



Physical-chemical properties and microstructural characterization of traditional mexican chili (*Capsicum annuum* L.) powders

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ABSTRACT

Objective: Evaluate the physical-chemical properties and characterize the microstructure of four varieties of traditional Mexican chili (*Capsicum annuum* L.) powders: "Arbol", "Guajillo", "Piquin" and "Mole ranchero" (Ancho chili).

Design/methodology/approach: Physical-chemical properties of chili powders were evaluated by means of moisture content, particle size, aerated and tapped bulk density, Carr index, Hausner ratio, angle of repose (flow properties), capsaicin, and carotenoids content. Microstructure of samples was characterized by Confocal Laser Scanning Microscopy and Scanning Electron Microscopy. ANOVA analysis and Tukey test were performed to evaluate the significant statistical difference between samples at 95% of confidence level.

Results: "Arbol", "Guajillo", "Piquin" and "Mole Ranchero" chili powders presented a cohesive behavior respect to its flow properties related to aerated and tapped bulk density, angle of repose, Carr Index, and Hausner ratio values under moisture content between 6.59-14.48 g H₂O/100g d.s. "Arbol" and "Piquin" chili powders presented the higher capsaicin content, while "Guajillo" and "Mole ranchero" showed the higher carotenoids content. FTIR spectra confirmed the presence of secondary amide, phenolic groups, alkanes, and aliphatic chains that belong to capsaicin structure at specific absorption bands. Microstructure of chili powders presented particles with surface imperfections as cracks and dents, and smooth surface that influence physical-chemical and flowability properties.

Limitations on study/implications: Hight moisture content affect the physical-chemical properties, flowability and microstructure of traditional Mexican chili powders.

Findings/conclusions: Moisture content between 6.59 and 14.48 g H₂O/100g d.s. influences the physicalchemical properties, flowability and microstructure of traditional Mexican chili powders. To improve physicalchemical properties and flowability behavior of chili powders is required that moisture content be lower than 6.59 H₂O/100g d.s.

Keywords: Mexican chili powders, physical-chemical properties, flowability, capsaicin, carotenoids, microstructure.

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INTRODUCTION

The chili (Capsicum annuum L.) (Solanaceae) is native of America, is consumed as vegetable, spice, and as source of vitamins A, C and E, carotenoids, and capsaicin which produce the characteristic pungency of *Capsicum* species. The chili in Mexico is usually processed in dried form to conserve its color and flavor. Dehydrated chili is employed in instant soups, frozen pizzas, salad dressings and a great variety of sauces and foods. During the dehydration process, water content and water activity are partially or totally reduced and, consequently, microbial growth and the enzymatic activity are limited, vielding an extended shelf-life of the product, beside of protect the bioactive compounds and conserve the physical-chemical properties of chili-based products (Domian & Poszytek, 2005; De Marino et al., 2006; Cisneros-Pineda et al., 2010; Yaldiz et al., 2010; Valdez-Fragoso et al., 2013). Processing and handling of powders have increasingly in the food and pharmaceutical industries. Knowledge of food powder properties has grown to the extent that increases product value, and processes complexity as well as development of new formulations. In this sense, flow properties are very important in many unitary operations which involve powders flowability, such as: pneumatic transport from specific equipment or silo, mixing and packaging. Flowability is affected by many factors such as particle size, shape, density, chemical composition, and moisture content. Powder properties are commonly determined under loading conditions of gravity, by means of angle of repose, standardized flow rate, aerated and tapped bulk density values, beside of Carr Index and Hausner ratio (Zou and Brusewitz, 2002; Thalberg et al., 2004; Cavalcante-Alves et al., 2008; Emery et al., 2009; Perea-Flores et al., 2010). Density is a basic property for materials and industrial processes characterization, storage selection, packaging, and distribution conditions. Aerated and tapped bulk density are generally used to characterize final products obtained by mean of milling or drying, weight estimation to fill containers and to relate the powder density before and after compression (Abdullah & Geldart, 1999; Barbosa-Cánovas et al., 2005; Fitzpatrick, 2005). Angle of repose is the steepest angle of descent relative to the horizontal plane to which a material can be piled without slumping. This parameter indicates that interparticulate friction has been used to characterize flow behaviour of the powder and granular materials respect to flowability (Fraczec et al., 2007; Xinde et al., 2007; Ileleji & Zhou, 2008). Powder compressibility is commonly used as a flowability indicator and is often expressed using the Hausner ratio, by means of aerated/tapped bulk density ratio and is appropriate to estimate cohesion in powders. In addition, the Carr index is other compressibility indicator to evaluate powder flow properties (Thalberg et al., 2004; Barbosa-Cánovas & Juliano, 2005; Cavalvante-Alves et al., 2008; Khandai et al., 2014). Nowadays, the study of food powder microstructure is relevant and has grown in parallel with the development of microscopy and image processing techniques (Wang, 2006; Pérez-Alonso et al., 2009). Some methods analyze food microstructure and correlate it with chemical composition and properties: Light Microscopy (LM), Scanning Electron Microscopy (SEM), and Confocal Laser Scanning Microscopy (CLSM), covering the whole dimensional scale from microstructural to macrostructural level (Kim et al., 2009; Perea-Flores et al., 2010). Therefore, the aim of this research was evaluating the physicalchemical properties and characterize the microstructure of four traditional Mexican chili powders: "Árbol", "Guajillo", "Piquin" and "Mole ranchero" (Figure 1).

