NUTRACEUTICAL VALUE OF FRESH AND DRIED CHILI (Capsicum annuum L.) IN DIFFERENT STAGES OF MATURITY

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ABSTRACT

The wide diversity of species and varieties of chilies existing in Mexico demands the determination of their antioxidant properties to be recommended as food of high nutraceutical value, so far unknown in most chilies species, especially when the fruits are dehydrated. This information may provide economic alternatives to the producer and promote more efficient agro-industrial use of the fruits. The objective of the study was to evaluate the content of ascorbic acid, phenolic compounds, flavonoids, β -carotene and antioxidant activity of fruits of *Capsicum annuum* L. cultivar chili ancho, at two maturity stages of fresh and dehydrated chili. Results demonstrate that it is convenient to harvest shortly before horticultural maturity occurs so as to take advantage of the high content of ascorbic acid and DPPH radical scavenging. The content of phenolic compounds and flavonoids in the mature dehydrated (MD) chili was higher (898.58 and 345.78 mg·100 g⁻¹ DW, respectively) compared with the premature fresh chili (PMF), mature fresh (MF) and premature dehydrated (PMD). Significant differences were found in the content of β -carotene between the treatments PMF and MF (5.8 and 27.1 µg·g⁻¹DW) and the treatments PMD and MD (347.8 and 483.0 µg·g⁻¹ DW, respectively). Opposite effect was observed in the ascorbic acid content. The antioxidant activity of the chili PMF was significantly higher than that of MD. The process of dehydration did not affect the antioxidant activity in the premature chilies, nor in the mature ones. These nutraceutical characteristics provide an added value to the product.

Additional keywords: Antioxidant activity, ascorbic acid, β-carotene, dehydrated chili, flavonoids, maturity, phenolics

RESUMEN

Valor nutracéutico del chile (Capsicum annuum) fresco o deshidratado en diferentes estados de madurez

La amplia diversidad de especies y cutivares de chile que existen en México demanda la determinación de las propiedades antioxidantes de los frutos para ser recomendados como un alimento de alto valor nutracéutico, hasta ahora desconocidas en la mayoría de las especies de chile, especialmente en los frutos deshidratados. Esto proporcionará alternativas económicas para el productor y un eficiente uso agroindustrial. El objetivo del presente estudio fue evaluar los contenidos de ácido ascórbico, compuestos fenólicos, flavonoides, β -caroteno y la actividad antioxidante del cultivar chile ancho (*Capsicum annuum* L.) en dos estados de maduración del fruto fresco y deshidratado. Los resultados indicaron que es conveniente cosecharlo tempranamente antes de la madurez comercial por su alto contenido de ácido ascórbico y actividad inhibidora de radicales DPPH. Los contenidos de compuestos fenólicos y flavonoides en el chile maduro deshidratado (MD) fueron más altos (898,58 y 345,78 mg·100 g⁻¹ P.S., respectivamente) comparados con los chiles premaduros frescos (PMF), maduro fresco (MF) y premaduro deshidratado (PMD). Se encontraron diferencias significativas en el contenido de β -caroteno entre los tratamientos PMF y MF (5,8 y 27,1µg·g⁻¹ P.S.) y los tratamientos PMD y MD (347,8 y 483,0 µg·g⁻¹ P.S., respectivamente). Un efecto opuesto se observó con el contenido de ácido ascórbico. La actividad antioxidante en el chile PMF fue significativamente más alta que la del MD. El proceso de deshidratación no afectó la actividad antioxidante de los chiles premaduros, ni los maduros. Estas características nutracéuticas proporcionan un valor agregado al producto.

Palabras clave adicionales: Ácido ascórbico, antioxidantes, β-caroteno, chile deshidratado, fenólicos, flavonoides, madurez

INTRODUCTION

There is now a preference in the consumer for

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nutraceutical foods, which has stirred great interest in the investigators to study the content of phenolic compounds, flavonoids, vitamin C,

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carotenes (Bernal et al., 2011; Tiwari and Cummins, 2013), and their alteration as a consequence of the technologies of processing and postharvest preservation, during which physiological and biochemical changes occur which degrade these phytochemicals (De Ancos et al., 2009). These components in various fruits are known to provide important nutraceutical benefits to consumers (Tsao and Akhtar, 2005). Consumption of these foods, due to the presence of certain metabolites (phenolic compounds, flavonoids, vitamin C and natural pigments), prevents some chronic-degenerative diseases (Soetan, 2008; Das et al., 2012; Gul et al., 2016). Phenolic compounds are a group of secondary metabolites that protect plants from oxidative stress, and in human food, and contribute to preventing diseases in humans. Flavonoids are another group of secondary metabolites that are important due to growing evidence of their versatile health benefits through epidemiological studies. As their occurrence is directly associated with human daily dietary intake of antioxidants, it is important to evaluate flavonoid sources in food (Yao et al. 2004).

