The effect of citric acid on the phenolic compounds, flavonoids and antioxidant capacity of wheat sprouts

Efecto del ácido cítrico sobre los compuestos fenólicos, los flavonoides y la capacidad antioxidante en brotes de trigo

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Abstract

Wheat sprouts are a source of carbohydrates, fiber, vitamins, essential nutrients and bioactive compounds that have been linked to the prevention and treatment of illnesses. The presence of these compounds in wheat sprouts may be increased by means of the application of organic acids during the growth stage. The objective of this investigation was to evaluate the effect of a sprayed application of citric acid (0, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M) on the total antioxidant capacity and total content of phenolic compounds and flavonoids in wheat sprouts. A completely randomized design was used, consisting of six repetitions. The results showed that phenolic compounds, flavonoids and antioxidant capacity increased significantly with the application of a 10^{-2} M foliar spray as compared to the control. As such, the spraying of low concentrations of secondary metabolism-inducing molecules during the sprout development process is a useful and effective method to stimulate the biosynthesis of bioactive phytochemicals and improve the nutraceutical quality of wheat sprouts.

Keywords
Nutraceutical quality • nutrition • food • germinated

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Resumen

Los brotes o germinados son una fuente de carbohidratos, fibra, vitaminas, nutrimentos esenciales y compuestos bioactivos, los cuales se han relacionado con la prevención y tratamiento de enfermedades. La presencia de estos compuestos, en brotes de trigo, pueden incrementarse mediante la aplicación de ácidos orgánicos durante la etapa de crecimiento. El objetivo de la presente investigación fue evaluar el efecto de la aplicación por aspersión de ácido cítrico (0, 10^{-2}, 10^{-3}, 10^{-4} y 10^{-5} M) en la capacidad antioxidante total, contenido de compuestos fenólicos totales y flavonoides totales en brotes de trigo. Se utilizó un diseño completamente al azar con seis repeticiones. Los resultados mostraron que los compuestos fenólicos, flavonoides y la capacidad antioxidante se incrementaron significativamente con la aspersión foliar 10^{-2} M en relación al testigo. Por lo tanto, la aspersión de bajas concentraciones de moléculas inductoras del metabolismo secundario durante la etapa de desarrollo de brotes es un método útil y efectivo para estimular la biosíntesis de fitoquímicos bioactivos y mejorar calidad nutracéutica de los brotes de trigo.

Palabras clave
calidad nutracéutica • nutrición • alimento • germinados

Introduction

Wheat sprouts are a fresh food product that provides carbohydrates, fiber, vitamins, nutrients and a high content of phytochemical compounds with a bioactive effect and "nutraceutical" qualities, including antibacterial, anti-diabetic, anti-inflammatory, anti-cancer and antioxidant effects (6, 17). Wheat sprouts are widely consumed in countries such as Spain, Japan and the United Kingdom due to their quality and composition; however, they are not commonly consumed as part of the gastronomic culture in developing countries (21). On the other hand, these countries present high rates of malnutrition (11) and chronic degenerative illnesses such as obesity, diabetes and hypertension (18). Due to the above, societies increasingly demand foods that not only satisfy hunger, but that also contain bioactive compounds with antioxidant effects (5, 22). The antioxidant effect of foodstuffs is related to their capacity to slow or prevent the oxidation of biological molecules, preventing their oxidation and reducing aging and the emergence of illnesses (7). In this sense, it is prudent to develop technologies that increase the antioxidant content and overall production of foods, which can be of use to both the food and pharmaceutical industries (8, 9). Several studies have demonstrated that the nutraceutical properties of sprouts of various species can be increased with the application of compounds that function as elicitors, promoting the synthesis of derivative compounds of phenylpropanoids and activating signal cascades that increase antioxidant activity (28). The application of hydrogen peroxide, yeast hull extracts, various abiotic stresses and minerals such as selenium and sulfur are all treatments that have been studied in relation to improving the content of health-promoting compounds (27).

