

Effect of water stress on functional and marketable properties of roasted Big Jim chili pepper (*Capsicum annum L.*) in Southern USA

Efecto del estrés hídrico en las propiedades funcionales y comerciales del chile rostizado Big Jim (*Capsicum annum L.*) en el sur de Estados Unidos

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ABSTRACT

This study aimed to evaluate the effect of water stress on spiciness, fatty acids, and aroma compound profile of roasted Big Jim chili. A flooded furrow irrigation system for chili peppers production was utilized with 4 irrigation treatments: every 7, 9, 11, and 13 days for W1, W2, W3, and W4, respectively, in a completely randomized block design. Capsaicinoid content was increased (~160%) by increasing water stress ($P < 0.05$). However, the roasting process reduced the capsaicinoids content ($P < 0.05$). Contents of linoleic, palmitic, and arachidonic acids were not affected. Water stress reduced hexanal and linalool content by approximately 64 and 72%, respectively ($P < 0.05$), whereas 2-isobutyl-3-methoxypyrazine content increased ($P < 0.05$). Water stress increased pungency and capsaicinoids content in chili, while the roasting process reduced pungency.

KEYWORDS

drought, capsaicinoids, volatile compounds, fatty acids, roasting process

RESUMEN

Este estudio evaluó el efecto del estrés hídrico en el picor y los perfiles de ácidos grasos y compuestos aromáticos del chile rostizado de la variedad Big Jim. Se incorporó un diseño de bloques completamente al azar con 4 variaciones en la frecuencia de riego: cada 7, 9, 11 y 13 días para los tratamientos W1, W2, W3 y W4, respectivamente. El contenido de capsaicinoides se incrementó (~160%) con el estrés hídrico ($P < 0.05$). No obstante, el proceso de rostizado reduce la concentración de capsaicinoides ($P < 0.05$). Los contenidos de ácido linoleico, palmítico y araquidónico no fueron afectados por el estrés hídrico ($P > 0.05$). El estrés hídrico redujo los contenidos relativos de hexanal y linalol aproximadamente 64 y 72%, respectivamente; por otro lado, el 2-isobutil-3-metoxipirazina aumentó ($P < 0.05$). El estrés hídrico incrementa el picor y el contenido de capsaicinoides en el chile, mientras que el proceso de rostizado reduce el picor.

PALABRAS CLAVE

sequía, capsacionoides, compuestos volátiles, ácidos grasos, proceso de rostizado

INTRODUCTION

The United States of America (USA) ranks sixth in the world for large chili pepper production, accounting for 3.6% of the world's total production, just behind China, Mexico, Indonesia, Turkey, and Spain (FAO 2017). New Mexico produces approximately 50% of the chili peppers grown in the USA, with a market value of 162.85 million dollars (USDA 2017). Big Jim chili pepper, which was developed in New Mexico, USA, belongs to the *Capsicum annuum* L. species (Erwin 1937), and it is commonly used in the preparation of local and regional food (Nakayama 1975). Roasting chili peppers when in season and then freezing for later use is standard practice. The roasting process has been reported to highlight certain flavors, soften the texture, and improve the taste through the inactivation of specific compounds (Srisajalertwaja et al. 2012).

Marketable quality for chili peppers also includes pungency and volatile compounds. It is important to note that pungency and volatile compounds concentrations present variations with ripening and growing conditions (Arora et al. 2011; Othman et al. 2011). Moreover, chili peppers could also be considered as a good source of fatty acids (Pérez-Gálvez et al. 1999). The capsaicinoids content in chili peppers is directly related to fruit spiciness and pungency. Its bioactive compounds confer the chili pepper specific pharmaceutical properties, such as analgesic effects against arthritis pain and inflammation, gastro-protection properties, energy enhancer properties and its usefulness for the treatment of neuralgia (Backonja et al. 2010; Deal et al. 1999; Inoue et al. 2007; Mozsik et al. 2005; Othman et al. 2011; Wahyuni et al. 2012). However, the consumption of high levels of capsaicin may be toxic for health (Othman et al. 2011).

In the foreseeable future, the competition for water use among agricultural, industrial, municipal, and tourism sectors is expected to increase (Pereira et al. 2002). Also, an increasing demand for food, fiber, and fuel from a growing global population will boost the demand on water supply for irrigation (Gadissa and Chemedda 2009). The oldest and most common irrigation system in certain zones of the USA (i.e., New Mexico) is flooded furrows (Herrera and Sammis, 2000). In some regions, crop irrigation by flooded furrow is usually done every 5-7 days. Furthermore, the irrigation activity in this region uses about 85% of the total water demand in the state, which is intensified by higher temperatures due to climate change (Hurd and Coonrod 2008). Different conventional methods are increasingly being used to

detect water stress tolerance among cultivars (Penella et al. 2014). Nevertheless, certain compounds and metabolites produced in the plant may be affected due to water stress, which may modify its marketable characteristics and consumer acceptance.

