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RESEARCH ARTICLE

Ultrasonic extraction increases the extraction of antioxidant and citrulline in damaged watermelons in the Comarca Lagunera, México

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ABSTRACT

In this study, the content of lycopene, total phenolics, total flavonoids, antioxidant activity and citrulline in watermelon from different localities of the Comarca Lagunera were determined. In the Comarca Lagunera, watermelon is the second most important crop, representing 9% of the cultivated area at the national level, with a production value of $9,000,000. Watermelon is known to contain high contents of flavonoids and phenolic compounds, which have antioxidant and anti-inflammatory properties; these compounds include metabolites such as lycopene and citrulline. The determination of these components may vary according to the method of cultivation, fertilization, irrigation, and location, as well as the extraction method used. A completely randomized experimental design was used, analyzing three repetitions for each sample obtained. The data obtained in the present study varied according to the method of extraction, for lycopene (41.22 - 8.48 mg g^{-1} FW), citrulline (161.44 - 71.11 mg g^{-1} FW), flavonoids (5.05 - 0.17 mg g^{-1} FW) and phenolics (10.3 - 0.89 mg g^{-1} FW). The results indicated that there were no significant differences ($p \leq 0.05$) for the effect of the variables degree of damage and locality, but there were significant differences for the extraction methods. Therefore, the ultrasound extraction method could be an alternative for the industry due to the increase of active biocompounds that can be obtained, reducing the time of obtaining, the low process costs and the contribution to a sustainable development.

Keywords: Flavonoids; Lycopene; Phenolics; Watermelon defects.

INTRODUCTION

A problem that characterizes some regions of Mexico is the temporary oscillation of prices, due to periods of excess supply, this variation causes a contraction in the price received by the producer due to market saturation (Ramírez-Barraza et al., 2015). In the Comarca Lagunera, located in Mexico in different municipalities of the states Durango and Coahuila, this problem occurs specifically in the watermelon crop, since, at certain times of the year, the market is saturated to such a degree that the price is so low that the producer loses less by leaving the product in the field than by harvesting it, which brings serious economic losses, those prices oscillate between 0.50 cents to 1.00 peso in national coin per piece of frut while in supermarket is sold for a price 8 times more than the price achieved with the producer (SNIIM, 2014). In the Comarca Lagunera, watermelon is the second most important crop, representing 9% of the cultivated area at the national level, with a production value of $9,000,000 (SIAP-SAGARPA, 2016). Watermelon production starts in May and ends in October; in the months of June, July, August and September there are oversupplies since 80% of production is obtained in these months, this causes low prices at the wholesale level affecting the producer's profit (Ramírez-Barraza et al., 2015). One of the measures proposed to avoid the seasonal drop in prices and therefore economic losses for producers has been to alternate with other crops during the course of the year but most of them do not have the economic resources to produce after the loss due to the cultivation of watermelon. (García et al., 2011). Another alternative is to take advantage of watermelon

