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POLYPHENOL CONTENT IN *Solanum lycopersicum* UNDER THE ACTION OF A STATIC MAGNETIC FIELD

Contenido de polifenoles en *Solanum lycopersicum* L. bajo la acción de un campo magnético estático

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ABSTRACT. Polyphenols contribute to antioxidant properties of *Solanum lycopersicum* L. (tomato); plant in which they have demonstrated positive effects of magnetic fields (MF) on germination and productivity. So it was necessary to study the content of polyphenols in tomato fruits from plants irrigated with water treated with static magnetic field (SMF) to 150 mT. A completely randomized experimental design, where the control plants were irrigated with normal water and secondary treatment plants were irrigated with SMF to 150 mT was used. From ripe fruits aqueous ethanolic extracts and Folch mixture (chloroform:methanol, 2:1) were prepared. Total polyphenols were measured by the Folin - Ciocalteu and identified with high performance liquid chromatography (HPLC). Simple classification ANOVA and multiple range comparison with least significant difference were performed. Aqueous extracts had a higher content of polyphenols relative to the ethanol and made with the Folch mixture, with statistically significant differences. All extracts from fruits receiving irrigation with treated water with SMF showed most polyphenols. In aqueous extracts prepared from fruit irrigated with SMF, the polyphenol content was 1,25 times higher compared to the control treatment aqueous extracts. Polyphenols rutin, quercetin and gallic acid were identified, of which has been reported significant contribution in the antioxidant capacity of *S. lycopersicum* L.

Key words: gallic acid, HPLC, quercetin, rutin, tomato

RESUMEN. Los polifenoles contribuyen a las propiedades antioxidantes de *S. lycopersicum* L. (tomate); planta en la cual se han evidenciado los efectos positivos de los campos magnéticos (CM) en la germinación y la productividad. De manera que fue necesario estudiar el contenido de polifenoles en frutos de tomate provenientes de plantas irrigadas con agua tratada con campo magnético estático (CME) a 150 mT. Se utilizó un diseño experimental completamente aleatorizado, donde las plantas control se irrigaron con agua normal y las plantas del segundo tratamiento se irrigaron con CME a 150 mT. A partir de los frutos maduros se prepararon extractos acuosos, etanólicos y con mezcla Folch (cloroformo:metanol, 2:1). Se cuantificaron los polifenoles totales por el método de Folin-Ciocalteu y se identificaron con la Cromatografía Líquida de Alta Resolución (HPLC) siglas en Inglés. Se realizó un ANOVA de Clasificación Simple y la Comparación de Múltiples Rangos con Diferencia Mínima Significativa. Los extractos acuosos presentaron un mayor contenido de polifenoles con relación a los etanólicos y a los elaborados con la mezcla Folch, con diferencias estadísticamente significativas. Todos los extractos procedentes de los frutos que recibieron el riego con agua tratada con CME mostraron una mayor cantidad de polifenoles. En los extractos acuosos elaborados a partir de frutos irrigados con CME, el contenido de polifenoles fue 1,25 veces mayor con relación a los extractos acuosos del tratamiento control. Se identificaron los polifenoles rutina, quercetina y ácido gálico, de los cuales se ha reportado su contribución significativa en la capacidad antioxidante de *S. lycopersicum* L.

Palabras clave: ácido gálico, HPLC, quercetina, rutina, tomate

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INTRODUCTION

Tomato, *Solanum lycopersicum* L., is considered a nutraceutical plant, due to its secondary metabolites that can prevent human diseases (1, 2). Polyphenols are within this group of compounds, which are secondary metabolites that allow plant defense against stress situations, besides being antioxidants (3, 4). In human beings, polyphenols play its role against free radicals and attack oxidative stress, a causal agent of several diseases (5, 6, 7). Regarding *S. lycopersicum* L. plant growth, several researchers have demonstrated electromagnetic field (EMF) potentialities in a range between 100 and 200 mT (8, 9, 10, 11). Yield and productivity have Increased (12, 13), whereas seed germination has been favored by static magnetic inductions from 15 to 300 mT (11, 14). Studies at metabolic level point out secondary metabolite content variation in fruits (15, 16). However, the information is not enough in spite of these evidences, so this research was aimed to study polyphenol content in *S. lycopersicum* L. fruits from plants irrigated with static magnetic field (CME) treated water at 150 mT.

