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Modification of the nutraceutical characteristics of jalapeño chili peppers in response to hormones

Modificación de las características nutracéuticas del chile jalapeño en respuesta a las hormonas

H. Medina[1], L. González-Cruz[1], R.G. Olan-Villegas[2], G. Acosta-García[1], N.L. Flores-Martínez[2], A. Bernardino-Nicanor[1]*

[1]*Tecnológico Nacional de México/IT Celaya, Antonio García Cubas Pte #600 esq. Av. Tecnológico. Celaya, Gto. México, C.P. 38010. A.P. 57.*

[2]*Universidad Politécnica de Guanajuato. Avenida Universidad Sur 1001. Comunidad Juan Alonso, Cortazar, Gto. C.P. 38483. México.*

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Abstract

Pot experiments were conducted in a greenhouse of the National Technological of Mexico in Celaya during two summer seasons, in 2019 and 2020, to study the changes in the concentration of nutraceutical compounds and protein expression patterns in jalapeño chili pepper in response to two phytohormones (gibberellic acid and auxins) and two drying processes (lyophilization and drying by convection oven). Jalapeño chili pepper (*Capsicum annuum* L.) plants were treated with the phytohormones separately (gibberellic acid, 5 μM solution and auxins (2 mL/L solution)) and mixed together. The obtained results indicated that the freeze-drying process significantly preserved the concentration of nutraceutical compounds and antioxidant activity. The individual application of phytohormones led to significant increases in the concentrations of all nutraceutical compounds and antioxidant activity in the jalapeño chili peppers. The application of exogenous giberellins modified the proteins expression. An antagonist effect of the phytohormone mixture on jalapeño chili pepper production was observed, and for this reason, no jalapeño chili pepper samples were obtained. In conclusion, drying and phytohormonal treatment influence the preservation of nutraceutical compounds and antioxidant activity.

Keywords: jalapeño chili pepper, carotenoids, nutraceutical compounds, gibberellic acid, auxins.

Resumen

Experimentos en maceta fueron llevados a cabo en un invernadero del Tecnológico Nacional de México en Celaya, durante dos temporadas de verano (2019 y 2020), para analizar los cambios en la concentración de compuestos nutracéuticos y en los perfiles de expresión de proteínas en chile jalapeño en respuesta a la aplicación de dos fitohormonas (ácido giberélico y auxinas) y dos procesos de secado (liofilización y secado en horno de convección). Las plantas de chile jalapeño (*Capsicum annuum* L.) , fueron tratadas con las hormonas de forma separada (disolución de ácido guberélico 5 μM y disolución de auxinas (2 mL/L) y una mezcla de ambas. Los resultados obtenidos indican que el proceso de secado por liofilización preserva significativamente la concentración de compuestos nutracéuticos y la actividad antioxidante. La aplicación individual de fitohormonas lleva a un incremento significativo en la concentración de todos los compuestos nutracéuticos y la actividad antioxidante del chile jalapeño. La aplicación de giberelinas modificó la expresión de proteínas. Se observó un efecto antagonista en la mezcla de fitohormonas sobre la producción de chile jalapeño, lo que origin, que no se obtuvieran muestras con este tratamiento, el secado y el tratamiento fitohormonal influyen en la preservación de compuestos nutracéuticos y actividad antioxidante.

Palabras clave: chile jalapeño, carotenoides, compuestos nutracéuticos, ácido giberélico, auxinas.

* *Corresponding author. E-mail*: aurea.bernardino@itcelaya.edu.mx
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1 Introduction

The most important genus in the Solanaceae family is the *Capsicum* genus due to its important food and economic value. The Capsicum genus was originated and initially diversified in South America (Pickersgill, 2016). In Mexico, the domestication of chili pepper was carried out in the central-eastern part of the country, with *Capsicum annuum* being the most cultivated species due to the nutritional characteristics of its fruits, principally regarding their mineral and vitamin contents (Khan *et al*. 2019), and their use as a spice to color and flavor food. In Mexico, *Capsicum annuum* cv. jalapeño accounts for approximately one-third of the total production of chili pepper (Peña-Yam *et al*. 2016), mainly due to its pungency. However, the degree of pungency varies according their cultivar type, origin, growing conditions and cultivation practices (Li *et al*. 2016).