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production and/or leftovers by extracting compounds such as carotenoids and amino acids, including lycopene and citrulline, respectively (Maroto et al., 2002). Watermelon can be a valuable source of these compounds and can be utilized at any post-harvest time. Despite bruised, deformed or discolored exteriors, external appearance has no effect on nutrient content (Al-Sayed and Ahmend, 2013). This vegetable is the fruit that contains the highest amount of water (93%), so its caloric value is very low, just 20 calories in 100 grams. The levels of vitamins and mineral salts are not very relevant, with potassium and magnesium being the most important, although in lower amounts compared to other fruits (Akashi et al., 2001). Its carotenoids and amino acids content is possibly what gives it added value not only for fresh consumption, but also using in functional food and health industry (Zhao et al., 2020). The red color of its pulp is due to the presence of the pigment lycopene (Cruz-Bojórquez et al., 2013). Lycopene belongs to the family of carotenoids such as beta-carotene, a substance that is not synthesized by the human body, but vegetables and some microorganisms do it (García-Layana et al., 2007). Lycopene has antioxidant properties and acts to protect human cells from oxidative stress, produced by the action of free radicals, which are one of the main causes of cardiovascular diseases, cancer and aging (Waliszewski and Blasco, 2010). On the other hand, watermelon is the richest known source of citrulline, a non-essential amino acid first identified from watermelon rind. Citrulline is used in the synthesis of nitric oxide in humans and has antioxidant and vasodilatory functions. In addition, citrulline is used in dietary supplements to improve sexual stamina and erectile function (Durán et al., 2017). In addition, watermelon contains high content of flavonoids and phenolic compounds, which have antioxidant and anti-inflammatory properties (Kim et al., 2014a). The content of lycopene, citrulline, and flavonoids in watermelon can vary according to the cultivation method, fertilization, irrigation, and location, as well as the extraction method used (Choudhary et al., 2015). On the other hand, the extraction of these compounds can lead to the creation of business models oriented to the agri-food industry in watermelon producing areas (Rico et al., 2020). In this regard, there are extraction processes that can vary in the yield obtained, economic profitability and environmental viability (Padma and Srivastava, 2010). Ultrasound extraction improves the extraction efficiency of bioactive compounds, due to the effect of cell alteration and cavitation that favor solute/solvent contact (Benavides et al., 2020), in addition to improving extraction efficiency by shortening the time and reducing the volume of solvents needed (Vankar and Srivastava, 2010) all of these features are advantages over the traditional solvent extraction method. In this context, the objective of the research was to compare the best extraction method (traditional and ultrasonic) for different bioactive compouds (lycopene, total phenolics, total flavonoids, antioxidant activity and citrulline) in damage watermelon from five different localities of the Comarca Lagunera.

MATERIALS AND METHODS

Study area

The study was carried out in the municipalities of the Comarca Lagunera in Durango (Gomez Palacio, Bermejillo, San Pedro del Gallo, Mapimi and Tlahualilo) located at coordinates 25 33' 00" and 25 32' 27" north latitude and 103 18' 27" and 103 40' 30" west longitude, at an altitude of 1,150 meters above sea level, with a very dry or steppe climate. The average annual temperature is 20ºC, with an annual rainfall of 200 millimeters. The soil composition corresponds to the xerosol type. The study was carried out in september 2019.

Sample collection and characteristics

Watermelon samples of the Charleston variety grown in the Laguna Region of Durango were collected from the municipalities of Gómez Palacio (M1), Bermejillo (M2), San Pedro del Gallo (M3), Mapimí (M4) and Tlahualilo (M5) in the spring - summer cycle. Samples were taken after the harvest (10 days after) according to the degree of damage corresponding to the classification reported by Valdiviezo (2010) in rejection watermelon. Where number 1 represents post-harvest rejection fruit with minimal physiological damage and number 4 represents the most damaged fruit according to the physiopathologies presented in Table 1. For this study there were collected samples with the four different damage in the five different places mentioned previously (Figs. 1-4).

Sampling and transfer of samples

Using simple random sampling, 6 watermelon samples of each of the types established in the previously mentioned classification were taken and transferred to the physicochemical analysis laboratory of the Universidad Politécnica de Gómez Palacio, Durango.

Sample preparation

The rind was separated from the fruit and then cut into 2 cm cubes, separating the seeds from the fruit. The peel was cut into 1 cm cubes. The pulp and peel were liquefied

Table 1: Damage to reject watermelon

Category	Defects
1	Flattening [Fig. 1]
2	Flattening and cracking [Fig. 2]
3	Cracking type 1 [Fig. 3]
4	Cracking type 2 [Fig. 4]

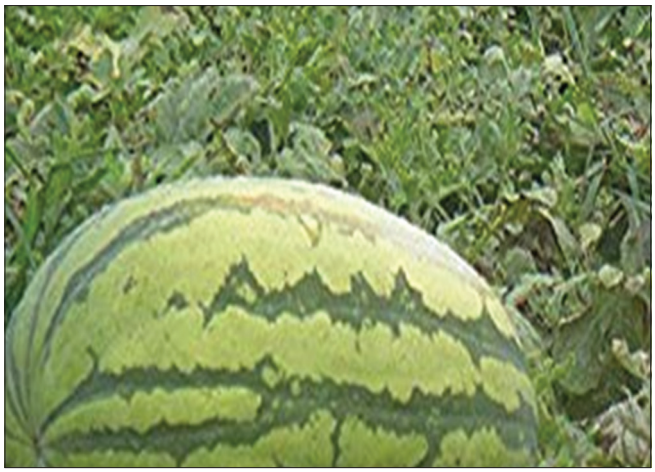

Fig 1. Fruit flattening.