The objective of this study was to determine the effect of water stress and commercial roasting processes on the concentration of capsaicinoids, fatty acids content, and variation of volatile compounds in Big Jim chili peppers.

MATERIALS AND METHODS

Plant material and experimental conditions

Big Jim chili pepper seeds were donated from a local plant breeder in New Mexico, USA. Seeds were sown in greenhouses on the New Mexico State University (NMSU) campus in Las Cruces, NM, USA, into 12-celled planting containers with Redi-Earth plug and potting mix (Sungro, MA, USA). During this process, plants were watered with a hose twice a day until saturated. Seedlings were transported to the Sustainable Agriculture Science Center of the NMSU at Alcalde, NM, USA, in the fall of 2013, just 32 days after emergence and immediately transplanted. Fertilization consisted of nitrogen and phosphorous at 224.2 and 112.1 kg ha⁻¹, respectively (Joukhadar and Walker 2018). Afterward, plants were watered following regional standard practices for 42 days after transplanting. After this, plants were subjected to water stress conditions for 120 days according to intervals selected on the availability of water in the acequia ditches. A flooded furrow irrigation system was implemented with 4 treatments with a different frequency of irrigation events: every 7, 9, 11, and 13 days for W1, W2, W3, and W4, respectively, where W1 is the control treatment.

Plants were harvested entirely, and randomly-selected pods were roasted under commercial conditions (60 RPM, 250°C, 25 lb/cycle, 8 min/cycle) in a commercial roaster (Barbacoa-2, Santa Barbara Chili Roaster Co, Ca, USA). Roasted and non-roasted samples were vacuum-sealed into polyethylene bags and stored at -20°C for further analysis. Once the remaining analysis was ready to be developed, samples were freeze-dried in a Freezemobile 25 XL unit, peeled ground (< 100 µm) (Hamilton-Beach, model 80335R), and packaged into polyethylene bags containing another bag with silica gel. Then they were stored at 10°C in a dry and dark place for one week before using.

Laboratory analysis

The extraction of capsaicinoids from pods before and after the roasting process was performed as described by Srisajjalertwaja et al. (2012), using ethanol instead of methanol. Afterward, the extract was centrifuged, and approximately a 5 μ l aliquot was sampled and injected via autosampler for analysis in a GC/FID equipment (Varian CP-3900, Varian Inc, Ca, USA). GC oven temperature was programmed from 80°C (held constant for 1 min) to 200°C at a rate of 20°C/min, next to 320°C at a rate of 10°C/min (held constant for 5 min). The injector temperature was 250°C. Capsaicinoids separation was carried out in a 30m length and 0.25mm ID Rxi-5Sil MS column (Rustek, USA).

Fatty acid extraction was performed as described by Patil et al. (2012), using 0.2N KOH in methanol for extraction and direct methylation underwater bath conditions at 50°C for 1h. Fatty acid methyl esters (FAME) were then extracted with hexane as an organic phase. About 5 μ l aliquot was injected via autosampler in a GC/MS (CP-3800 Varian Inc, Ca, USA). The oven temperature was programmed from 70°C (held constant for 3 min) to 210°C at a rate of 8°C/min, next to 280°C at a rate of 4°C/min (held constant for 10 min). Monitored ions were 41-360 amu. The ion trap temperature was 100°C. FAME separation was carried out in a 30m length and 0.25mm ID Rxi-5Sil MS column (Rustek USA).

For the analysis of volatile compounds, freeze-dried samples were placed in 15 mL vials, sealed and stored at 60°C for 1 h. Afterward, a SPME 50/30 μ m DVB/CAR/PDMS (Sigma-Aldrich, USA) fiber was inserted in the vial headspace and exposed for volatile compounds absorption. After a 30-min-exposure period, fiber was conducted and exposed into a GC/MS (CP-3800 Varian Inc, CA, USA) in a splitless mode for 2 min at 220°C as the injector temperature for volatile compounds desorption. Volatile compounds separation was

carried out in a 30m/0.25mm ID Rxi-5Sil MS column (Rustek, USA). GC oven temperature was programmed from 60°C (held constant for 2 min) to 280°C at a rate of 3°C/min and held for 10 min. Monitored ions were 41-360 amu. The ion trap temperature was 100°C. External standards were acquired and used to identify compounds (Sigma-Aldrich, USA).

