. *Genetic Resources and Crop Evolution* 60, 225-232.
- Luning, P.A., de Rijk, T., Wichers, H. J., Roozen, J. P. (1994). Gas Chromatography, Mass Spectrometry, and Sniffing Port Analyses of Volatile Compounds of Fresh Bell Peppers (*Capsicum annuum*) at Different Ripening Stages. *Journal of Agricultural and Food Chemistry* 42, 977-983.

- Maity, R., Sharma, J., Jana, N. R. (2010). Capsaicin induces apoptosis through ubiquitin-proteasome system dysfunction. *Journal of Cellular Biochemistry* 109, 933-942.
- Mazida, M.M., Salleh, M. M., Osman, H. (2005). Analysis of volatile aroma compounds of fresh chilli (*Capsicum annuum*) during stages of maturity using solid phase microextraction (SPME). *Journal of Food Composition and Analysis* 18, 427-437.
- Mohsenin N.N., (1980). Physical Properties of Plant and Animal Materials. *Gordon and Breach Sci. Publ.*, New York.
- Moreno, E., Fita, A., Gonzáles-Mas, M., Rodríguez-Burruezo, A. HS-SPME. (2012). Study of volatile fraction of *Capsicum* accessions and hybrids in different parts of the fruit. *Scientia Horticulturae* 135, 87-97.
- Montoya-Ballesteros, L.C., Gardea-Béjar, A., Ayala-Chávez, G.M., Martínez-Núñez, Y.Y., Robles-Ozuna, L.E., (2010). Capsaicinoids and color chiltepín (*Capsicum annuum* var. *aviculare*). Processing effect on sauces and pickles. *Revista Mexicana de Ingeniería Química* 9, 197-207.
- Perva-Uzunalić, A., Škerget, M., Weinreich, B., Knez, Ž. (2004). Extraction of chilli pepper (var. Byedige) with supercritical CO<sub>2</sub>: Effect of pressure and temperature on capsaicinoid and colour extraction efficiency. *Food Chemistry* 87, 51-58.
- Pino, J., Sauri-Duch, E., Marbot, R. (2006). Changes in volatile compounds of Habanero chile pepper (*Capsicum chinense* Jacq. cv. Habanero) at two ripening stages. *Food Chemistry* 94, 394-398.
- Pino, J., Fuentes, V., Barrios, O. (2011). Volatile constituents of Chachucha peppers (*Capsicum chinense* Jacq.) grown in Cuba. *Food Chemistry* 125, 860-864.
- Rao, T.V. R., Gol, N. B., Shah, K. K. (2011). Effect of postharvest treatments and storage temperature on the quality and shelf life of sweet peppers (*Capsicum annum* L.). *Scientia Horticulturae* 132, 18-26.
- Rochín-Wong, C. S., Gámez-Meza, N., Montoya-Ballesteros, L. C., and Medina-Juárez, L. A., (2013). Effect of drying and pickling processes on antioxidant capacity of phytochemicals of chiltepín (*Capsicum annuum* L. var. *glabriusculum*). *Revista Mexicana de Ingeniería Química* 12, 227-239.
- Rodríguez-Burruezo, A., Kollmannsberger, H., González-Mas, M. C., Nitz, S., Nuez, F. (2010). HS-SPME Comparative Analysis of Genotypic Diversity in the Volatile Fraction and Aroma-Contributing Compounds of *Capsicum* Fruits from the *annuum-chinense-frutescens* Complex. *Journal of Agricultural and Food Chemistry* 58, 4388-4400.
- Rodríguez-del Bosque, L. (2005). Preferencia del consumidor por el chile Piquín en comparación con otros chiles en el Noroeste de México. *Revista Chapingo Serie Horticultura* 11, 279-281.
- Serment-Moreno, V., Barbosa-Cánovas, G., Torres, J. A., Welti-Chanes, J. (2014). High-pressure Processing: Kinetic Models for Microbial and Enzyme Inactivation. *Food Engineering Reviews* 6, 56-88.
- Sousa, E. T., Rodrigues, F. M., Matins, C. C., de Oliveira, F. S., Pereira, P. A., Andrade, J. B. (2006). Multivariate optimization and HS-SPME/GC-MS analysis of VOCs in red, yellow and purple varieties of *Capsicum chinense* sp. peppers. *Microchemical Journal* 82, 142-149.
- Tadesse, T., Hewett, E. W., Nichols, M. A, Fisher, K. J. (2002). Changes in physicochemical attributes of sweet pepper cv. Domino during fruit growth and development. *Scientia Horticulturae* 93, 91-103.
- Valdez-Fragoso, A., Soto-Caballero, M.C., Soria-Hernández, C.G., Valiente-Banuet, J.I., Welti-Chanes, J. y Mújica-Paz, H. (2013). Efecto de las variables de encurtido en los parámetros de transferencia de masa, estabilidad y calidad de chile piquín. *Revista Mexicana de Ingeniería Química* 12, 1-10.