MATERIALS AND METHODS

Samples: Four samples of traditional Mexican chili powders: "Arbol", "Guajillo", "Piquin" and "Mole ranchero" (Ancho chili), were purchased in a local market from Toluca, State of Mexico, Mexico. Capsicum oleoresin standard was provided by Sensient Colors Company, S.A. de C.V. (Lerma, State of Mexico, Mexico). All reagents used in the study were purchased from Sigma-Aldrich, S.A. de C.V. (Toluca, State of Mexico, Mexico).



Figure 1. Traditional mexican chili powders (Capsicum annuum L.) Source: Created by the authors.

Aerated and tapped bulk density: samples were gently poured into a 100 mL graduate cylinder. Aerated bulk density (ρ_a) was calculated as the ratio between the weights (g) of the sample contained in the cylinder and the filled volume (100 mL). Tapped bulk density (ρ_b) was estimated by tapping the cylinder (100 times) until no measurable change in volume was noticed (León-Martínez *et al.*, 2010; Gallo *et al.*, 2011).

Particle size: was determined using a particle size analyzer Malvernsizer 2000 (Malvern Instruments, Ltd., Malvern, Worcetershire, UK). The average particle size was obtained using the software Malvernsizer 5.6 integrated to the equipment (Pérez-Alonso *et al.*, 2009).

Moisture content: was determined according to AOAC method (2005).

Angle of repose: was determined by pouring a pre-defined mass of 50 g of chili powder sample through a funnel located at a fixed height on a graph paper flat horizontal surface and measuring powder conical pile height (h) and radius (r) formed. The tangent of the angle of repose is given by the h/r ratio (Gallo *et al.*, 2011).

Carr index and Hauser ratio: were evaluated by means of the relationship between aerated bulk density and tapped bulk density, using equations (1)-(2) (Ganesan *et al.*, 2008; Gallo *et al.*, 2011):

$$CI\% = \left[\frac{(\rho_b) - (\rho_a)}{\rho_b}\right] \times 100 \tag{1}$$

$$HR = \left[\frac{\left(\rho_{b}\right)}{\left(\rho_{a}\right)}\right] \tag{2}$$

CI% is the Carr index and *HR* is the Hausner ratio, (ρ_a) is the aerated bulk density in g/mL and (ρ_b) is the tapped bulk density (g/mL).