Fig 2. Flattening and cracking.

Fig 3. Type 1 crackin.

Fig 4. Type 2 cracking.

separately and the sample was refined using a strainer with an opening diameter of 3.0 mm. The pulp was used for the determination of lycopene, total flavonoids and total phenolic compounds. The peel was used to determine citrulline content. Both watermelon pulp and rind were subjected to two extraction methods: The traditional method (TM) with 80% ethanol and the ultrasonic extraction method (UM).

Extraction methods for lycopene and citrulline

TM. Two grams of sample was weighed into a test tube, adding 10 ml of 80% ethanol at boiling temperature. The mixture was shaken for 25 min until homogenization. It was left to stand for 24 hours and 2 phases were formed, the upper organic phase and the lower one. Subsequently, the organic solvent solution used was recovered and cooled to obtain lycopene crystals; the same procedure was carried out with the peel to obtain citrulline. The solutions were filtered to recover the compounds. The extracts obtained were heated to evaporate the solvent and stored in amber-colored vials for later analysis (Valdiviezo, 2010).

UM. Two grams of sample was weighed into a test tube by adding 10 ml of 80% ethanol. The mixture was shaken for 25 min until it was homogenized, then it was introduced in the ultrasonication equipment for 10 min with 50% adjustment of the sound power to 80 W with a frequency range of 20 KHz (Ultrasonics, ultrasonic processor 2100®). After being subjected to this process, the oleaginous phase of the pulp was extracted and stored in amber vials for analysis. The above procedure was repeated with watermelon rinds for citrulline determination (Vazquez et al., 2020).

Extraction method for flavonoids, total phenolics and antioxidant capacity

TM. To obtain extracts, 2 g of fresh sample were mixed in 10 ml of 80% ethanol in screw-capped plastic tubes, which were placed in rotary shaker (ATR Inc., USA) for 24 h at 20 rpm at 5 °C, this methodology was adequate based on the method used by Chen and Chang (2015). The tubes were then centrifuged at 3000 rpm for 5 min, and the supernatant was extracted for analysis.

UM. Two g of sample was weighed into a test tube by adding 10 ml 80% ethanol. The mixture was shaken for 25 min until homogenized, then it was introduced in the ultrasonication equipment for 10 min with 50% adjustment of the sound power to 80 W with a frequency range of 20 KHz (Ultrasonics, ultrasonic processor 2100®). After being subjected to this process, they were stored in amber colored vials for analysis (Chen and Chang 2015).

Determination of lycopene content

To the extracts obtained, a solution of tetrahydrofuran and methanol (1:1 v/v THF: MeOH) was added and the suspension was filtered under vacuum. The filtrate was transferred to a separatory funnel and petroleum ether and 10% NaCl solution were added, then mixed with careful stirring. The top layer of petroleum ether was washed with 100 mL of water. The ether fraction was transferred to a 50 mL flask and evaporated to dryness in a Napco brand vacuum oven for 12-14 h at a pressure of 60 mm Hg and at 50 °C. The residue was redissolved to a final volume of 6 mL with hexane. It was filtered and analyzed by high performance liquid chromatography (HPLC) on an Agilent 1100 Series chromatograph, on which a C18 Supelco Discovery column (15 cm x 4.6 mm and 5 μm) was installed in reverse phase. An isocratic mobile phase system consisting of acetonitrile: methanol: 2 propanol (38:60:2 v/v/v) was used. The flow rate was 1 mL/min and 20 μL of the sample was injected. Lycopene was quantified at a wavelength of 470 nm. Lycopene identification was based on the retention time of the Sigma brand lycopene standard (Candelas et al., 2006). The concentration of the standard was 50 μg/mL. The whole process was carried out under reduced light. The calculation of lycopene concentration was performed based on the ratio between the known concentration of the standard and the area of the corresponding peak and reported as mg of lycopene per gram of fresh weight (mg g^{-1} FW).