The application of organic acids has also been demonstrated to increase the presence of the compounds in question (30). As the subject of this study, citric acid has been shown to increase the antioxidant content of foods, being that it stimulates greater synthesis of nutraceutical compounds that function as antioxidants, including phenolic compounds, flavonoids and tannins (15, 23). As such, the application of citric acid during the production of wheat sprouts could be a useful tool to increase the synthesis of bioactive compounds. Wheat seeds contain high levels of nutrients and antioxidants (13), presenting moderate levels of phenolic compounds that complement those found in fruits. On the other hand, no studies are currently available that have explored the effect of citric acid applications on the nutraceutical quality of wheat sprouts. As such, the objective of this investigation was to evaluate the effect of a sprayed application of citric acid in varying concentrations on total antioxidant capacity and the total content of phenolic compounds and flavonoids in wheat sprouts.

Materials and methods

The study was carried out in the Biotechnology Laboratory in the Gómez Palacio Polytechnical University, located in in Gómez Palacio, Durango, Mexico. Wheat seeds (*Triticum aestivum L*) were used, with a germination percentage of 95%. Temperature conditions were 15-18°C. Distilled water was used through the study. Four stages can be identified in the process of obtaining sprouts: washing and disinfection, pregermination, germination and sowing, and growth. In the first stage, seeds were submitted to washing through immersion in water containing sodium hypochlorite (NaCIO, 1 mL L^{-1}) for 15 minutes for the purpose of disinfection. Following this, the seeds were rinsed twice in order to remove excess NaClO and drained prior to pregermination.

The pregermination phase consisted of immersing the seed in water for 6 hours (6). Next, the seeds were drained and rinsed prior to germination. The germination stage consisted of placing the pregerminated seeds in 15 x 10 x 5 cm polystyrene foam containers, perforated in the base to allow percolation and aeration. The containers were covered with a number one cloth and placed in darkness. This stage lasted 6 hours. After this time began the growth stage, for which the cloth was removed and the containers were placed on shelves with natural lighting. In this stage the treatments were applied and which lasted 6 days.

A completely randomized experimental design with six repetitions per treatment, consisting of the application of citric acid ($C_6H_8O_7$) in concentrations of 0, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M (1) was used. During the germination and sowing stage irrigation was applied every 3 hours, exclusively with water, through the spraying of 3 ml per application. The application of the treatments took place at the growth stage with the same irrigation dosage. The variables evaluated were total antioxidant capacity (TAC), total phenolic content (TPC), and total flavonoid content (TFC).

Extracts were prepared in order to quantify the variables evaluated in the study, mixing 2 g of fresh sample in 10 ml of ethanol at 80 % in screw-top plastic tubes which were placed in a rotating agitator (ATR Inc., US) for 24 hours at 20 rpm and 5°C. The tubes were then centrifuged at 3000 rpm for 5 minutes, and the overdrain extracted for analysis.

The TAC was determined with the *in vitro* DPPH+ method (2, 3). To carry out the method, a solution of DPPH+ (Aldrich, St. Louis, Missouri, USA) in ethanol was prepared, adjusting the solution absorbance to 1.100 ± 0.010 at a wavelength of 515 nm. A standard curve was obtained with Trolox (Aldrich, St. Louis, Missouri, EU), and the results were reported as equivalent antioxidant capacity in µM equivalent in Trolox per 100 g base fresh weight (M equiv Trolox•100 gm^{-1}FW).

The TPC was determined with a modified Folin-Ciocalteu method (26). To calculate TPC, a calibration curve was created using gallic acid as a standard, and the results were registered as mg of gallic acid equivalent per 100 g base fresh weight (mg GAE/100 g FW). To quantify the concentration, a calibration curve was created using a quercetin standard. The results were expressed as mg quercetin equivalent per 100 g base fresh weight (mg g^{-1} FW).