A challenge for breeders is the characterization of agronomic and biochemical modifications that impact the phenotypic traits of chili pepper due to the focus of growers who on high yields, disease resistance, adequate color and a high level of pungency. For this reason, several methods for improving the yield, fruit weight, number of fruits per plant, color and pungency level of the chili pepper have been established, such as the inoculation of plants with different strains of bacteria and the use of fungal endophytes, composted greenwaste or different types of mulches (Peña-Yam *et al*. 2016; Kumara *et al*. 2016; Moreno-Salazar *et al*. 2020; Reddy and Crohn, 2018). On the other hand, the application of natural steroid hormones involved in various plant growth and development processes has been reported; for this reason, several studies on the regulation of plant development through approaches such as optimizing natural hormones and analogs used as plant growth regulators for agricultural production, have been conducted with the goal of increasing productivity in horticulture (Pérez-Jiménez *et al*. 2015).

However, few studies have considered the level of pungency and concentration of pigments in pepper fruit resulting from the addition of hormones as final quality parameters. Pichardo-González *et al*. (2018) reported that the application of gibberellins in combination with low fertilization increased the production of *Capsicum annuum* L, and Sandoval-Oliveros *et al*. (2017) previously indicated that plants of *Capsicum annuum* treated with a combination of cytokinins and gibberellic acid showed an increased crop yield. These results suggest that exogenous phytohormones application modifies molecular mechanisms, such as protein expression, that determine the crop yield Other authors used brassinosteroids or brassinosteroid analogs to evaluate the effects on the yield and quality parameters of pepper (Serna *et al*. 2012).

Capsicum annuum is an important agricultural crop not only due to its economic value but also due to its technological importance as a spice for improving the color and flavoring of foodstuffs. The goal of this study was to evaluate the effects of the application of two phytohormones (auxins and gibberellic acid) on the yield and final quality parameters (pungency level and carotenoid content) of the fruits and changes in the protein expression patterns of the plants.

2 Materials and methods

2.1 Plant material

The experiment was carried out in a greenhouse using *Capsicum annuum* L. cv. jalapeño (jalapeño chili pepper) under controlled conditions (30 °C and relative humidity of 38.75%). The seedlings were grown in a combination of peat moss-perlite-vermiculite (3:1:1) as growth medium in the nursery during the month of June.

2.2 Experimental conditions and hormonal treatments

The experiment was conducted under a completely randomized 2 x 2 factorial design with three replicates. The experiment was evaluated with respect to hormone application according to the results shown in Table 1. For each treatment, 26 plants were used, and the plants were watered every 3rd day throughout the production period. The plants in all treatments were fertilized with Miracle-Gro (NPK 15-15-30) (Scotts Company, Marysville, OH) at a rate of 0.5 g per week.

Hormone addition in all treatments was conducted two times at 30 and 45 days after seed germination.

Table 1. Hormonal treatments according to the experimental design.

Treatment	Specifications/concentration
Control	Without application of hormones
Auxins 5	μM solution
Gibberellic acid	5 μM solution
Gibberellic acid/auxin	2 mL/L- 5 μM solution

2.3 Collection, conditioning and characterization of jalapeño chili pepper fruits

2.3.1 Collection and conditioning of fruits

The jalapeño chili pepper fruits subjected to all hormonal treatments were collected at 40 days after anthesis; the fruits collected from each treatment were divided into two equal groups to determine the effect of the drying process on the nutraceutical characteristics of the fruits. The first group from all treatments was subjected to lyophilization, and the second group was subjected to convection drying. The fruits of jalapeño chili pepper were cut into 5 mm square pieces and stored until the drying procedure, in an LG Model GR-452SH refrigerator (LG Electronics, Mexico) at at 4ºC, for the drying process and in a Torrey Model FG-600 congelator (Torrey, Mexico) at -20ºC for the lyophilization process.

2.3.2 Lyophilization process

Square pieces of the jalapeño chili pepper fruits of 5 mm were lyophilized using a laboratory freeze dryer (Scientz-10N, Ningbo, China). During the lyophilization process, the prefreezing temperature was set at -40 °C. When the cold trap temperature reached -55 °C and the system pressure was reduced to 6 Pa, the lyophilization process was initiated. The dried samples were milled into a fine powder and sieved through a size 40 mesh (425 m). The jalapeño chili pepper powder was packaged in 25-g glass bottles and stored until use.