Determination of citrulline content

The citrulline extract was neutralized at pH 7.0 with dilute NaOH, then filtered through Whatman No. 1 paper. After filtration, the residue was washed with water and concentrated to 5 ml under vacuum. This material was filtered through a 0.22 μm membrane and injected into the HPLC. Extraction and analysis of all samples were performed in triplicate.

Watermelon samples (5 μl) were injected to the HPLC (Ridwan et al., 2018). The concentration of L-citrulline was calculated on the basis of linear calibration functions and with respect to the dilution factor. The L-citrulline content was expressed in milligrams per gram of watermelon sample. HPLC separation was carried out at a flow rate of 0.5 ml/min using a mobile phase of (A) 3 mM phosphoric acid, (B) acetonitrile (Rimando and Perkins, 2005). Detection was carried out using 263 nm wavelength, 70-0% A gradient elution and results were reported as mg citrulline per g fresh weight (mg g^{-1} FP).

Determination of total phenolic compounds

Total phenolic content was measured using a modification of the Folin-Ciocalteu method (Singleton et al., 1999). 30 μl of extract was mixed with 270 μl of distilled water in a test tube, then 1.5 ml of diluted (1:15) Folin-Ciocalteu's reagent (Sigma-Aldrich, St Louis MO, USA) was added and vortexed for 10 s. After 5 min, 1.2 ml of sodium carbonate (7.5% w/v) was added and stirred for 10 s. The solution was placed in a water bath at 45 °C for 15 min, and then allowed to cool to room temperature. The absorbance of the solution was read at 765 nm in a UV spectrophotometer (Genesys 10®). To calculate the phenolic content, a calibration curve was performed using gallic acid as a standard with a linear regression value of $R^2 = 0.995$ ($y = 0.0013x + 0.004$), and the results were recorded in mg of gallic acid equivalent per g on a fresh weight basis (mg g^{-1} FP).

Colorimetric measurement of total flavonoids

To determine the total flavonoid content, the technique described by Lamaison and Carnet (1990) was used, taking 250 μL of the ethanolic extract supernatant and then adding 1.25 mL of distilled water and 75 μL of 5% NaNO2, vortexing the mixture and allowing it to react for 5 min. Subsequently, 150 μL of 10% AlCl-H2O was added, vortexing the mixture and allowing it to react for 6 min. Then 500 μL NaOH 1M and 275 μL water were added, vortexing. The absorbance was read in a UV spectrophotometer (Genesys 10) at a wavelength of 510 nm. For concentration quantification, a standard curve (y= 0.0122x-0.0067; R2= 0.9653) prepared with quercetin was performed. Results were expressed as mg quercetin equivalents per 100 g on a fresh weight basis (mg g^{-1} FW).

Determination of total antioxidant capacity

Antioxidant capacity was determined by the in vitro DPPH+ method (Brand-Williams et al., 1995). For this, a solution of DPPH+ (Aldrich, St. Louis, Missouri, USA) in ethanol was prepared, adjusting the absorbance of the solution to 1 100 ± 0.010 at a wavelength of 515 nm. For the determination of antioxidant capacity, 50 μl of sample and 1950 μl of DPPH+ solution were mixed, and after 30 min of reaction the absorbance of the mixture was read at 517 nm in a UV spectrophotometer (Genesys 10). The readings were taken in triplicate and ethanol was used as a blank. A standard curve ($y = -0.0007x + 0.7328$; $R^2 = 0.992$) was prepared with Trolox (Aldrich, St. Louis, Missouri, USA), and the results are reported as antioxidant capacity in μM Trolox equivalent per g on fresh weight basis (μM equiv Trolox g^{-1} FW).

Statistical analysis

Results were analyzed by analysis of variance in SAS statistical software (1999), the experimental design was completely randomized with a factorial arregement with three replications. The results were evaluated according to data for location and extraction method. The comparisons between means were made by Tukey's test with a $p \leq 0.05$ significance.