For the statistical analysis, the SAS software version 9.0 (25) was used. A Tukey test at 0.05 probability was applied for the comparison of averages. Furthermore, the coefficient of determination analysis ($p \leq 0.05$) was made in order to detect association between the evaluated variables.

Results and discussion

Different concentrations of citric acid led significant differences ($p \leq 0.05$) in TAC in the wheat sprouts. A concentration of 10^{-2} M provoked the greatest antioxidant capacity, whereas the lowest value was obtained with the 10^{-4} to 10^{-5} M solution (table 1). The antioxidant capacity of a foodstuff depends on the nature and concentration of the different compounds and the natural antioxidants present within (4). In the case of this study, the antioxidant capacity can be attributed to the action of the citric acid in stimulating the accumulation of antioxidant compounds (16).

On the other hand, Swieca and Baraniak (2014) highlight that greater or lesser antioxidant activity is not always accompanied by the concentration of phenolic compounds. These authors stress that, when capacity for free radical capture is being assessed, TPC is not always as important as the position of the hydroxyl group. Another factor that may increase antioxidant activity in wheat sprouts are the additive and synergetic affects between other phytochemicals and phenolic compounds altered by the elicitation effect of citric acid.

Table 1. Average values obtained in wheat sprouts under different molar concentrations of citric acid.

Tabla 1. Valores promedio obtenidos en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

Citric Acid	TAC	TPC	TFC
M	Meq TOLOX/100 gr PF	mg AGE/100 gr PF	mg QE/100 gr PF
10^{-2}	115.04 a	189.97 a	150.03 a
10^{-3}	105.56 b	154.27 b	138.62 b
10^{-4}	99.50 d	142.70 c	129.84 c
10^{-5}	99.73 d	136.22 d	112.91 d
0	100.93 c	136.33 d	102.17 e

In order to determine the relationship between antioxidant capacity and phenolic compound content, in this study the coefficient of determination analysis was performed (figure 1A, page 124), in which it was shown that there was a strong positive correlation between both variables (r^2=0.96), suggesting that these phenolic compounds are antioxidant in nature. This correlates with other studies in which it was found that the potential health benefits of phenolic compounds can be primarily attributed to their antioxidant activity through the donation of a hydrogen atom from the aromatic hydroxyl group to the free radicals (14).

Regarding the TPC, a significant increase was found that can be attributed to the application of citric acid ($p \leq 0.05$). The 10^{-2} M treatment surpassed the control treatment by 72 % (table 1, page 122).

The lowest concentration of TPC was obtained with the lowest citric acid dosage (10^{-5}) and the control. While there continues to be a deficit of studies into the effect of citric acid application, results can be found that indicate the possibility of obtaining an increase in phenolic compound content due to the effect of treatments that cause the degradation of polyphenols such as tannins to simple phenols (16). It is also known that some phenolic compounds accumulate in cellular vacuoles (29), and that hydrolization by citric acid can liberate these otherwise unavailable phenols.

The increase found as a result of the citric acid application may be attributable to the liberation of phenolic acids following the decomposition of cellular components and walls. This suggests a disassociation of polyphenolic forms due to citric acid hydrolization, possibly followed by aglycosylation, polymerization or oxidation of the phenolic components (30), which was responsible for the increase in total phenolic compound content found in this study. Similarly, the coefficient of determination was made between the TPC and that of the flavonoids (figure 2, page 125), as these were the principal phenolic compounds identified and reported in the sprouts (24). The results showed a positive correlation between the TPC and the TFC (r^2=0.92), demonstrating that the flavonoids belong to the polyphenolic group (14).

TFC found in the wheat sprouts was significantly affected by the different concentrations of citric acid ($p \leq 0.05$). The values obtained fluctuated between 102 and 150 mg of quercetin (QE) / 100 g fresh weight (table 1, page 122). The greatest flavonoid content was found in the wheat sprouts that were sprayed with a 10^{-2} concentration of citric acid, compared to the control in which a value 68 % smaller was obtained.