2.3.3 Convection oven drying process

Square pieces of the jalapeño chili pepper fruits of 5 mm were dried in an air convection heat oven (Binder, Model FD115-UL, USA) at 50 °C for 48 h. The dried samples were milled into a fine powder and sieved through a size 40 mesh (425 m). The jalapeño chili pepper powder was packaged in 25-g glass bottles and stored until use.

2.4 Quantification of nutraceutical compounds

2.4.1 Quantification of capsaicinoids

One gram of a dry sample of jalapeño chili pepper was mixed with 15 mL of absolute methanol in a 100 mL glass reactor (15 min; 35 °C). Then, the mixture was filtered using Whatman no. 40 filter paper; the obtained liquid was centrifuged (Hermle Z200A, Germany) at 6000 x g for 10 min at 25ºC; and the supernatant was recovered. The supernatant was filtered again, and the absolute methanol was evaporated in a bath at 50 °C. The capsaicinoid extracts were stored at 4 °C until use.

The capsaicin content of the extracts was estimated with a UV-Vis spectrophotometer (Genesys 2, Thermo Fisher Scientific, Inc. Waltham, MA, USA). The optical density was recorded at 280 nm. The capsaicin concentration in the samples was calculated using capsaicin as an external standard (Sigma, Aldrich) and was expressed as mg/g of jalapeño chili pepper powder and finally converted to Scoville heat units.

2.4.2 Total carotenoids

Samples of 0.3 g of jalapeño chili pepper powder were mixed with 30 mL of cold acetone and vacuum filtered. The residual sample was re-extracted with cold acetone (20 mL) and vacuum filtered. The two filtrates were combined and transferred to petroleum ether by adding water in a separatory funnel. The petroleum ether phase was recovered and washed four or five times with water until the total elimination of acetone. The recovered extract was saponified with a solution of KOH (10%) in methanol for 1 h. Thereafter, the sample was washed with water until reaching neutral pH and then dried with Na_2SO_4.

Quantification was performed via the external standard method using β-carotene as the external standard. The concentration of the external standard was determined spectrophotometrically. According to Campos-Herrera *et al*. (2018).

$$\text{Total content (mg)} = \frac{AY10^6FD}{B100} \qquad (1)$$

Where:
Y is the capacity volume (mL); FD is the dilution factor; A is the absorbance at 450 nm, B is specific absorption coefficient of β-carotene in petroleum ether (2592).

2.5 *Free radical-scavenging capacity*

2.5.1 *DPPH method*

The DPPH method was performed according to Brand-Williams *et al*. (1995), in which the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA) was used. To determine the % DPPH values, 100 mg/mL of each sample was vortex-mixed for 2 min, then centrifuged at 1000 x g for 20 min at 25ºC, and 0.05 mL of the supernatant was transferred to vials. Then, 2 mL of DPPH in methanol (6×10^{-5} M) was added. The reduction of absorbency was determined at 515 nm using a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) beginning at time 0 and then every 10 min until the reaction was completed.

The % DPPH radical calculation was performed using the following equation:

$$\%DPPH = \frac{A_0 - A_s}{A_0} \times 100\% \quad (2)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the test sample.

2.5.2 *ABTS method*

The scavenging capacity of ABTS radicals was evaluated according to Re *et al*. (1999). In brief, the ABTS radical cation ($ABTS^{*+}$) was obtained through the reaction of 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 7 mM) with K2S2O8 (2.45 mM) under incubation at room temperature in darkness for 16 h. The $ABTS^{*+}$ solution was then diluted with absolute ethanol to obtain an absorbance of 0.700 nm. The $ABTS^{*+}$ solution (3 mL; absorbance of 0.700 nm) was added to 0.03 mL of the test sample, followed by mixing vigorously. The reaction mixture was allowed to stand at room temperature (25 °C) for 30 min, and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using a Trolox standard solution at various concentrations (ranging from 0 to 400 μM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard, and the results were expressed as Trolox equivalents.