RESULTS

The compounds evaluated in the present work are among the compounds known as functional and originated from the secondary metabolism of plants. Their utilization from watermelon that does not reach the market can be an alternative for producers. According to Choudhary et al. (2015) the results of this work agree with the results obtained by the author, due to this it is deduced that the degree of damage of the fruit has no effect on the phytochemical content of the fruit; since the results obtained in the different analyses performed on the fruits with damage or already in waste classification showed similar concentrations in terms of total phenolic compounds, flavonoids and lycopene. The results differences between localities in each studied municipality showed no significant differences, being possibly the similar edaphological, hydrological and management conditions in each municipality, which affect the content of functional compounds. On the other hand, for extraction method, there were highly significant differences ($p \leq 0.05$) for the evaluated variables.

Lycopene

Lycopene content varied both between municipality and extraction method ($p \leq 0.05$). Lycopene levels in the samples taken from the different municipalities presented highly significant differences being the municipality of Tlahualilo with the highest most significant value of 41.22 and 23.5 mg g^{-1} FW (ultrasound method and traditional method, respectively). The municipality of Tlahualilo was followed with similar values by the municipality of Gómez Palacio (28.38 and 13.39 mg g^{-1} FW) and San Pedro del Gallo (27.46 and 11.91 mg g^{-1} FW). Finally, the municipalities of Bermejillo (19.18 and 9.19 mg g^{-1} FW) and Mapimí (19.53 and 8.48 mg g^{-1} FW) resulted with less significant difference the lowest values of lycopene content. In all cases, using the ultrasonic method almost double quantity of lycopene was obtained than using the traditional solvent extraction method (Fig. 5).

Citrulline

Citrulline content varied among municipalities (Fig. 6). The levels of citrulline in the samples taken from the different municipalities showed highly significant differences, with the municipality of San Pedro del Gallo having the highest value (169.88 and 98.37 mg g^{-1} FW, using the ultrasound and traditional methods, respectively). It was followed with similar values by the municipality of Mapimí (161.44 and 83.38 mg g^{-1} FW) and Tlahualilo (154.77 and 79.85 mg g^{-1} FW). Finally, the municipalities of Gómez Palacio (125.21 and 88.71 mg g^{-1} FW) and Bermejillo (105.12 and 79.11 mg g^{-1} FW) had the lowest values of citrulline content. According to the extraction method, there were highly significant differences, obtaining up to 72% more citrulline in the highest sample.

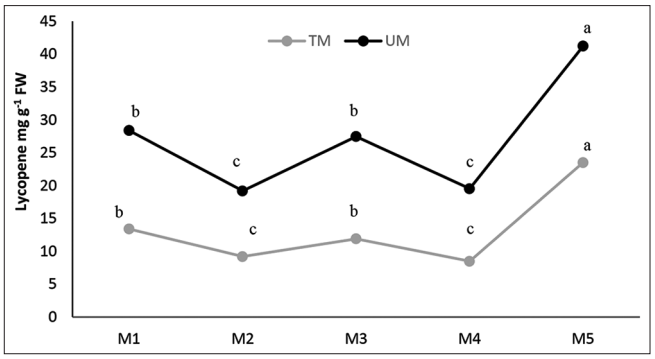

Fig 5. Lycopene content in watermelon from different municipalities of the Comarca Lagunera of Durango, using different extraction methods.

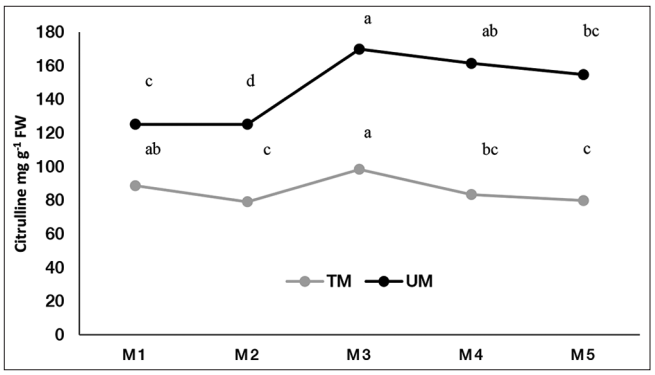

Fig 6. Citrulline content in watermelon from different municipalities of the Comarca Lagunera of Durango, using different extraction methods.