Statistics

All experimental data were analyzed according to a completely randomized block design with three replications using the GLM procedure (SAS Institute Inc., Cary, NC, 2002). The model used was: $Y = \mu + T_i + e$, where μ is overall mean, T_i is the treatment effect, and e is the error term. Differences between means were identified and considered significant at $P < 0.05$ using Tukey's test.

RESULTS AND DISCUSSION

Pungency

Water stress affected capsaicinoids content among treatments (Table 1). Total and individual capsaicinoids content increased among treatments due to water stress ($P < 0.05$). The highest contents for capsaicinoids were observed in treatment W2 ($P < 0.05$), representing a rise of about 160% when compared to W1. Also, W3 and W4 showed an increase of about 71 and 115%, respectively. Moreover, a comparison between total capsaicinoids was developed before and after the roasting process (Table 2). Reductions in total capsaicinoids due to the roasting process were approximately 70% in the control treatment (W1). In the same way, high diminutions were noticed under the other irrigation treatments, representing reductions of about 66, 77, and 43% for W2, W3, and W4, respectively.

Table 1. Capsaicin, dihydrocapsaicin, and total capsaicinoids content (μ g/g, DM) in roasted Big Jim chili pepper under different irrigation treatments.

Treatment	Capsaicin	Dihydrocapsaicin	Total capsaicinoids
W1	89.3 \pm 3.46d	32.4 \pm 0.90d	121.7 \pm 4.36d
W2	238.4 \pm 7.75a	98.8 \pm 3.69a	337.3 \pm 11.42a
W3	146.7 \pm 4.66c	61.2 \pm 2.74c	208.0 \pm 7.40c
W4	178.1 \pm 3.73b	84.7 \pm 1.98b	262.8 \pm 5.62b
SED	14.68	7.19	21.73

^{ab}Means with different letters within the same column are statistically different ($P < 0.05$); SED = Standard error of the difference between means.

Sung et al. (2005) reported an increase in enzymatic activity for capsaicinoid synthesis under water stress conditions. These authors also reported a reduction in the degradation of capsaicinoids by peroxidation, decreasing the activity of peroxidase when plants were under water stress. Similarly, several studies (Bernal et al. 1993, 1994, 1995) reported that peroxydation uses caffeic and ferulic acids as substrates obtained from the phenylpropanoid pathway. However, peroxidation decreases under water stress conditions, and this permits precursors to synthesize more capsaicinoids, thereby avoiding oxidation. Nevertheless, in this study, as water stress increased, capsaicinoids concentrations were reduced. This phenomenon may be attributed to the inactivation or reduction in the synthesis of capsaicinoids. These results suggest a synergy between kinetics for synthesis and degradation of capsaicinoids without specifying whether synthesis is increased or degradation is reduced. However, when plants were under conditions in W3 and W4, total capsaicinoids concentration was reduced when compared to those values obtained in W2. Thus, an inactivation of capsaicinoids synthase is more likely to occur.

Similar results are presented by Ruiz-Lau et al. (2011), who reported a reduction in capsaicinoids degradation by peroxidation. Nevertheless, in the same study, the synthesis of capsaicinoids was reported to be similar among treatments. Additionally, the results presented in Table 2, demonstrate that performance among experimental treatments remained identical before and after roasting.

The latter explains a reduction in pungency over 50% during the roasting process. Also, Contreras-Padilla and Yahia (1998), and Bernal and Barceló (1996) reported a reduction in the capsaicinoids content under drying processes due to a more extended exposition of capsaicinoids to hydrogen peroxide, which leads to a peroxidation. Daood et al. (2006) dried chili peppers at different temperatures below 110°C and found out that capsaicin was stable below 90°C but affected at a higher temperature. However, they suggested that a reduction in capsaicinoids content was due to the protective effect of carotenoids by capsaicinoids.

Pungency is expressed in Scovill Heat Units (SHU) for all treatments before and after the roasting process (Figure 1). Water stress resulted in a considerable increase in pungency in green pods, resulting in an increase of approximately 160% in W2 ($P<0.05$). However, when chilis were roasted, considerable reductions $>50\%$ in pungency were observed in SHU for every treatment ($P<0.05$).

Coon et al. (2009) classified the pungency of the Big Jim variety within a range of 2000-6000 SHU. In this study, as water stress was increased, marked changes in pungency were observed. These changes in pungency locate Big Jim variety in another pungency level, which may affect consumer acceptance and marketability. However, these changes may be diminished by submitting pods to a roasting process. In this way, pungency values can be relocated to their habitual range and classification.