The chili pepper Capsicum (Solanaceae) has a long cultural tradition in Mexico. It has been speculated that chili was the first domesticated crop in Mesoamerica (Long-Solís, 1998), and has been an obligatory ingredient in Mexican food for thousands of years (Laborde and Pozo 1984; Paredes et al., 2006). Although it is a perishable material, and does not have good preservation, evidence has been found of the existence of chili in the Prehispanic era (7 000 and 5 000 B.C.) in various archaeological sites (Valley of Tehuacán, Puebla, Mexico) (Laborde and Pozo, 1984). Presently, Mexico is the country with widest genetic diversity, although it occupies sixth place in production after China, Spain, Turkey, Nigeria and India (Paredes et al., 2006). Despite the great production and genetic diversity of these fruits in the country, the contents of nutraceutical components in most chili species are unknown.

Approximately 90 species of *Capsicum* are known, 12 are used by humans and of these only five are cultivated and domesticated (Paredes et al., 2006). Among these five are *Capsicum annuum* which includes cultivars such as ancho or poblano, jalapeño, serrano, pasilla,

guajillo, chile de árbol, piquin; *C. baccatum, C. chinense* (habanero), *C. frutescens* (Tabasco) and *C. pubensces* (manzano) (Paredes et al., 2006). The fruit of *C. annuum* cultivar ancho is of a large size (12 cm length) and moderately hot; in immature state (dark green) is known as poblano and when mature (red) it is known as ancho (Aguilar, 2010); it is commercialized fresh (50 %) and dehydrated or dry (red) for the preparation of diverse Mexican culinary specialties (Núñez, 1996; Paredes et al., 2006).

There are studies that point out the variation of vitamin C (Daood et al., 1996; Howard et al., 1999; Martínez et al., 2005), carotenes (Osuna et al., 1998; Daood, 2009), phenolic compounds (Sukrasno and Yeoman, 1993; Howard and Wildman, 2007) in different stages of maturation (Osuna et al., 1998; Márkus et al., 1999; Howard et al., 2000) and processing (Márkus et al., 1999; Guerra et al., 2001) of some species of chili. However, affectation of the nutraceutical compounds (such as phenolic compounds, flavonoids, carotenes) from the dehydration process of some species and cultivars of chili cultivated in Mexico is unknown. The objective of the study was to evaluate the content of ascorbic acid, phenolic compounds, flavonoids, β-carotene and antioxidant activity of Capsicum annuum L. cultivar chili ancho in two maturity stages of fresh and dehydrated chili, with the purpose of documenting more efficient agroindustrial use.

MATERIAL AND METHODS

Collection of plant material. The fruits of cultivar chili ancho were collected in the locality of Lázaro Cárdenas (Rancho Grande), situated in the municipality of Fresnillo, in the state of Zacatecas (state with highest chili production in Mexico) (Paredes et al., 2006). The site is located at 23° 27' N, 102° 57' W; 2010 m altitude.

Dehydration of the chilies. Samples of ten fruits free of pathogens were randomly harvested from each plant (experimental unit). The harvest was made in two development stages: a) in physiological prematurity (15 d before physiological maturity, green fruit color), identified as PMF, and b) in physiological maturity (green-red fruit color), identified as MF. The seeds and the placenta were removed from all of the harvested chilies, and the mesocarp was treated with liquid nitrogen to suspend the enzymatic activity; it was maintained in freezing conditions at -20 °C until their analysis. Fifty percent of the fresh chilies in the two development stages (PMF and MF) were analyzed. For the dehydration, the remaining 50 % of the samples in the two development stages (PMD and MD) were placed separately in aluminum trays. They were exposed to a current of hot air at 70 °C and a velocity of 2.5 m·s⁻¹ in a tray dehydrator (Jersa, model L) The material was weighed at 15 min intervals during the first hour and then every 30 min until concluding the dehydration time.

