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MICROBIOLOGY

Obtaining polyphenolic extracts from pomegranate peel (*Punica granatum*) to evaluate the bactericide and antioxidant activity

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Abstract: Pomegranate (*Punica granatum*) contains secondary metabolites with antioxidant and bactericide activity; however, the study of the peel in the endemic varieties of Mexico has not been deepened. The polyphenols extraction of peel pomegranate endemic to the state of Michoacan, Mexico could be used in the formlulation of healthy food due contains antioxidant compounds or could be used like drugs due contains antibactericide compunds.In this work 3 varieties of pomegranate were analyzed; *Wonderful, Apaseo* and *Tecozautla* harvested in 2017 and 2018, carrying out a physicochemical characterization to establish the ripening, application of an experimental design of response surface for drying the peel and extracting polyphenols using two solvents (acetone and ethanol) by the Soxhlet method. As a result, the pomegranates were in the correct ripening, in the drying an optimal point of operation was found without affecting the metabolites (36 h at 55 °C) and in the extraction, the bactericide and antioxidant activity was evaluated observing that in the ketone extracts the best results were obtained in the *Apaseo* variety being; ABTS$^{\cdot+}$ technique of 150.78 ET mM/g, DPPH$^{\cdot}$ 109.8 ET mM/g and 11.82 EAG mg/g in dry extract. For the bactericide activity measured by inhibition halos in *S. aureus* and *E. coli* it was had; 20.03 mm and 14.05 mm respectively for the *Apaseo* variety, which is why it is convenient to extract polyphenols under this method in peel of Mexican pomegranate varieties.

Key words: poliphenols, extraction, Soxhlet, peel pomegranate.

INTRODUCTION

Pomegranate (*Punica granatum*) belongs to the *Punicaceae* family and is a plant native to Iran 'Sarkhosh et al. 2006'. The pomegranate is considered a shrub, which has abundant foliage, with a thin trunk, crooked and slightly spiny branches reaching a height around 5 m, which generally adapts to Mediterranean climates. The leaves are green, elongated, with a smooth and shiny surface, slightly wavy. The flower is flared and consists of 5 to 8 bright orange petals 'Al-Maiman & Ahmad 2002. Chemically, pomegranate is made up of chemical compounds that result from its metabolism, either primary or secondary, receiving the name of metabolites. A primary metabolite is one that a plant synthesizes and uses to develop, grow and reproduce; whose molecular weight is high 'Díaz 2009'. Among the primary metabolites are: carbohydrates, proteins, lipids, nucleic acids, vitamins and structural polymers; Like cellulose. On the other hand, a secondary metabolite usually has a low molecular weight and participates in the process of adaptation of the plant, as well as in the symbiosis with other organisms

and in the attraction of pollinating insects for the dispersion of seeds and fruits 'Sepúlveda-Jiménez 2003. Secondary metabolites are considered as defense compounds and among them are polyphenols, terpenes, alkaloids, latex, gums and waxes 'Vilela et al. 2011; however, because chemical analyzes in foods are usually based on primary metabolites, due to their high nutritional value, secondary metabolites are used to make functional biotechnological products 'Chirinos et al. 2007'. Chemical analyzes of the fruit of the pomegranate have been reported by dividing them into three fractions, characterizing the compounds present in the shell, seed and arils. Of the total weight of the pomegranate fruit, about 40% of the weight corresponds to the aryls, which contains 85% of water, 10% of total sugars such as glucose, fructose and sucrose mainly, 1.5% of the weight corresponds to pectins, 0.2 to 1% are polyphenolic compounds, and the rest a mixture of organic acids, minerals such as calcium, magnesium, sodium, potassium, zinc, and cobalt, and other products of the secondary metabolism of the plant. The seeds contained in the aryls represent 10% of the total weight of the fruit, where, from 12 to 20% it is constituted by oils, of which, 80% corresponds to conjugated fatty acids with high contents in cis 9, trans configuration 11 and cis 13, such as punic acid and linoleic acid. The fatty acids of the seeds constitute 95% of the oil, of them, 99% are triacylglycerols, the rest that make up the oil are: sterols, steroids, tocopherols and some cerebrosides. The other compounds that make up the total weight of the seed are lignins, protein, raw fiber, sugars, some vitamins, minerals, pectins, phytoestrogens such as comestrol and some polyphenols and terpenes 'Sai & Prakash, 2011. Finally, the pomegranate peel represents 50% of the total weight of the fruit and in it you can find some polyphenolic compounds such as: elagitannins, proanthocyanins, as well as alkaloid and glycoside compounds 'Viuda-Martos et al. 2010. Polyphenols, like terpenes, are the most abundant chemical compounds in nature. These are compounds with great structural diversity; however, they have hydroxylated aromatic rings in common. The classification of polyphenols is based on: the number of hydroxyl groups in the structure, where phenol is the simplest molecule and can be substituted with more than one hydroxyl radical, the following classification is based on its chemical composition; that is, based on the number of simple phenols bound in a molecule, there being, therefore, mono, di, oligo and polyphenols 'Kabera et al. 2014. It has been observed that the polyphenols contained in pomegranate juice have an antioxidant effect 'Tezcan et al. 2009' as well as chemopreventive and anticancer activity for those who consume it 'Syed et al. 2007' as well as bactericidal and even antiviral and antifungal activity 'Gautam et al. 2012' so it is important to evaluate these same properties but in the peel of pomegranate which has no use during theprocessing of the fruit to edible products and is only discarded.