Previous studies indicate that application of citric acid increases the synthesis of secondary metabolites such as flavonoids (19), which correlates with the results of the present study, in which the citric acid application considerably increased the presence of these metabolites. It is possible that a synergistic reaction occurred with the application of the acid, which activated the transduction pathways that conduct the secondary signals produced by plants (20).

Several investigations have shown that flavonoids are implicated in the prevention of chronic non-transmissible diseases due to their antioxidant activity (10), which agrees with the correlation obtained in the current study (figure 1B, page 124) between antioxidant capacity and flavonoid content (r^2=0.97). This suggests that flavonoids may be implicated in the prevention of chronic non-transmissible diseases as a result of their antioxidant activity (12).

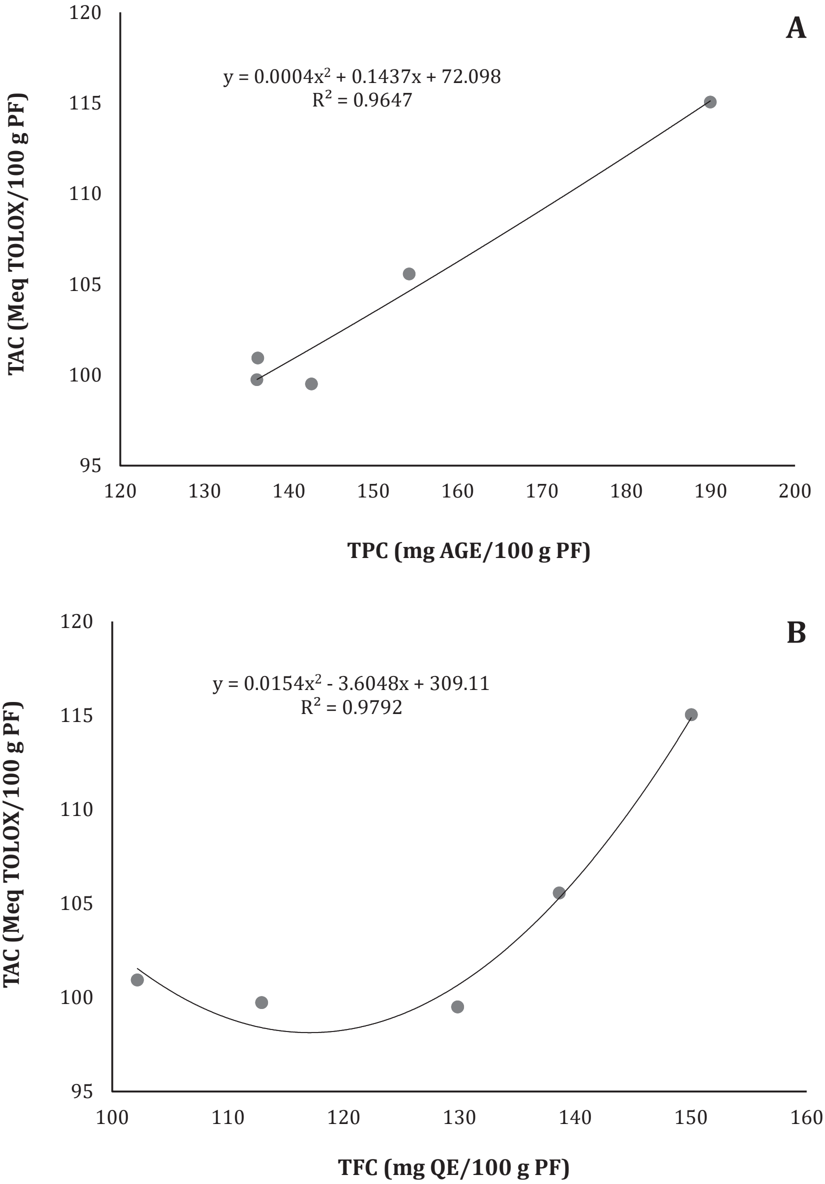

Figure 1. A) Pearson correlation (r^2) between total antioxidant capacity (TAC) with total phenolic content (TPC) and B) total antioxidant capacity (TAC) with total flavonoid content (TFC) in wheat sprouts under different concentrations of citric acid.