MATERIALS AND METHODS

A working stage was carried out at the National Center of Applied Electromagnetism (CNEA) in Santiago de Cuba using tomato (*Solanum lycopersicum* L.) Aegean hybrid. Seeds showed a good phytosanitary status and were supplied by the Provincial Seed Laboratory from the Ministry of Agriculture (MINAG) in Santiago de Cuba. Plants were grown in “Veguita” protected growing house under semicontrolled conditions. Substrate characteristics fulfilled the requirements established by the Ministry of Agriculture and cultural farming followed the standards for this species. A specimen was placed at the Eastern Center of Ecosystems and Biodiversity (BIOECO) in Santiago de Cuba, which was registered by BSC 21509.

Plants were irrigated by drip and aerial microjet, depending on its growing stage. A randomized complete experimental design with two treatments and three replicates was used. Plants from the first treatment (control) were irrigated with normal water; meanwhile those from the second one were irrigated with CME treated water at 150 mT. Then, 10 ripe fruits were randomly selected per each treatment.

Fruits were dried in an oven (MWL-200, VEB, Germany) at 60 °C for 72 h. Later, they were converted into powder in an electric mill (IKA MHD 2000, China). All operations enabled to extract active principles according to Public Health Branch Standard 310/91 (17).

Aqueous extracts were made at 100 mg mL^{-1} by distilled water infusions; they were cooled and centrifuged for 10 minutes at 2000 rpm in a centrifuge (Centribio, MLW T24D, Germany). The supernatant was filtered through a Whatman No. 4 paper at a 0.45 µm Millipore filter. Ethanolic extracts were prepared from 100 mg mL^{-1} *S. lycopersicum* L. powder with ethanol (70 %). Mixtures were macerated at room temperature in the dark for seven days periodically stirred and further concentrated up to dryness in a Rotoevaporator (Heidotpin 4011).

Concentrated extracts were resuspended in bidistilled water, then centrifuged and supernatants were filtered like in the aqueous extract.

So as to prepare extracts with Folch blend, *S. lycopersicum* powder was mixed with a solution containing chloroform and methanol (2:1, v/v).

Table I describes the extracts employed. Total polyphenol content was determined in each extract according to the established method (18). Extracts were mixed with 0.2 mL Folin-Ciocalteu reagent (1N) and later 0.4 mL sodium carbonate was added (7.5 %).

Reaction mixture was kept at 45 °C for 40 minutes. Absorbance was measured at a wavelength (Λ) of 765 nm on a spectrophotometer (1650PC: uv-visible Shimadzu). A calibration curve was drawn with standard gallic acid (2,5-20 µg mL^{-1}) and concentrations were expressed as mg total polyphenols per gram dry mass (mg PFT g dry mass).

Table I. Description of *S. lycopersicum* L. extracts prepared

Extracts	Description
C Folch	Extracts of Folch mixture and *S. lycopersicum* irrigated without CME
T Folch	Extracts of Folch mixture and *S. lycopersicum* irrigated without CME
C Ethanolic	Ethanolic extracts of *S. lycopersicum* irrigated without CME
T Ethanolic	Ethanolic extracts of *S. lycopersicum* irrigated with treated water with CME
C Aqueous	Aqueous extracts of *S. lycopersicum* irrigated without CME
T Aqueous	Aqueousextracts of *S. lycopersicum* irrigated with treated water with CME

To identify some polyphenols, a high resolution liquid chromatography (HPLC, Shimadzu) was performed with a Teknokroma Tracer Extrasil Cromasil column (C18, ODS2 5 µm). The mobile phase consisted of three eluents: A (water and acetic acid, 99:1, v/v), B (acetonitrile) and C (water, acetonitrile and acetic acid, 67:32:1, v/v/v). The injection volume was 20 µL with a flow of 0,8 mL min^{-1}.

Polyphenols were identified with an ultraviolet wavelength of 330 nm. Chromatographic peaks were confirmed by comparing its retention time (tr) with those obtained in commercial reference standards. Rutin, quercetin and gallic acid polyphenols were identified. All chromatographic operations were performed at room temperature and in triplicate.

Kolmogorov-Smirnov test was used for statistical processing, in order to check delivery pattern. A Single Classification Variance Analysis and the Comparison Test of Multiple Ranges of Fisher’s Significant Minimum Difference (LSD) were performed to determine differences between treatments.

RESULTS AND DISCUSSION

All extracts from fruits that were irrigated with CME treated water showed a higher polyphenol content. Among the six extracts analyzed, aqueous extracts had a higher polyphenol content compared to ethanolic extracts and those elaborated with Folch blend. (Table II).