2.5.3 *FRAP assay*

The antioxidant activity of the jalapeño chili pepper fruits was determined using the ferric reducing/antioxidant power (FRAP) assay of Benzie and Strain (1996). The FRAP reagent contained 2.5 mL of 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer at pH 3.6. Freshly prepared FRAP reagent (3.0 mL) was mixed with 100 μL of jalapeño chili pepper extract, and the mixture was incubated at 37 °C for up to 30 min. Aqueous solutions of known Fe(II) concentrations in the range of 200 - 1000 μM ($FeSO_4 \cdot 7H_2O$) were used as standard. The absorbance was determined at 593 nm.

2.5.4 *Total Phenolic Content (TPC)*

The extraction of phenolic content was carried out according the method indicated by Alvarez-Padilla *et al*., 2011. To 0.5 g of the sample was added 10 mL of 80% methanol, the solution was sonicated (Jeken PS-30, USA) during 30 min, at 60ºC. The supernatant was recovered and then newly were added 10 mL of 80% methanol to the sediment, both supernatant recovered were combined and filtered. The extract was evaporated to dryness and suspended in 10 mL of distilled water. A 0.5 mL aliquot of the extract was added to 0.5 mL of the Folin-Ciocalteu reagent according described Vega *et al*., (2009), the mix was left to stand at room temperature for 5 min before to added 2 mL of 20% Na_2CO_3 solution. Then the absorbance was measured at 725 nm in spectometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The results were expressed as the gallic acid equivalents (GAE) in milligrams per gram of sample using a gallic acid (Sigma-Aldrich, St. Louis, MO, USA) standard curve.

2.6 *Protein extraction and quantification*

Samples of jalapeño chili pepper were pulverized to obtain a fine powder, and 0.7 g of the powder was mixed with 3 mL of 50 mM Tris-HCl pH 8.0, 10 mM NaCl, 1% sodium dodecyl sulfate (SDS, 0.1 mM dithiotreitol (DTT), 0.5% β-mercaptoethanol (PEB buffer) and 0.6 μL of 0.1 M phenylmethanesulfonylfluoride (PMSF). The mixture was stirred and heated in boiling water for 8 min in a water-bath. After the samples were cooled, 7.5 μL of protease inhibitor cocktail (PIC; Sigma 200-664-3; 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF) inhibits serine proteases, such as trypsin and chymotrypsin; 1,10-Phenanthroline inhibits metalloproteases; Pepstatin A inhibits acid proteases, such as pepsin (human or porcine), renin, cathepsin D, chymosin (bovine rennin), and protease B (*Aspergillus niger*); Leupeptin inhibits

both serine and cysteine proteases, such as calpain, trypsin, papain, and cathepsin B; Bestatin inhibits aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase; E-64 inhibits cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L.) was added, followed by gentle stirring for 2 h. The samples were centrifuged at 12,000 x g for 15 min at 4 °C, and the supernatant was recovered (total protein extracts). Protein quantification was carried out according to Bradford's method (1976).

2.6.1 *Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE)*

SDS-polyacrylamide gel electrophoresis was performed in 10 g/100 mL separating gels with 4 g/100 mL stacking gels according to the method of Laemmli (1970), using a Mini Protean 3Cell (Bio-Rad Laboratories, Hercules, CA 94547 USA) vertical unit. The molecular masses of the polypeptides were calculated using the following standard proteins (Bio-Rad Laboratories Hercules, CA 94547, USA): phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). The total protein samples were dissolved in sample buffer (0.1 mol/L Tris-HCl, pH 6.8, 20 mL/100 mL glycerol, 2 g/100 mL SDS, and 0.05 g/100 mL bromophenol blue). Gels were fixed and the protein were staining with Coomassie Brillant Blue.

2.7 *Statistical analysis*

The quantitative data are expressed as the mean ± standard deviation, and the analysis of variance (ANOVA) was performed, followed by Tukey's test. SAS software was used for the data analysis, and all experimental determinations were performed in triplicate.

3 Results and discussion

3.1 *Effect of the drying process on the nutraceutical compounds of jalapeño chili pepper*

The recovery of the nutraceutical compounds content of the jalapeño chili pepper fruits dried via two different drying methods is shown in Table 2.

The capsaicinoid, carotenoid and phenolic compound contents of the dried jalapeño chili peppers were significantly different among the samples. The freeze-dried jalapeño chili peppers exhibited the highest values. The capsaicinoid, carotenoid and phenolic compound contents of the freeze-dried jalapeño chili peppers were 32, 48 and 15% higher than hot air-dried jalapeño chili pepper respectively.