The biotechnological importance of this research is in the microbiology area because with the extraction of components, such as polyphenols, with antioxidant and bactericidal activity, it is possible to use them both for the formulation of healthy foods and as disinfectant products or some medicines, due to the bactericidal activity they possess. In addition, the use of the shell of the endemic pomegranate of Mexico as extraction material allows to treat and take advantage of this residue generated during the processing of the product.

Abbreviations

$ABTS^{\cdot+}$; 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical.

$DPPH^{\cdot}$; 2,2-diphenyl-1-picrylhydrazyl radical

EAG; gallic acid equivalents
ET; Trolox equivalents

MATERIALS AND METHODS

Vegetable material

Three varieties of pomegranate in ripening coming from the states of Hidalgo and Guanajuato were analysed. The varieties were: *Wonderful, Apaseo* and *Tecozautla* harvested in August of the years 2017 and 2018 where, to evaluate the degree of ripening a physicochemical characterization was made with the objective of estimate the ripening state of the non-climacteric fruit since there is not norms about its parameters. The parameters measured were the following:

Physical parameters

Size

The representative weight fruit of each varieties was expressed in grams. Subsequently, the equatorial and longitudinal diameters were measured and expressed in centimeters. The longitudinal diameter was measured partially and totally way, where, the total diameter included the crown or calyx of the fruit and the partial longitudinal diameter did not include it. Finally, the peel thickness was measured with a Vernier and the results expressed in mm.

Color

This parameter was measured with a ColorFlex to obtain the coordinates of the CIE color system (for the acronym in English of the Commission Internationale de l´Éclairage), was calculated L * a * b * coordinates where the brightness is representing with the L * coordinate, green tones for negative values with the a * coordinate and yellow tones for positive values with the b * coordinate.

Hardness

This parameter was measured with a TA-XT2i texturometer where a deformation of 3 mm was made to the fruit, following the internal manual for hardness determination of green and ripening guava of the Biotechnology Laboratory of the Faculty of Chemistry Pharmacobiology of the Universidad Michoacana de San Nicolás de Hidalgo. Hardness is an important parameter because depending of the force applied to the fruit, it reflects the ripening, if the force applied is less the ripening was completed but if the force applied is mayor the ripening is early. The results were expressed as force applied to the fruit in Newtons (N).

Chemical parameters

The pH was measured with a potentiometer, the total soluble solids were measured to the juice extracted from each fruit with an ABB refractometer expressed in ° Brix, the titratable acidity was measured to the juice and were expressed the results as citric acid concentration (mg/ml) following Mexican norms (NMX-F-102-S-1978).