Figura 1. A) Coeficientes de determinación (r^2) entre la capacidad antioxidante total (CAT) con los compuestos fenólicos totales (CFT), y B) capacidad antioxidante total (CAT) con el contenido de flavonoides totales (FT) en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

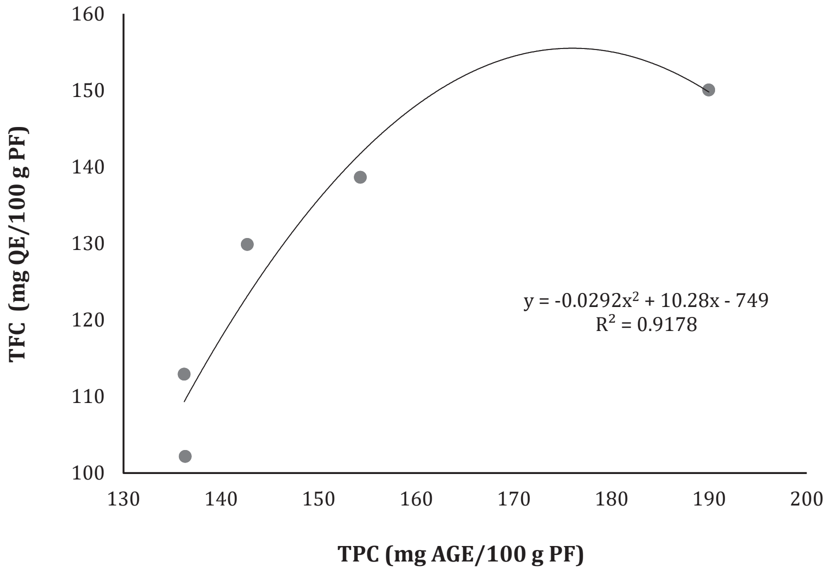

Figure 2. Pearson correlation (r^2) between total phenolic content (TPC) and total flavonoids (TFC) in wheat sprouts under different concentrations of citric acid.

Figura 2. Coeficientes de determinación (r^2) entre el contenido de compuestos fenólicos totales (CFT) y flavonoides totales (FT) en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

The results suggest a positive increase in each variable evaluated by the application of citric acid. However, it is not conclusive that the content of phenolic compounds, flavonoids and the subsequent antioxidant capacity in the evaluated germinates can always be linear with the increase in the application of citric acid.

Therefore, it could be interesting to investigate the range of application of this acid to determine if there is a quadratic response and to relate the molar concentration of citric acid and the production of phenolic compounds and total flavonoids. Likewise, it remains to be resolved if the increase in the concentration of citric acid modifies other quality variables and the development of the sprouts.

Conclusions

The total phenolic and flavonoid content of the wheat sprouts increased significantly as a result of the 10^{-2} M citric acid solution treatment, by 205 % and 184%, respectively, while the TAC increased by 123 %.

As such, citric acid favors the biosynthesis of phytochemical compounds in wheat sprouts when sprayed during the growth stage.

However, it could be interesting to increase the range of application of this acid to determine if there is a quadratic response and to relate the molar concentration of citric acid and the production of phenolic compounds and total flavonoids.

These results indicate that the application of low concentrations of secondary metabolism-inducing molecules is a useful and effective means of stimulating the biosynthesis of bioactive phytochemicals and improving the antioxidant capacity of wheat sprouts, opening up the possibilities of using this ingredient for consumption in functional foodstuffs.

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