As shown the experiment included the following four treatments: premature fresh chili (PMF), premature dehydrated chili (PMD), mature fresh chili (MF) and mature dehydrated chili (MD). They were studied under a completely randomized experimental design where each selected sample was considered a treatment with four replicates.

Preparation of the extract for total phenolic compounds and flavonoids. For the analysis the samples were homogenized in a food processer. One gram of ground fresh or dehydrated fruit was weighed, dissolved in 25 mL of ethanol at 95 % v/v by means of sonication (Cole Parmer 8892) for 15 min. After 24 h the volume was adjusted to 25 mL with ethanol at 80 % v/v, and the mixture was centrifuged at 1409 g.

Quantification of total phenolic compounds. The content of total phenols was quantified according to the method proposed by Waterman and Mole (1994). An aliquot of 0.5 mL of ethanolic extract was mixed in a vortex with 10 mL 10 % (p/v) sodium carbonate. Each sample was placed in a water bath at 38 °C for 15 min, after which 3 mL distilled water and 1 mL Folin-Ciocalteu solution and water (1:1, v/v) were added to 1 mL of the mixture. The mixture was left to repose for 15 min in the dark at room temperature. Absorbance was read in a spectrophotometer Genesys 10s at 760 nm. The concentration of phenols was calculated from the equation adjusted by linear regression of the standard curve based on gallic acid (y= 0.000212x-0.0142; r² = 0.995). The total content of phenols in the extract was expressed in mg equivalent of gallic acid per 100 g of dry matter.

Quantification of flavonoids. The flavonoid content was quantified according to the method proposed by Chang et al. (2002). An aliquot of 0.5 mL of ethanolic extract was mixed in a vortex with 1.5 mL 95 % (v/v) ethanol, 0.1 mL 10 % (w/v) aluminum chloride, 0.1 mL 1.0 M potassium acetate solution, and 2.8 mL distilled water, and then the mixture was incubated for 30 min at room temperature. Absorbance was read in the spectrophotometer at a wavelength of 415 nm. The concentration of flavonoids was calculated from the equation adjusted by linear regression of the standard curve based on guercetin (Sigma-Aldrich) (y = 0.0047x - 0.0007; r² = 0.999). The results were expressed in mg equivalent of quercetin per 100 g of dry weight.

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Quantification of ascorbic acid. One gram of sample was homogenized in 3 mL metaphosphoric acid at 3 % v/v for 3 min, the mixture was filtered and 1 mL supernatant was diluted to 10 mL with metaphosphoric acid at 3 % v/v. Then, 2 mL of the mixture were added with 2 mL buffered solution (glacial acetic acid: sodium acetate at 5 % w/v, 1:1) (pH = 4), 3 mL dichloroindophenol and 15 mL xylene, stirred vigorously in a vortex (Thermolyne Type 6700) and absorbance was measured in the spectrophotometer at 520 nm. The curve was built with ascorbic acid standard (y = -2.666x + 0.567; r² = 0.995) and results were expressed in mg equivalent to ascorbic acid in 100 g fresh weight.

Ouantification of **β-carotene.** For the quantification of β -carotene, 0.5 g of mesocarp of fresh and dehydrated chilies without seeds in the two development stages were macerated with a solution of acetone, ethanol and hexane in a proportion of 5:5:10, the mixture of solvents was evaporated by passing a stream of nitrogen gas. Next, the extract was gauged to 1 mL with hexane grade HPLC (Merck) and was filtered with microdiscs of 0.5 µm of pore diameter. A volume of 20 uL was injected in the HPLC (Perkin Elmer Series 200) equipped with a C18 Thermo column (250 mm x 4.6 mm internal diameter), particle size of 5 µm. The analysis was carried out in reverse phase, isocratic absorption, the mobile phase was acetonitryl: methanol: 2-propanol (44: 54:2), flow velocity 0.5 µL min⁻¹ detector UV-Vis, wavelength at 470 nm. As reference an authentic sample of β-carotene was used (Sigma-

Aldrich).