Capsaicin content: 0.1 g of sample and 0.025 g for capsicum oleoresin standard, were extracted during 4 hours at 25 °C with 50 mL of acetone, with a slight modification respect to Hornero & Mínguez spectrophotometric method (2001). Absorbance was measured in a UV spectrophotometer GENESYS2-UV/visible (Spectronic, Rochester, NY, USA) at λ =460 nm and using acetone as blank (Hornero & Mínguez, 2001; Braga & Oliveira, 2007).

Carotenoids content: determined according to Hornero & Mínguez (2001) spectrophotometric method with a slight modification. Absorbance measurements were made in a UV spectrophotometer GENESYS 2-UV/visible (Spectronic, Rochester, NY, USA) at $\lambda = 472$ and 508 nm. To obtain isochromic fraction and total carotenoids content, the following equations (3)-(5) were used:

$$CR = \left[\frac{(A_{508}) \times (2144) - (A_{472}) \times (403.3)}{270.9}\right] (\mu g / mL)$$
(3)

$$CY = \left[\frac{(A_{472}) \times (1724.3) - (A_{508}) \times (403.3)}{270.9}\right] (\mu g \mid mL)$$
(4)

$$CT = CR + CY(\mu g / mL)$$
⁽⁵⁾

 A_{508} is the absorbance of samples at $\lambda = 508$ nm, A_{472} is the absorbance of samples at $\lambda = 472$ nm of the samples *CR* represents the red isochromatic fraction content, *CY* represents the yellow isochromatic fraction content, and *CT* represents the total carotenoids content.

Extractable color (ASTA units): were determined according to ASTA 20.1 method. The absorbance of each sample was measured against acetone blank at λ =460 nm in a UV spectrophotometer GENESYS 2-UV/visible (Spectronic, Rochester, NY, USA). Extractable colour values were expressed in ASTA units and calculated by the Ec (6) (Topuz *et al.*, 2009; Rascón *et al.*, 2011):

$$ASTA units = \left[\frac{A \times 164 \times If}{W_{sample}}\right]$$
(6)

A is the absorbance of sample at I = 460 nm, If is the deviation factor of the spectrophotometer and W is the sample weight (g) in dry basis.

FTIR Spectroscopy: chemical groups associated to capsaicin and carotenoids were identified by FTIR Spectroscopy, using a Micro-Raman Spectrometer (Lab RAM HR800, Horiba Jobin-Yvon, France) coupled to Fourier Transform Infrared Spectroscopy and a Charge Detector, using an Attenuated Total Reflectance objective (ATR-FTIR) with 36x magnification. Spectra were acquired from 4000 to 400 cm⁻¹ and the baseline spectra adjusted with Origin Pro 8.0 software (Origin Lab Corporation, MA, USA) and compared with capsicum oleoresin standard spectrum.

Scanning Electron Microscopy (SEM): microstructure was characterized using a Dual Beam SEM Microscope model (Quanta 3D, FEG, FEI, Holland). Samples were fixed with double-sided carbon adhesive tape on aluminum stubs and directly observed at 15 kV, using a low vacuum secondary electron detector to minimize charging. Micrographs were acquired at 250x and 1000x (Quintanilla-Carvajal *et al.*, 2011; De la Rosa-Millán *et al.*, 2014).

Confocal Laser Scanning Microscopy (CLSM): samples were characterized with CLSM equipment (CLSM 710 NLO, Carl Zeiss, Germany) using a plan-Apochromat 40x/1.3 oil DIC M27 objective, and were excited at $\lambda = 405$, 488 and 561 nm. Autofluorescence intensity measurement was performed using the software ZEN coupled to the equipment. Were acquired 3D images of samples (x, y and z) in sections of different focal planes (Quintanilla-Carvajal *et al.*, 2011).

Statistical analysis: ANOVA and Tukey test were performed to evaluate the significant difference between samples at 95% of confidence level.