Peel drying

For the peel drying a response surface design of central compound was made: the dising was 2^2 with star points evaluanding the effects of 2 factors (temperature and time) in 11 experimental runs to conclude if there is an effect with respect to the antioxidant activity as a response variable, since the interest compounds are sensitive to elevated temperatures; However, as it is possible to conserve the samples of plant tissue with this method, the best drying condition can be concluded without affecting the metabolites in the peel. The experimental design was only applied in the *Wonderful* variety of the harvest of August 2017.

Polyphenols extraction

To obtain the liquid extracts with polyphenols from the pomegranate peel, the metabolites were first extracted in the August 2017 harvest by Soxhlet method applying a response surface design of central compound: 2^2 with star points where it was studied the effect of 2 factors, the first factor was the solvent concentration and in which we used two solvents; solvent 1 was acetone and solvent 2 was ethanol, for which as a lower limit of the concentration for both solvents in the design a concentration in the solution was used based on the percentage v/v of the solvent in water, the low point was 70%, central point 80% and high point 90%, moreover was used axial points to find the optimum extraction point using 66% as the lower axial point and 94% as the upper axial point. Regarding the extraction time for all experimental runs, the lower limit was 1 h, the central point 2 h and the upper point 3 h; the axial points were 50 min and 3 h with 40 min. The total of experimental runs were 11 for solvent, with a total of 22 experimental runs for variety.

For the storage and remotion of the solvent in the extracts obtained, a rotary evaporator was used where, for the acetonic extracts was used 60 °C of temperature with an extraction time of 20 min at 60 rpm. For ethanolic extracts was used 80 °C of temperature with an extraction time of 20 min and at 60 rpm. It is important to mention that all the extracts were worked under the same conditions since otherwise this could be a noise factor at the time of data analysis.

Once evaluated the effect of both factors considered in the design of response surface of the August 2017 harvested, the optimal conditions were calculated and validated with the August 2018 harvested so that reproducibility could be analyzed and if it was possible to get the estimated concentrations with the optimization process.

Antioxidant Activity

$ABTS^{\bullet+}$ radical method

A solution was prepared with the $ABTS^{\bullet+}$ radical aqueous (7 mM), ammonium persulfate aqueous solution (2.5 mM) and a Trolox stock (80 µM/ml), then a calibration curve was made in µM Trolox equivalents to calibrate the spectrophotometer at 734 nm with ethanol. Once the calibration curve was constructed, the inhibition percentage of the ABTS•+ radical was calculated when in contact with the Trolox solution and subsequently the Trolox concentration equivalents was calculated. For the determination in the polyphenolic extracts, a volume of 10 µL of sample was diluted with 990 µL of $ABTS^{\bullet+}$ adjusted solution was used and the corresponding readings and calculations were treated to express the results in mM of Trolox equivalents/ml or grams of extract dry. The abbreviation used for Trolox equivalents was ET (Trolox Equivalents).

DPPH• radical method

A solution was prepared with the $DPPH^{\bullet}$ radical dissolved in methanol at a concentration of 0.023 g/L and a Trolox stock of 80 µM/ml concentration, then a calibration curve was performed in µM of Trolox equivalent by calibrating the spectrophotometer at 515 nm with methanol. Once the calibration curve was constructed and with the equation of the straight line calculated in the linear regression of the data, the percentage of $DPPH^{\bullet}$ radical inhibition by the Trolox solution was calculated and subsequently the concentration in Trolox equivalents was calculated. For the determination in the polyphenolic extracts, a volume of 100 µL of sample diluted with 3900 µL of $DPPH^{\bullet}$ adjusted solution was used and the corresponding readings and calculations were treated to express the results in mM of

Trolox equivalents/g of dry extract. The same abbreviation for ET was used for the results.