Table 2. Total capsaicinoids content ($\mu\text{g/g}$, DM) in Big Jim chili pepper under different irrigation regimes before and after the commercial roasting process under different irrigation treatments.

Treatment	Total capsaicinoids		SED
	Before roasting*	After roasting	
W1	408.1 \pm 15.39a	121.7 \pm 4.36b	22.62
W2	997.2 \pm 38.2a	337.3 \pm 11.42b	56.39
W3	928 \pm 124.08a	208.0 \pm 7.40b	175.79
W4	463.7 \pm 16.97a	262.8 \pm 5.62b	25.28

^{ab}Means with different letters within the same row are different; SED = Standard error of the difference between means.

*Data obtained from Joukhadar & Walker (2018)..

Similarly, the roasting process decreased capsaicinoids content significantly. Srisajjalertwaja et al. (2012) observed a reduction in the capsaicinoids content of about 50% when thai green chilis were baked at 180-210°C for 20 min, reporting a pungency loss at higher temperatures. In this study, chili pods were exposed to higher temperatures than those presented by Srisajjalertwaja et al. (2012).

Fatty acids content

Four predominant fatty acids were identified (Table 3). Linoleic acid (C18:2) is the major fatty acid found in samples among treatments with means above 8.7 $\mu\text{g/g}$ of dry chili pepper. In contrast, the other fatty acids showed concentrations $< 2.5 \mu\text{g/g}$ of dry chili pepper ($P<0.05$).

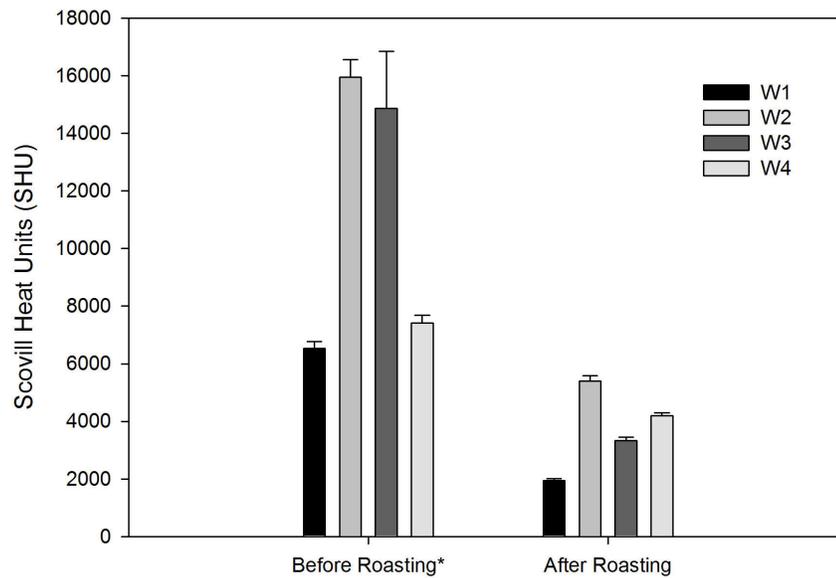


Figure 1. Pungency content in Big Jim chili pepper at different irrigation treatments before and after the roasting process. *Data obtained from Joukhadar & Walker (2018).

Table 3. Fatty acids content ($\mu\text{g/g}$, DM) in roasted Big Jim chili pepper under different irrigation treatments.

Treatment	Fatty acids			
	C16:0	C17:0	C18:2	C20:0
W1	2.5 \pm 0.11a	0.6 \pm 0.02b	8.7 \pm 0.56a	1.6 \pm 0.06a
W2	2.0 \pm 0.05a	0.6 \pm 0.00b	8.9 \pm 1.73a	2.4 \pm 0.37a
W3	2.3 \pm 0.15a	0.7 \pm 0.01a	9.0 \pm 1.15a	2.5 \pm 0.32a
W4	2.4 \pm 0.45a	0.6 \pm 0.00b	9.3 \pm 0.79a	2.1 \pm 0.20a
SED	0.70	0.03	3.25	0.76

^{ab}Means with different letters within the same column are statistically different ($P < 0.05$); SED = Standard error of the difference between means.

However, no differences were found in palmitic (C16:0), linoleic (C18:2), and arachidonic (C20:0) among experimental treatments ($P > 0.05$). Heptanoic acid (C17:0) increased by approximately 16% when plants were under water stress in treatment W3 ($P < 0.05$).