RESULTS AND DISCUSION

Aerated and tapped bulk density: Density is influenced by particle size; at lower particle size, aerated and tapped bulk density increase because there is more surface contact area available for cohesive and frictional forces to resist flowability and the influence of compaction capability and the shape of particles of chili powders, this behavior is showed in "Guajillo", "Piquin" and "Mole ranchero", meanwhile "Arbol" chili exhibit the higher particle size and the lower density values (Table 1). "Mole ranchero" showed the highest aerated and tapped bulk density values. In the other hand, particle size and shape influence the aerated and tapped bulk densitiy due to the compaction capability that powders present by the presence of surface imperfections as cracks and dents that "Arbol", "Guajillo" and "Piquin" presented and by the smooth surfaces that "Mole Ranchero" presented.

Angle of repose: to classify flowability of powders, Gallo *et al.* (2013), give a classification between 25° and 30° as an excellent flowing and greater than 31° as poor flowing, while Santomaso *et al.* (2003), give values between 30° and 45° to powders that free flowing and values between 45° and 60° to powders fairly to free flowing. "Arbol"chilli", "Piquin" and "Mole ranchero" showed a behavior near to free flowing, while "Guajillo" showed a behavior fairly to free flowing, and these values indicate that chili powders are cohesive and tend to form agglomerates (Table 1, Figure 2). Angle of repose involves a compaction and tapping process, which produce a little deformation of agglomerates and reflect the surface properties, including the degree of agglomerates, particle morphology, friction, and cohesive forces between particles, whereby the angle of repose could be used as a flowability index of chili powders. Figure 2 illustrates the flow pattern of the conical pile formed to measure angle of repose from traditional mexican chili powders.

Carr index and Hausner ratio: these parameters express the compaction and compressibility capabilities related to frictional forces between powder particles. Gallo *et al.* (2013) give Carr index values between 10 and 25 to identify powders with excellent and acceptable flowability. Santomaso *et al.* (2003) give Hausner ratio values between 1 and 1.25 to identify powders with excellent and near to free flowing, while values between 1.25 and

| Samples | Moisture content (gH ₂ O/100g d.s) | Particle size d _{4,3} (µm) | Aerated bulk density (ρ _a) (g/mL) | Tapped bulk density ($ ho_b$) (g/mL) | Carr index (au)* | Angle of repose a_r (°) | Hausner ratio (au)* |
|-----------------|--|---|---|--|--------------------------|---------------------------------|---------------------------|
| Arbol chilli | 7.92 ± 0.10^{a} | 643.68 ± 0.00^{a} | 0.40 ± 0.00^{a} | 0.57 ± 0.01^{a} | 29.82 ± 0.95^{a} | 37.9 ± 0.09^{a} | 1.43 ± 0.01^{a} |
| Guajillo chilli | $9.58 \pm 0.10^{\rm b}$ | 330.99 ± 0.00^{b} | 0.42 ± 0.02^{b} | 0.61 ± 0.00^{b} | 31.14 ± 0.00^{b} | 46.68 ± 0.08^{b} | 1.45 ± 0.00^{b} |
| Piquin chilli | $6.59 \pm 0.10^{\circ}$ | $247.49 \pm 0.00^{\circ}$ | 0.44 ± 0.02^{c} | $0.69 \pm 0.01^{\circ}$ | $36.23 \pm 1.00^{\circ}$ | $44.31 \pm 0.40^{\circ}$ | 1.56 ± 0.02^{c} |
| Mole ranchero | 14.48 ± 0.10^{d} | 342.53 ± 0.00^{d} | 0.52 ± 0.05^{d} | 0.77 ± 0.01^{d} | 32.46 ± 0.01^{d} | 41.20 ± 0.04^{d} | 1.48 ± 0.01^{d} |

Table 1. Physical-chemical properties of traditional mexican chili powders.

*(au): adimensional units.

The values with different letter in the same column present a significant difference between every sample.