The freeze-drying method significantly improved the contents of all nutraceutical compounds tested in the dried jalapeño chili peppers compared to those in the hot air-dried jalapeño chili peppers, apparently because of the thermal degradation of all three types of compounds (capsaicinoids, carotenoids and phenolic compounds). It has been reported that thermal processing causes a loss of the capsaicin content (Ahmed *et al*. 2002, Montoya-Ballesteros *et al*., 2010, Montoya-Ballesteros *et al*., 2017). In addition, it has been reported that the dehydration conditions of chili peppers might improve the extractability of capsaicinoids (Toontom *et al*. 2016), and freeze drying improves the extraction of capsaicinoids. The results are in accordance with the claim of Park and Kim (2007) that freeze drying is the best processing method for preserving dried chili pepper quality.

With respect to the variation in the carotenoid content of the dried jalapeño chili peppers, Daood *et al*. (2006) demonstrated that the loss of carotenoids in red peppers increased as the drying temperature was raised in increments of 10 °C, and boiling and grilling have been reported to consistently decrease the concentrations of all forms of carotenoids (Cervantes-Paz *et al*., 2014).

Table 2. Nutraceutical compound concentrations in dried jalapeño chili peppers.

Drying process	Capsaicinoids	Carotenoids	Phenolic compounds
Hot air drying	0.463 ± 0.00^{b}	0.468 ± 0.01^{b}	1.596 ± 0.01^{b}
Freeze drying	0.613 ± 0.01^{a}	0.697 ± 0.01^{a}	1.830 ± 0.00^{a}

Means followed by same superscript within a column do not differ significantly ($p < 0.05$).

Table 3. Antioxidant activity of hot air =-dried and freeze-dried jalapeño chili pepper.

Drying process	Minimum concentration to reach the IC_{50} (mg/mL)		
	DPPH	ABTS	FRAP
Hot air drying	$46.775 \pm 0.00^{a}_{A}$	$15.926 \pm 0.06^{a}_{B}$	$15.762 \pm 0.02^{a}_{C}$
Freeze drying	$34.954 \pm 0.03^{b}_{A}$	$13.601 \pm 0.01^{b}_{B}$	$11.916 \pm 0.01^{b}_{C}$

Values followed by different lower-case letters in the same column are significantly different at p= 0.05 level according to Tukey. Values followed by different capital letters in the same row are significantly different at p= 0.05 level according to Tukey.

Table 4. Concentration of nutraceutical compounds in dried jalapeño chili pepper after phytohormonal treatments.

Treatment	Capsaicinoids mg/g of jalapeño chili pepper	Carotenoids mg/g of jalapeño chili pepper	Phenolic compounds mg gallic acid/ g of jalapeño chili pepper
Control*	0.583 ± 0.00^{c}	0.613 ± 0.01^{c}	1.830 ± 0.00^{b}
Auxins	0.637 ± 0.00^{b}	1.164 ± 0.04^{b}	1.574 ± 0.00^{c}
Gibberellic acid	0.739 ± 0.00^{a}	1.733 ± 0.09^{a}	2.430 ± 0.00^{a}
Gibberellic acid/auxin	WS	WS	WS

WS: Without Sample. Means followed by same superscript within a column do not differ significantly. The significance level is represented by * at 0.05 according to Tukey.

Other authors have indicated that convective dehydration and smoke drying decrease the carotenoid content by approximately 40% in relation to that of fresh red chili peppers (Campos-Hernández *et al*., 2018). According to some authors, heat exerts a significant effect on the total carotenoid content of dried mango, with the highest levels of carotenoids being retained in lyophilized samples; the obtained results are in accordance with previous reports indicating a declining trend in carotenoid content values when driers are operated at higher temperatures (Sogi *et al*., 2015).