Evaluation of antioxidant activity. The analysis was made using the free radical DPPH method (2,2-diphenil-1-pycrylhydracil,), described by Amico et al. (2008). Next, 10 g of sample were macerated in methanol and each sample was maintained in a bath with sonication during 20 min. The solution was filtered and the supernatant was concentrated in a Büchi R-210 rotoevaporator. By serial dilutions, from the highest concentration of the methanolic extract, the following dissolutions were prepared in methanol to obtain the concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 $mg \cdot mL^{-1}$. Then 1 mL of each concentration of the extracts was taken and 3 mL of a solution of DPPH (0.1mM) were added. They were left at room temperature during 30 min and later the absorbance reading was made at 516 nm. The low absorbance of the reaction mixture indicated high antioxidant activity. The percentage of inhibited DPPH was determined using the formula % DPPH = $(A_{control} - A_{sample}) \cdot 100 / A_{control}$ where; $A_{control}$ was the absorbance of the control (DPPH 0.1mM). A_{sample} was the absorbance obtained after 30 min of each sample with DPPH 0.1 mM. Quercetin was used as reference, and the same procedure was applied as that of the samples. The values of the mean inhibitory concentration of the samples and quercetin (IC_{50}) were obtained by graphing the inhibition percentages as a function of the concentrations of the extract of each sample and the reference. The IC_{50} was the concentration required by the sample to reduce the absorbance 516 nm of the DPPH by 50 %.

For the construction of the standard curve of DPPH, 3.93 mg of DPPH were dissolved in 100 mL of methanol to obtain a concentration of 0.1 mM. From this solution the following concentrations were prepared by dilution: 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mM of DPPH. The absorbance was measured at 516 nm in the spectrophotometer. The standard curve was constructed with the absorbance of the radical DPPH as a function of its concentrations and the adjusted equation was obtained by linear regression (y = 9.7723x-0.0027; $r^2=0.999$).

Statistical analysis. The results were analyzed by means of Anova and Tukey test for comparison of means using the program Statistical Analysis System, SAS (Cary, NC, USA), version 9.1.3.

RESULTS AND DISCUSSION

Content of total phenols. No significant differences were found (P>0.05) of the content of phenolic compounds among the treatments PMF, MF and PMD, with the exception of MD where the chili presented the highest concentration $(P \leq 0.05)$ (Figure 1A). Sgroppo and Chávez (2009) mention that high temperatures accelerate the plant metabolism, which partially explains the results of MD. The heating may disrupt the cell membranes, leading to the release of membranebound these phytochemicals, the development of Maillard browning reactions simultaneously with others effects as the of the phenol-sugar glycoside bonds, leading to the formation of phenolic aglycons, although the accumulation of some metabolites depending on the plant species (Minatel et al., 2017). Similarly, De Ancos et al. (2009) point out that the phenolic compounds accumulate in the seeds, thus the low levels of phenolic compounds found may be due to the absence of seeds in the samples of the analysis, in the present study. Velioglu et al. (1998) mention that the seeds of various vegetables are rich in these metabolites, which may contribute significantly to the antioxidant activity.

The results obtained in the present study suggest that the chili is an important source of phenolic compounds as indicated by Howard and Wildman (2007). Chili can be used as nutraceutical foods and its consumption could help in the prevention of some chronic or degenerative diseases.

Sukrasno and Yeoman (1993) report the presence of phenolics (hydroxycynamic and ferulic acids) and their respective glycosides, as well as flavonoids (glycosides of quercetine, lueoline and apigenine) in C. annuum, but some free phenolics (cinnamate, p-coumarate, caffeate, ferulate, vanillin and vanillylamine) are sometimes detected in small quantities in development stage because they suggest being intermediaries in the synthesis of capsaicinoids and lignin and the concentration of the latter increases during maturation (Sukrasno and Yeoman, 1993), which could explain the little variation of these metabolites between premature and mature fruits of the present study.

Flavonoids content. The content of flavonoids

was significantly higher ($P \le 0.05$) in the MD chili than in the chilies PMF and MF (Figure 1B). The higher content of these phytochemicals in the dehydrated chilies may be due to the activation of the enzyme phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5.) during dehydration process, considered a defense mechanism.

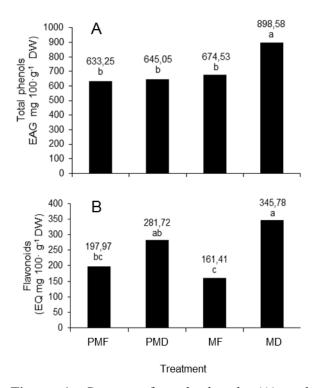


Figure 1. Content of total phenols (A) and flavonoids (B) in chili ancho (*Capsicum annuum*). Different letters in the bars indicate significant differences according to Tukey test ($P \le 0.05$). PMF: premature fresh; PMD: premature dehydrated; MF: mature fresh; MD: mature dehydrated