Figure 2. Angle of repose evaluated from traditional mexican chilli powders (*Capsicum annuum* L.) Source: created by the authors

1.4 indicate a behavior fairly to free-flowing powders. "Arbol", "Guajillo", "Piquin" and "Mole ranchero" showed a cohesive behavior fairly to free flow with significant difference between Carr index and Hausner ratio values. "Mole ranchero" is a very cohesive powder due to its higher moisture content of 14 gH₂O/100g d.s. respect to "Arbol", "Guajillo" and "Piquin" chili at moisture contents of 7.92, 9.58 and 6.59g H₂O/100g d.s. respectively.

Chemical group's composition: FTIR spectra of chili powders presented in Figure 3, showed a characteristic band of absorption of axial deformation of C-H aromatic and aliphatic chains at 2900 cm⁻¹, a band of asymmetric and symmetric axial deformation of C-O-C bonds at 1690 cm⁻¹, a band at 1652 cm⁻¹ associated to a secondary amide, a band of axial deformation of C=C of the double ring at 1590 cm⁻¹, and a band of absorption of axial deformation of C=O bonds at 1158 cm⁻¹ related to a phenolic group. These results are agreed with capsicum oleoresin standard spectra and with the FTIR spectra reported for chili samples by Toshimasa *et al.* (2003) and De Marino *et al.* (2006), which confirms the chemical groups related to capsaicin molecule and carotenoids structure.

Capsaicin and carotenoids content: results are shown in Table 2. "Piquin" and "Arbol" presented the higher capsaicin content, meanwhile "Guajillo" and "Mole ranchero" showed the lower capsaicin content, respect to the capsicum oleoresin standard content. In the other hand, "Guajillo" and "Mole ranchero" presented the higher carotenoids content respect to the capsicum oleoresin standard content. "Guajillo" showed the higher carotenoids content and a lower capsaicin content, while "Piquin chili" presented the higher capsaicin content and the lower carotenoids content. "Guajillo" and "Mole Ranchero" presented the higher the higher carotenoids content and the lower carotenoids content. "Guajillo" and "Mole Ranchero" presented the highest red fraction, yellow fraction, and total fraction of carotenoids, and the lowest capsaicin content. Experimental values obtained are in accordance with the values reported in chili samples by Rodríguez-Maturino *et al.* (2012).

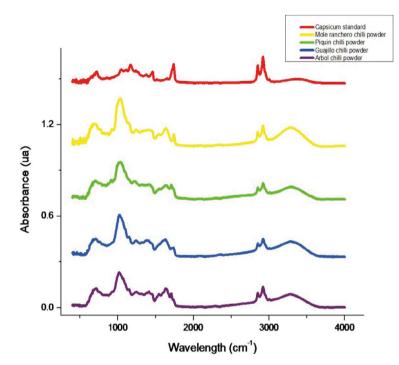


Figure 3. FTIR spectra of traditional mexican chilli powders (*Capsicum annuum* L.) Source: created by the authors.

Extractable color (ASTA units): "Guajillo" and "Mole Ranchero" showed the higher extractable color value, while "Arbol" and "Piquin" showed the lower extractable color values. All samples were compared with capsicum oleoresin standard extractable color values (Table 2). The significant difference of extractable color in the samples is related to the Red (CR) and Yellow (CY) fractions of carotenoids, where "Guajillo" and "Mole Ranchero" presented the higher red and yellow fractions of carotenoids, while "Arbol" and "Piquin" presented the lower red and yellow fraction (CR). Results obtained are agreed with the values reported by Rodríguez-Maturino *et al.* (2012).