3.2 Impact of the drying process on the antioxidant activity of jalapeño chili pepper

The analysis of the antioxidant activity of jalapeño chili pepper extracts showed that the hot air-dried jalapeño chili peppers exhibited lower antioxidant activity than the freeze-dried jalapeño chili peppers (Table 3). Thus, greater preservation of the antioxidant activity was observed in the freeze-dried jalapeño chili peppers considering that the amount of the sample required to reach the IC_{50} for the antioxidant activity of freeze-dried jalapeño chili pepper was lower than the amount of hot air-dried jalapeño chili pepper necessary to reach the IC_{50}. The DPPH and FRAP values showed the greatest differences between the two drying processes (approximately 33%), while the difference was smaller for the ABTS value (approximately 17%). However, the amount of the sample necessary to reach the IC_{50} value was lower for the ABTS and FRAP methods than for the DPPH method.

In almost all cases, the hot air-drying process increased antioxidant activity. It has been reported that thermal treatment can release many antioxidant compounds in green pepper, and some other authors indicate that during thermal treatment, thermal chemical reactions occur that produce more potent radical-scavenging antioxidants (Makris and Rossiter, 200; Yamaguchi *et al*. 2001; Vázquez-Cárdenas *et al*., 2015, Medina-Torres *et al*., 2021) that are different from the tested compounds. Apparently, capsaicinoids contribute greatly to the antioxidant activity of peppers; however, the ripening stage and drying method are determinant for the contribution level (Alvarez-Parrilla *et al*. 2001; Rochín-Wong *et al*., 2013).

3.3 Effect of phytohormonal treatment on the nutraceutical compounds of jalapeño chili pepper

The application of phytohormones increased the contents of all nutraceutical compounds significantly over those in the control (Table 4), with the carotenoid content showing increases of 1.8-fold and 0.8-fold in the jalapeño chili peppers treated with gibberellic acid and auxins, respectively, in comparison with the jalapeño chili peppers that did not receive any

phytohormonal treatment (control). The combination of phytohormones showed an antagonist effect on jalapeño chili pepper production; for this reason, no production of jalapeño chili pepper was observed.

It has been reported that a large number of phytohormones are capable of inducing carotenoid accumulation; however, the increase in carotenoids is determined by the specific phytohormonal treatment (Chen *et al*. 2020). In higher plants, a phytohormone that acts as a protective signaling molecule shifting physiological responses to resist abiotic stresses can apparently inhibit cell growth (Kozlova *et al*. 2017). Previous results showed that treatment with gibberellic acid reduced the fall of flowers and fruits in chili pepper, which increased the production of fruits. The improvement in the growth and performance of chili peppers under gibberellic acid application have been previously reported and is apparently due to the more efficient use of assimilates, leading to a higher photosynthetic efficiency, an increase in the resources of the plant, an increase in translocation and, consequently, an increase in the concentration of sugars and other metabolites, such as capsaicinoids and carotenoids (Abd and Faten, 2009; Pichardo-González *et al*. 2018).

On the other hand, some studies have shown that auxins are able to modify carbohydrate demand in the fruit and thus influence fruit quality parameters (Roussos *et al*. 2021). In addition, auxins have been shown to have an interplay with gibberellins and this participates in the induction of early growth of fruit development and its subsequent development. Recent reports have shown the participation of auxins in the regulation of cell division and the involvement of auxin-gibberellin interactions in cell expansion and fruit set, being key in determining fruit size and shape and therefore the crop yield.

3.4 Impact of phytohormonal treatment on the radical-scavenging activity of jalapeño chili pepper

The radical-scavenging activity of the jalapeño chili peppers under different phytohormonal treatments was measured, and Table 5 shows the results regarding radical-scavenging activity. Significant changes in the antioxidant activity of jalapeño chili pepper were observed under the different phytohormonal treatments. Antioxidant activity measured in the DPPH, ABTS and FRAP assays was higher (lower IC_{50} values) than in the control, especially under both individual treatments (auxins and gibberellic acid).

During chili pepper development, an increase in capsaicinoid and carotenoid concentrations is observed, and consequently, antioxidant activity is increased. It has been reported that the capsaicinoid content progressively increases during fruit development (Contreras-Padilla and Yahia, 1998); apparently, plants under the different phytohormonal treatments show the activation of their two main antioxidant defense mechanisms, involving the nonenzymatic and enzymatic systems (Jaleel *et al*. 2009), which is reflected in an increase in the concentration of capsaicinoids and carotenoids and, consequently, an increase in the antioxidant activity of chili peppers.