Total phenolics

The content of total phenolic compounds varied both between municipality and extraction method ($p \leq 0.05$). The levels of phenolics in the samples taken from the different municipalities presented highly significant differences with the municipality of Mapimí having the highest value with 10.3 and 6.4 mg g^{-1} FW (ultrasound method and traditional method, respectively). The municipality of Mapimí was followed with significant differences by the municipalities of Tlahualilo (6.34 and 4.25 mg g^{-1} FW), San Pedro del Gallo (3.74 and

2.53 mg g^{-1} FW), Gómez Palacio (2.43 and 1.04 mg g^{-1} FW) and Bermejillo (1.98 and 0.89 mg g^{-1} FW). In all cases, using the ultrasonic method, a higher content of phenolic compounds was obtained (Fig. 7).

Total flavonoids

The content of total flavonoids varied both between municipalities and between extraction methods ($p \leq 0.05$). The levels of flavonoids in the samples taken from the different municipalities presented highly significant differences with the municipality of Mapimí which having the highest value with 5.05 and 2.92 mg g^{-1} FW (ultrasound method and traditional method, respectively). The municipality of Mapimí was followed with significant differences by the municipalities of Tlahualilo (3.80 and 1.67 mg g^{-1} FW), San Pedro del Gallo (1.94 and 0.95 mg g^{-1} FW), Gómez Palacio (2.30 and 0.67 mg g^{-1} FW) and Bermejillo (1.13 and 0.17 mg g^{-1} FW). In all cases, using the ultrasonic method, a higher content of total flavonoids was obtained (Fig. 8).

Antioxidant capacity

It has been reported that the levels of health-promoting bioactive compounds and antioxidant activity of fruits and vegetables are strongly influenced by genotype differences and external factors such as agro-technical processes, environmental conditions, ripening stage, and harvest and postharvest manipulations (Tlili et al., 2011). It has been reported that the protective and functional effects in watermelon are highly correlated with the presence of phytochemicals such as carotenoids and phenolic acids that have antioxidant and anti-inflammatory properties (Kim et al., 2014a) which coincides with the results acquired in the determination of antioxidant capacity in this study.

According to the data obtained, there were no differences between the municipalities studied, but there were differences between the extraction methods used. The ultrasound method presented 94.39 µM equiv Trolox g^{-1} FW and the traditional method obtained 63.40 µM equiv Trolox g^{-1} FW, expressing a difference of 33% (Fig. 9).

The content of phytochemical compounds in watermelon is higher when the extraction method is by ultrasound than when it is extracted by the traditional method. There are also variations among the municipalities studied according to the standard deviation values (Table 2).

DISCUSSION

The results by extraction method were significant with the use of ultrasound obtaining the highest content of bioactive compounds such as lycopene, citrulline, total phenolic compounds and total flavonoids.

Azuola and Vargas (2007) found that ultrasound extraction has higher efficiency compared to other extraction methods, up to 70 times higher than solvent extraction,

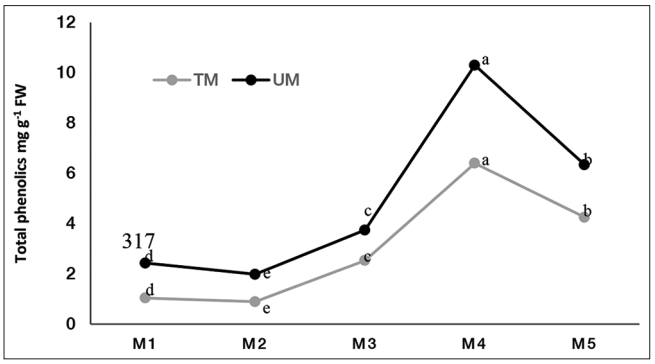

Fig 7. Total phenolics content in watermelon from different municipalities of the Comarca Lagunera of Durango, using different extraction methods.

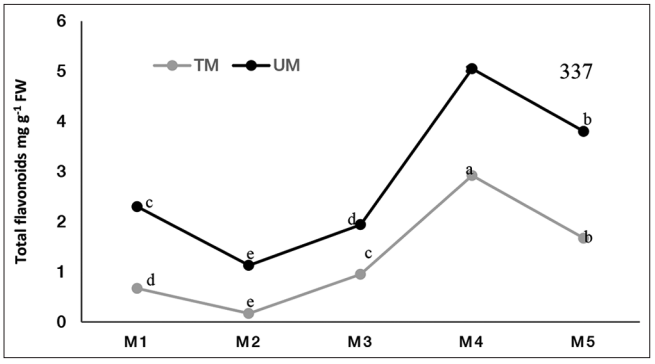

Fig 8. Total flavonoids content in watermelon from different municipalities of the Comarca Lagunera of Durango, using different extraction methods.