Howard and Wildman (2007) report the content of flavonoids in different cultivars of *C. annuum* in immature stage (17,17- 85,49 mg·kg⁻¹ FW) and mature (23,15-81,30 mg·kg⁻¹ FW); these authors found 31,0 mg·100 g⁻¹ in FW in chili *C. annuum* cultivar green and the difference in the concentrations with the present study may be due to the loss of moisture and soluble solids, which concentrate the sample (dry weight) or to the differences between cultivars. Howard et al. (2000) point out great variation in the content of flavonoids in diverse species of *Capsicum*,

specifically, the species *C. annuum* and *C. frutescens* presented the highest concentrations with respect to the species *C. chinense*. These metabolites are also important as they have antioxidant, anti-inflammatory, and anticancer properties (Crozier et al., 2009). Also, the results of this study can contribute knowledge of a food resource used ancestrally and still a part of the cultural identity of the Mexican Republic.

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Content of ascorbic acid. The content of ascorbic acid (vitamin C) was significantly higher in the samples of premature fresh chili than in the mature fresh chili (Figure 2).

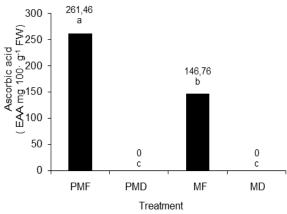


Figure 2. Content of ascorbic acid in chili ancho (*Capsicum annuum*). Different letters in the bars indicate significant differences according to Tukey test (*P*≤0.05). PMF: premature fresh; PMD: premature dehydrated; MF: mature fresh; MD: mature dehydrated

These results suggest that it would be convenient to harvest shortly before horticultural maturity to take advantage of the high content of this nutraceutical. To this respect, the content of vitamin C increases during the growth of the fruit, reaching its highest level in the stage which precedes total physiological maturity when the fruit is red in color (Monroy et al., 2007). Pérez et al. (2007) also indicate that the content of ascorbic acid in manzano chili (*C. pubensces*) increases according to the stage of maturity. In the present study the ascorbic acid was higher at PMF when the pigmentation of the fruit begins (Figure 2).

Howard et al. (1994) report the effect of ripening on the accumulation of ascorbic acid for a large number of chili varieties. However, there is no agreement on the magnitude of the changes, which seem to be dependent on chili cultivars. It has been reported that the vitamin C content during chili ripening increased by a factor that ranged from 1.2 to 4.1 (Howard et al., 1994; Martínez et al., 2005). Howard and Wildman (2007) report a lower content in chili *C. annuum* cultivar green San Luis ancho (168 mg·100 g⁻¹ FW). In chili Fresno de la Vega (*C. annuum*) the concentration of the metabolite was 159.63 mg $100 \cdot g^{-1}$ and $108.66 \text{ mg} \cdot 100 \text{ g}^{-1}$ FW in red and green fruits, respectively (Martínez et al., 2005). Osuna et al. (1998) report values between 14.8 and 276.6 mg·100 g⁻¹ in four different chilies cultivars of New Mexico, USA.

PMD and MD treatments had negative effect on ascorbic acid, possibly due to the dehydration process (Figure 2), because the principal cause of degradation of vitamin C in vegetables takes place during the postharvest phase as a result of storage and the high temperature (Lee and Kader, 2000; Howard and Wildman, 2007). To this respect, Martínez et al. (2005) point out that the largest percentage of loss of ascorbic acid was found in dehydrated samples, which contained only 12% of the initial content.

Howard and Wilman (2007) indicate that the content of ascorbic acid is influenced by the methods of drying. To this respect, they report a loss of 63 % for the fruit of Capsicum annuum when it was dried naturally; however, they point out a loss of 50 % when the fruit was dried with forced air. Furthermore, the authors report that the time and temperature can affect the content of ascorbic acid. The loss of ascorbic acid is due to its unstable structure and to the presence of oxygen, transforming it into dehydroascorbic acid, minority component of vitamin C. An extreme oxidation of the lactonic ring of dehydroascorbic acid degrades it into 2,3-dicetogulonic acid and promotes the loss of the activity of the vitamin C (De Ancos et al., 2009).

β-Carotene content. In the fresh chili there was an increase in the content of β-carotene in passing from the premature stage to the mature stage, and the same tendency was observed in the dehydrated chili (Figure 3). The 100-fold increase in the carotenoid content of the ripening pods is an evidence of the occurrence of the new synthesis (Daood et al., 1996; Hornero and Mingueza, 2000). Transformation of the carotenoids existing in the chloroplasts (mainly lutein and β -carotene) together with chlorophylls occurs first, followed by a new synthesis of carotenoids at the time when the chloroplasts are converted to chromoplasts (Daood, 2009).