3.5 Changes in the protein expression patterns

In order to explore the exogenous gibberellins effect on the protein expression, the total protein extracts were fractionated by SDS-PAGE. In the electrophoretic patterns of the samples (Figure 1), modifications in the number of bands were observed. Five bands corresponding to proteins of 73 kDa, 51 kDa, 40 kDa, 33 kDa, and 29 kDa, respectively, were over-expressed in all samples, including the control.

Table 5. Antioxidant activity of dried chili peppers after phytohormonal treatments.

Treatment	Antioxidant activity (IC_{50})		
	DPPH	ABTS	FRAP
Control	$34.95 \pm 0.03^{a}_{A}$	$13.60 \pm 0.01^{a}_{B}$	$11.91 \pm 0.01^{b}_{C}$
Auxins	$29.92 \pm 0.00^{a}_{B}$	$12.85 \pm 0.01^{b}_{C}$	$14.60 \pm 0.04^{a}_{B}$
Gibberellic acid	$20.18 \pm 0.07^{c}_{A}$	$9.96 \pm 0.04^{c}_{B}$	$9.10 \pm 0.02^{c}_{C}$
Gibberellic acid/auxin	WS	WS	WS

WS: Without Sample. Values followed by different lower-case letters in the same column are significantly different at p= 0.05 level according to Tukey. Values followed by different capital letters in the same row are significantly different at p= 0.05 level according to Tukey.

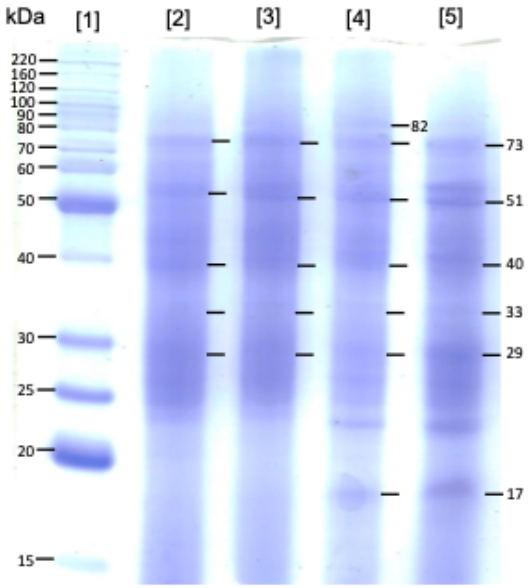

Figure 1. Protein expression patterns in response to exogenous phytohormones. [1] Molecular weight marker, [2] Control, [3] Auxins, [4] Gibberellic acid and [5] Gibberellic acid/auxins. SDS-PAGE of total protein extracts obtained from floral buds.

This apparently is due to that are proteins whose expression is not modified by effect of the addition of phytohormones; they surely are important for the physiological and molecular processes of plant development.

In the samples where gibberellic acid was added, three new bands corresponding to proteins of 82 kDa, 21 kDa, and 17 kDa, respectively, were observed (lane 4; Figure 1). They could be related to key components of the gibberellic acid signaling pathway, that have been previously described (Gao and Chu, 2020). On the other hand, the addition of auxins inhibits the synthesis of the proteins just mentioned suggesting that these could be related to the auxin regulation pathway (Slade *et al*., 2012; Wen *et al*., 2010). However, with the addition of the gibberellic acid/auxin mixture a new band were observed corresponding to proteins of 53 kDa around, that can be related auxin-gibberellins crosstalk (Weiss and Ori, 2007).

The results indicate that the synthesis of proteins expression involved in the metabolic plant development, mainly fruit formation, is affected in specific way by each phytohormone added and can be modified with the interaction between phytohormones. Many proteins have been reported to be specific of flowers, leaves, and seeds of *Capsicum annuum*, showing that they are involved in metabolic processes, cell organization, biogenesis, biological regulation, defense, and nutrient transport (Kozlova *et al*., 2017; Pérez-Jiménez *et al*., 2015; Pichardo-González *et al*., 2018).

Conclusions

The drying process has a significant effect on the preservation of nutraceutical compounds and antioxidant activity, in which freeze drying leads to more favorable results than drying in a convection oven. The combination of gibberellic acid and auxin has an antagonist effect on jalapeño chili pepper production; however, individual phytohormonal treatments improved the concentration of nutraceutical compounds and antioxidant activity. The exogenous phytohormones modify the protein expression patterns.

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