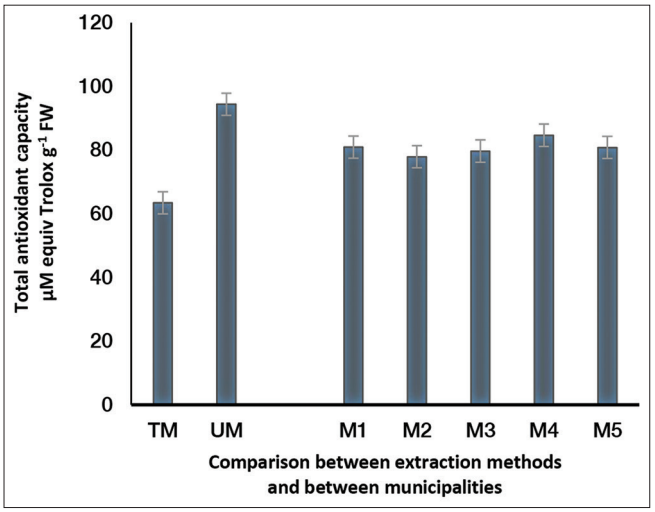

Fig 9. Mean values and error standard of total antioxidant capacity of watermelon determined with two extraction methods and different sampling municipalities.

Table 2: Concentration for lycopene, citrulline, phenolics and total flavonoids content obtained by two extraction methods in watermelon collected in different localities.

Extraction method	LC (mg g^{-1})	CT (mg g^{-1})	TPH (mg g^{-1})	TFL (mg g^{-1})
TM	8.06[b]	8.26[b]	1.36[b]	1.0[b]
UM	11.40[a]	37.40[a]	3.47[a]	1.51[a]
Municipality				
M1	8.6[b]	4.80[c]	0.76[c]	1.16[a]
M2	5.7[c]	8.87[c]	0.25[c]	0.63[b]
M3	15.50[a]	47.06[a]	0.36[c]	0.71[b]
M4	3.90[c]	45.68[a]	4.00[a]	1.23[a]
M5	9.90[b]	34.13[b]	1.20[b]	1.18[a]

(a) LC: Lycopene; CT: Citruline; TPH: Total phenolics; TFL: Total flavonoids with statistical significance of $\alpha = 0.05$
(b) Different letter in the same column indicate significant difference between the different extraction method and localities.

11 times higher than distillation and 35 times more efficient than soxhlet extraction. Malviya (2014), reported 3.18 mg 100 g^{-1} of lycopene in watermelon using a traditional method with benzene.

Durán et al. (2017) found the highest citrulline yield by solvent extraction to be 14.7 mg g^{-1} citrulline. Similar results were cited by Jayaprakasha et al. (2011) with red-fleshed watermelon rind (13.95 - 20.84 mg g^{-1}) employing methanol as solvent in a mixing and sonification process. In another study, Rimando and Perkins (2005) obtained watermelon rind citrulline yields of 15.6 mg g^{-1}, using ultrasonication with N2 bubbling and methanol as solvent; this results due to the efficiency of the method where they are using sound waves capable of breaking the molecular structure of the samples and making the bioactive compounds of interest more available.

The results of the experiment show agreement with those obtained by Rojas et al. (2014) who determined phenolic compounds in blackberry, having the best results in the extraction of these compounds in their ultrasound-assisted trials, obtaining 30 mg g^{-1} in dry weight, demonstrating the efficiency of sonicator-assisted extraction. The same was demonstrated by Corona et al. (2016), where the ultrasound-assisted technique proved to be the most reliable method for the extraction of phenolic compounds in chia.