Microstructure: "Arbol", "Guajillo" and "Piquin" presented particles with surface imperfections as cracks and dents, while "Mole Ranchero" showed a smooth surface, which

| Parameters Capsaicin | | | Color | | |
|----------------------|---------------------------|----------------------------|-------------------------------|---------------------------------------|----------------------------|
| Samples | Concentration (mg/L) | Red fraction CR (µg/mL) | Yellow fraction CY (µg/mL) | Total fraction CT=CR+CY (µg/mL) | ASTA units (au)* |
| Arbol chilli | 97.28 ± 0.18^{a} | 197.73 ± 0.00^{a} | 260.10 ± 0.00^{a} | 457.84 ± 0.00^{a} | 279.48 ± 0.00^{a} |
| Guajillo chilli | $18.67 \pm 0.00^{\rm b}$ | 846.44 ± 0.00^{b} | 1324.71 ± 0.00^{b} | 2171.15 ± 0.00^{b} | $1256.92 \pm 0.01^{\rm b}$ |
| Piquin chilli | $121.95 \pm 0.00^{\circ}$ | $117.07 \pm 0.00^{\circ}$ | $155.26 \pm 0.00^{\circ}$ | $272.33 \pm 0.00^{\circ}$ | $192.30 \pm 0.00^{\circ}$ |
| Mole ranchero | 46.23 ± 0.00^{d} | 316.94 ± 0.01^{d} | 427.43 ± 0.01^{d} | 744.37 ± 0.01^{d} | 476.41 ± 0.01^{d} |
| Capsicum std | 136.38 ± 0.00^{e} | 794.14 ± 0.00^{e} | 1477.24 ± 0.00^{e} | 2271.38 ± 0.00^{e} | 1405.64 ± 0.00^{e} |

Table 2. Capsaicin, carotenoids, and color content of traditional mexican chilli powders.

*(au): adimensional units

The values with different letter in the same column present a significant difference between every sample.

can be observed in Figure 4. The 3D CLSM images showed the autofluorescence (green and red) identified in traditional Mexican chili powders, which is related to capsaicin and carotenoids compounds (Vazquez-Gutiérrez *et al.*, 2011), where the red fluorescence is associated to capsaicin content at λ =460nm.

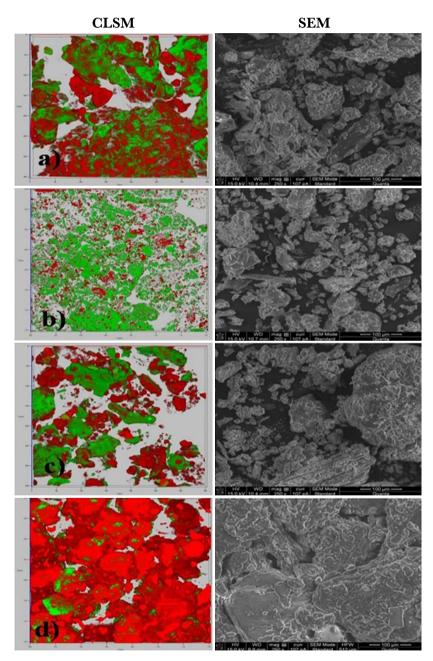


Figure 4. Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM) micrographs of traditional mexican chilli powders. a) Arbol chilli, b) Guajillo chilli, c) Piquin Chilli and c) Mole ranchero. Source: created by the authors.

CONCLUSIONS

"Arbol", "Guajillo", "Piquin" and "Mole Ranchero" presented a cohesive behavior respect to its physical-chemical properties and flowability. "Arbol" and "Piquin" present the higher capsaicin content while "Guajillo" and "Mole ranchero" showed the higher carotenoids content. FTIR spectra confirmed the presence of secondary amide, phenolic groups, alkanes, and aliphatic chains that belong to capsaicin structure from chili. Microstructure of chili powders presented particles with surface imperfections as cracks and dents, and smooth surface that influence physical-chemical properties. Moisture content between 6.59 and 14.48 g H₂O/100g d.s. influences the physical-chemical properties, flowability behavior and microstructure of traditional Mexican chili powders. To improve physical-chemical properties and flowability behavior of chili powders is required that moisture content be lower than 6.59 g H₂O/100g d.s.

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