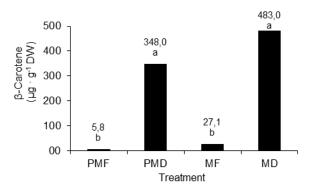


Figure 3. Content of β -carotene in chili ancho (*Capsicum annuum*). Different letters in the bars indicate significant differences according to Tukey test ($P \le 0.05$). PMF: premature fresh; PMD: premature dehydrated; MF: mature fresh; MD: mature dehydrated

The content of β -carotene was higher in the dehydrated chilies (Figure 3), which can be attributed to the fact that the heat processing increases the concentration of carotenoids (Howard et al., 1999). Drastic conditions of drying can degrade them; it has been reported that temperatures higher than 70 °C for more than 7 h can reduce this initial value of the carotene content by 50 % (Márkus et al., 1999; Daood et al., 2006). Quality, nutritive value and storage stability are dependent on the conditions at which drying is performed (Sun et al., 2007).

Bureau and Bushway (1986) and Howard et al. (1994) found that in different cultivars of *C. annuum* (green bell pepper, serrano and jalapeño) the concentration of β -carotene was 2-5, 5-8 and 3-5 $\mu g \cdot g^{-1}$ FW, respectively. Wall et al. (2001) point out that variation in pigment levels have been attributed to differences in cultivars, maturity, growing practices, climates and postharvest handling.

Antioxidant activity. Figure 4 shows that the stage of maturity of the fruits did not affect their antioxidant activity. The fruits of the treatment PMF presented higher antioxidant activity than those of treatment MD, by presenting a

significantly lower IC_{50} . There are few studies that indicate the antioxidant activity specifically in *C*. *annuum*.

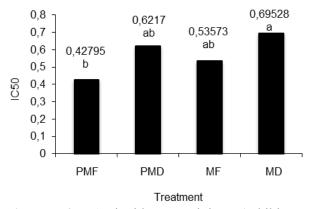


Figure 4. Antioxidant activity (Inhibitory concentration 50) in chili ancho (*Capsicum annuum*). Different letters in the bars indicate significant differences according to Tukey test ($P \le 0.05$). PMF: premature fresh; PMD: premature dehydrated; MF: mature fresh; MD: mature dehydrated

The higher antioxidant activity found in the premature fresh chili could be associated to the higher levels of ascorbic acid detected in this treatment, but this could not explain the antioxidant activity that was detected in the dehydrated chilies, due to the fact that the dehydration process practically destroyed this metabolite. Given that the content of β -carotenes, total phenols and flavonoids tended to increase, especially in the dehydrated chilies, possibly these phytochemicals are those that contributed in part to the antioxidant activity observed. To this respect, Ünver et al. (2009) report higher antioxidant capacity for the species C. annuum $(IC_{50} = 0.103 \text{ mg} \cdot \text{mL}^{-1})$ than that found in the present study, although the authors do not specify the type or variety that they studied.

It has been shown that dehydrated chili contains high levels of antioxidant compounds, and also high shelf life. It is affirmed that the antioxidant activity of fruits and vegetables is not only associated with the presence of the phenolic compounds and flavonoids, but also is attributed to other metabolites such as carotenoids, vitamin C (Delgado and Paredes, 2003; Materska and Perucka, 2005; Brat et al., 2007). According to the results obtained in the present study, the antioxidant activity of chili ancho (*C. annuum*) can be considered important (Howard and Wildman, 2007), principally in its fresh state because these nutraceutical characteristics provide an added value.

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CONCLUSIONS

The dehydration process with hot air provoked an increase in the content of total phenols and flavonoids, principally in the mature dehydrated chili. The stage of maturity affected the content of flavonoids present in chili cultivar ancho (*Capsicum annuum*). The β -carotene content was lower in the fresh than in dehydrated chilies. The stage of maturity of the chili did not affect the antioxidant activity of the fruits. Acid ascorbic was not observed in dehydrated chilies. The antioxidant activity of the mature dehydrated chili was higher than in the premature fresh chilies; therefore, these nutraceutical characteristics provide an added value. The dehydrated chili contains high levels of antioxidant compounds, and also high shelf life.

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