Total flavonoids have a beneficial effect on humans, so the determination of the nature of total flavonoid content in the material has a great effect on the future of the food industry (Perez and Fraga, 2018); the method for the determination of total flavonoids in watermelon by spectrophotometry is relatively simple, the reagents are easy to obtain, the requirements for environmental factors are low, and it is widely used in experiments (Wei et al., 2019). In agreement with Kim et al. (2014b), they found values of 14.1 mg 100 g^{-1} in flavonoid content in watermelon juice and 5.4 mg 100 g^{-1} in lycopene extracts; these results are similar to those obtained in this study with values of 1.0 - 1.5 mg g^{-1} in traditional extracts and by ultrasonication, respectively. It should also be noted that studies have been developed in which the flavonoid content is low compared to other published results such as those found in the experiment conducted by Ogbuji et al. (2012) where the values in these compounds vary between 171.7 - 905.7 μg g^{-1} where they conclude that the values so different from each other is given by the variety of watermelon analyzed. As cited, there are different reasons why the concentration of antioxidant compounds may vary, all phenotypic-chemical variation is attributable to the effects of genes, environment and the interaction of these, and this extends to phenotypic variation with ontogeny and phenology of the plant (Moore et al., 2014) due to this the results obtained for flavonoids in the samples of this study showed different values between them (Table 2).

All the compounds mentioned above are part of the group of secondary metabolites that give rise to the total antioxidant activity that a plant or fruit possesses; in the study by Choo and Sin (2012) established that the antioxidant activity of watermelon had values between 112 and 165 mg ml^{-1} these results are higher than those found in this study; however Adetutu et al. (2015) found values of 0.1 mg ml^{-1} which are representatively low. The results in the present study are between 70- 90 mg g^{-1} in watermelon FW closer to the results of Choo and Sin (2012); it is worth mentioning that as mentioned above the variation of these components is not predictable due to the particular characteristics of each sample analyzed.

For the results of this study, the effects generated by the ultrasound could cause the rupture of the pores of the cell wall, which improves the diffusion process, the rupture of the particles and the mass transfer through the membranes. With the rupture of the particles, the contact surface between the solid phase and the solvent is increased (Ji et al., 2019). By breaking the cell wall, ultrasonic cavitation effect allows the entry of the solvent into the inert areas of the plant material, which increases the yield of the extracts and the reduction of the time required for extraction (Rodríguez et al., 2014).

According to García et al. (2010) ultrasonic radiation accelerates several steps of the analytical process; this method helps in the pretreatment of solid samples, as it facilitates, the extraction of organic and inorganic compounds, homogenization and others. Ultrasound-assisted leaching is an effective way to extract analytes from different matrices in shorter times than with other extraction techniques (Dobiáš et al., 2010).

The use of ultrasound can improve existing extraction processes and enable new opportunities and commercial extraction processes; the main focus has been on obtaining polyphenols and carotenoids in aqueous and solvent extraction systems, such ultrasound extractions have shown improvements in extraction yields ranging from 6 to 35% (Vilkhu et al., 2008).

CONCLUSIONS

The results indicated a significant difference between extraction methods, with the use of ultrasound yielding the highest content of bioactive compounds such as lycopene, total phenolics, total flavonoids and citrulline. According to the municipality, the results indicated a significant difference for each variable evaluated but not for antioxidant capacity. Total antioxidant capacity was different only for the extraction methods used, but not for the municipality of collection.

Ultrasonic extraction in food processing technology is of interest to facilitate the extraction of components from plant materials. The higher yield achieved in these processes is of great importance from an industrial point of view, as the technology is an additional step to the process already in use with minimal disturbance, where the process can be performed in aqueous extracts and organic solvents can be replaced by solvents generally recognized as safe, in addition to reducing extraction time. The use of ultrasonic means for extraction purposes in high cost raw materials is an economical alternative to traditional extraction processes, being a demand from the industry for a sustainable development.

Author Contributions

Mercedes Georgina Ramírez Aragón: wrote the paper and performed HPLC analysis; Raúl Antonio Alvarado Arroyo and Tania Lizzeth Guzmán Silos: performed the critical reading of the manuscript; María del Rosario Moncayo Luján and Tania Breshkovskaya Ortiz Escobar: performed extract preparation and spectrophotometric analysis; Victoria Jared Borroel García: analysis and interpretation of results; José Luis García Hernández: defined the conception and the design of the research study and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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