The results obtained in the analysis of the fatty acid indicate that water stress did not affect the synthesis of the major fatty acids contained in the Big Jim variety. Water stress has been reported to result in fatty acids oxidation in plants, turning fatty acids into carbohydrates necessary for growth (Lisar et al. 2012). Arachidonic acid in the total fatty acid profile has a significant value and presents similar contents among treatments; however, it has not been reported in the literature as part of the chili pepper fatty acids profile. Oxidative stress promotes the production of reactive

oxygen species (ROS), and their presence causes the appearance of arachidonic acid, a defense mechanism against lipid peroxidation (Balboa and Balsinde 2006). Nederal et al. (2012) observed no significant difference in fatty acids content between roasted pumpkin seeds oil and non-roasted pumpkin seeds oil, obtained by a pressing process while oxidative stability of roasted oil may have been caused by antioxidative Maillard reactions products obtained in the roasting process.

Volatile and aroma compounds

Relative contents of three volatile compounds were calculated, and changes compared to W1 were reported (Table 4). There was a significant loss of hexanal due to water stress ($P < 0.05$).

Table 4. Relative loss and increase (%) of three volatile compounds in headspace fraction of roasted Big Jim chili pepper under different irrigation treatments compared to control treatment (W1).

Treatment	Hexanal	Linalool	2-isobutyl-3-methoxypyrazine
W1	-	-	-
W2	-67.3±4.14 b	-38.6±18.66 a	31.6±10.21 b
W3	-64.6±3.25 b	-72.6±3.48 a	76.8±5.61 a
W4	-21.9±10.05a	-73.6±3.58 a	24.8±7.42 b
SED	16.04	27.32	19.54

^{ab}Means with different letters within the same column are statistically different ($P < 0.05$); SED = Standard error of the difference between means.

Losses of approximately 67, 64, and 21% for W2, W3, and W4, respectively, were observed ($P < 0.05$). Similarly, changes in linalool were observed with reductions of about 38, 72, and 73% in W2, W3, and W4, respectively. Otherwise, 2-isobutyl-3-methoxypyrazine increased with water stress ($P < 0.05$). Also, increases of about 31, 76, and 24% for W2, W3, and W4 were observed ($P < 0.05$).

This study tried to track 6 different compounds reported in the literature for chili pepper samples (Mazida et al. 2005). However, only 3 of them were found to be present (hexanal, linalool, and 2-isobutyl-3-methoxypyrazine) in our samples. According to Plotto et al. (2004), odor descriptions for volatile compounds are (a) hexanal reported with a grassy odor; (b) linalool which odor is mentioned as floral, citric, sweet and fresh, and (c) 2-isobutyl-3-methoxypyrazine which is described with green pepper and spicy odor.

Plants with stress of any kind, including water stress, may lead to intricate patterns that occasionally overshadow the synthesis level emission controls for volatile compounds (Lisar et al. 2012). Aldehydes, like hexanal, are converted into carboxylic acid due to the presence of aldehyde dehydrogenases, which are produced when the plant is subjected to water stress. Additionally, aldehydes are also an intermediate product in the synthesis of osmolytes. Osmolytes have shown protective action during osmotic stress, which increases with water stress (Kotchoni and Bartels 2003). Wenda-Piesik (2011) reported that terpenoids contained in wheat were affected under water stress; the stress avoided the release of terpenoids, and therefore, compounds were not detected. Since linalool is cataloged as a terpenoid, the latter may explain its reduction across treatments; water stress avoided its release.

Similarly, 3-carene is a terpenoid, and its concentration in chili pepper is low (Mazida et al. 2005).

Thus, when plants were subjected to water stress, it could not be detected or traced. Plants that are subjected to water stress stimulate ethylene due to oxidative stress; thus, production and synthesis of volatile herbaceous compounds, like 2-isobutyl-3-methoxypyrazine, may be increased (Borsani et al. 2001; Narayana et al. 1991). Scheiner et al. (2012) reported similar results when modeling environmental impacts on methoxypyrazines contained in grapes for wine production; synthesis of these compounds increased when grapes were under water stress. Therefore, based on our results, it can be said that spicy and green pepper odors are increased, while floral, sweet, and grassy odors were diminished.

CONCLUSIONS

Water stress substantially increased Big Jim chili pepper pungency and capsaicinoids content, which may compromise its classification in the ASTA (American Spice Trade Association) ranking. However, the roasting process reduces pungency and capsaicinoids content, which results in limited changes in its usual classification in the ASTA ranking. Additionally, the roasting process releases some desirable volatile compounds, including spicy and green pepper odors that may increase consumer acceptance without affecting the fatty acid content.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in the present investigation.

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