Table II. Quantification of total depolyphenol content in *S. lycopersicum* L. fruit extracts irrigated with different water treatments

Extracts	Total Polyphenols Content (mg PFT g dry mass)
C Folch	101,233 ± 1,59
T Folch	111,689 ± 0,19
C Ethanolic	118,187 ± 5,19
T Ethanolic	114,776 ± 5,87
C Aqueous	120,976 ± 16,5
T Aqueous	151,623 ± 31,81*

Values represent means ± SD (n= 3)
Symbol (*) in the same column indicates significant statistical differences for the Multiple Range Comparison
Fisher’s Significant Minimum Difference (LSD) p<0.05

In general, aqueous extracts prepared from CME treated fruits had statistically significant differences compared to the other extracts for 95 % confidence. Polyphenols are quite soluble in water; therefore, aqueous solvent enabled a better metabolite extraction. Polyphenol content was 1,25 times higher in aqueous extracts from fruits irrigated with CME at 150 mT than in aqueous extracts from the control treatment. The highest polyphenol values slightly exceeded those reported in other research studies (19, 20, 21). It is stated that *S. lycopersicum* L. contains important amounts of antioxidant compounds with high levels of biological activity (2, 22, 23, 24). It can be considered that when CME treated water reaches *S. lycopersicum* L. plant cells, it modifies cell membrane potential, changing its polarization and permeability.

It has been reported that water magnetic treatment changes its physical-chemical properties, mainly in hydrogen bonds, polarity, surface tension, conductivity, pH and salt solubility (25, 26, 27). All modifications caused by magnetic treatment on water molecules are explained in literature (28, 29). It has been shown that water after passing through CM gets a more homogeneous structure. Consequently, nutrient absorption increases after applying magnetically treated water, as there is a greater access of these elements through cell membrane pores or channels, due to a better ion orientation. Such modifications may alter cell membrane characteristics and cell reproduction, among other processes (30). Thus, it can be considered that CM was able to increase metabolic processes and enzymatic activity of *S. lycopersicum* L. fruits with successive molecular and cell changes, which enabled to improve polyphenols. It has been reported that inductions close to 1T, such as the one in this research, do not have any toxicity to man or the environment (31, 32), so that plants exposed to this physical treatment are harmless.

Several researchers have experienced the action of magnetic and electric fields on polyphenol content of various plant species. *Zea mays* (maize) seedlings increased this group of metabolites when irradiated with CEM during their growth (33). In *Cicer arietinum* L. (chickpea), a favorable effect was obtained in total phenols after irrigating plants with CM (34). Similar results were observed in *Vicia faba* L. (green bean) (35), *Triticum* spp. (wheat), *Pisum sativum* (green pea), *Linum* and *Len culinaris* (lentil) plants (36). Satureja plants exposed to low frequency CEM increased its phenol levels (37). It has been shown that CEM can activate cell and biochemical functions to generate better bioactive compounds (16, 38); therefore, these results are in general confirmed in this investigation.

Rutin, quercetin and gallic acid polyphenols were identified in those six extracts prepared using HPLC (Table III). Despite applying CME, the three polyphenols were present in fruits. It can be considered that the action of this physical agent had no negative impact on the synthesis of these secondary metabolites. Specifically rutin and quercetin are flavonoids, whereas gallic acid is a phenolic acid, which have proved their antioxidant activity in several plants (39, 40). Concerning *S. lycopersicum* L., these polyphenols have a significant contribution to total antioxidant capacity in its fruits and nutritional supply (21, 41, 42).

Several articles report *S. lycopersicum* L. polyphenol benefits to human health (22, 24), which can be favored by applying CM. High concentrations of quercetin, carotenoids and ascorbic acid have been obtained, as well as lycopene by the influence of CEM (43) and CME (44). These results could be related to some reports about the increased antioxidant capacity in this species (45, 46).

This evidence denotes the significance of irrigating *S. lycopersicum* L. plants with CME at 150 mT, in order to enhance polyphenol synthesis. Taking into account these potentialities, there could be a favorable influence on a greater amount of metabolites and, in turn, on their pharmacological action as an antioxidant defense of human organism.

CONCLUSIONS

Polyphenol content in extracts depended on the magnetic treatment and solvent used for the extraction. A magnetic induction of 150 mT in irrigation water can increase 1.25 times polyphenol synthesis in *S. lycopersicum* L. fruits. Rutin, quercetin and gallic acid polyphenols were identified, which proved that CME at 150 mT had no negative influence on these polyphenols. The use of this powerful nutraceutical plant and CME application is supported as an alternative to enhance its polyphenol content.

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Table III. Polyphenols identified in fruit extracts of *S. lycopersicum* L.

Polyphenols	Retention time tr (min)	HPLC – PDA Λ maximum (nm)
Rutin	12,16	228
Quercetin	18,02	290
Gallic acid	2,51	296

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