Total phenols determination

A calibration curve was made with standard solution of gallic acid at 0.5 mg/ml of concentration, *Folin-Ciocalteu* reagent (1N) and $NaCO_3$ (20%). For the curve, the solution of gallic acid was reacted with the *Folin-Ciocalteu* reagent for 15 min in darkness, time passed, the $NaCO_3$ was added to the mixture of the two reagents (reacting for 1 h); the readings of the mixture were subsequently made on a Perkin Elmer spectrophotometer at a wavelength of 625 nm. As for the determination of the content of the total phenols in the extracts, dilutions were made with their respective solvents, since the spectrophotometer readings left the calibration curve; Once this was done, the readings were made and the corresponding calculations for to express the results in gallic acid equivalents in mg/ml for the 2017 varieties and mg/g of dry extract for the 2018 varieties because in the 2017 varieties only the effect of the operating conditions on the response variables was evaluated without being able to standardize dry weight extracts. For the 2018 varieties, as the effect of the factors was analysed and the optimal points were calculated, it was possible to standardize the extracts based on their dry weight. The abbreviation of both results were EAG (gallic acid equivalents).

Bactericide activity

Disk sensibility

The technique was made by means of the culture of *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria on Mueller-Hinton medium in Petri dishes. To evaluate the effect of polyphenolic extracts on the growth of both bacteria, 10 µL of extract at a concentration of 50 mg / ml on 5 mm Wotman filter paper discs were applied in the Petri dishes, as a positive control a volume of 10 µL of Erythromycin was used at 300 mg/ml of concentration and as a negative control a volume of 10 µL of solvent and water distilled were used. The dishes were incubated at 37 °C of temperature around of 24 h.

Statistical analysis

As mentioned earlier, several response surface designs were made, one for drying peel which was one of central compound: 2^2 with star points to evaluate the effects of 2 factors, the first factor was the temperature which as low point was 40 °C, as central point 50 °C, as an upper limit 60 °C and as axial points were 35.8 °C and 64.1 °C. For the drying time, it was used as a lower limit 24 h, center point 48 h, upper limit 72 h and as axial points 14.06 h and 81.9 h. Regarding the response surface design for Soxhlet extraction, the design was of central compound: 2^2 with star points studying the effects of 2 factors, the first factor was the concentration of solvent in which two solvents were worked solvent 1 being acetone and solvent 2 ethanol establishing as a lower concentration limit for both solvents 70%, as an average limit 80%, upper limit 90% and also axial points to find the optimum extraction point using 66% and another 94 %, all mixtures were with water. Regarding the extraction time for all the experimental runs, the lower limit was 1 h, the central point of 2 h and the upper limit 3 h, the axial points were 50 min and 3 h with 40 min. In each of the designs we worked with 11 experimental runs; however, for the extraction one, 11 runs were per solvent, so there were 22 experimental runs per variety. Once the optimal points for Soxhlet extraction in the 2017 varieties were calculated, the extractions were made in the 2018 varieties under the optimal conditions, in triplicate, for each of the varieties and the two solvents involved.

With the results of the response variables for each of the designs, analysis of variance and comparison of means for the bactericidal and antioxidant activity were performed, as well as for the quantification of total phenols, using as a significance value of 0.005 and using as evidence Post hoc the Tukey HSD test, so that it could be concluded which was the best working condition. For the analysis of fruit characterization data, analysis of variance and comparison of means were made in the same way to know if the fact that they were different varieties statistically were different or not and to be able to infer in how these characteristics could or could not be related to the analysis of the compounds of interest. The software used for the response surface design was StatgraphicsCenturion VXI and JMP and Origin 6.0 professional.

RESULTS AND DISCUSSION

Ripening fruit state

Physical parameters

The weight between the species analyzed in 2017 and 2018 is presented at the end of the work in table I and II with which it can be seen that it was similar only for the *Wonderful* and *Apaseo* varieties (405.36±34.1 g and 460±50.49 g respectively). The *Tecozautla* variety being the one with the lowest weight (95.99±5.17 g) because it had a shorter period of cultivation and was considered as partially green fruit since, based on comparisons of other pomegranates and growing conditions, the weights oscillate above 250 g; however, for the year 2018 (table I) the physical parameters were similar, so the variability observed in Figures 1, 2, 5 and 6 for the different responses does not depend on the ripening state of the fruit if not of the variety.

Table I. Different weighs of mexican pomegranate varieties (*P. granatum*). The data analyzed have a significance value (α) of 0.05 and are expressed as the mean ± standard deviation, n=10.

Variety	Weigh (g)			
	2017 harvest		2018 harvest	
Wonderful	405.36 ± 34.1	a	294.18 ± 55.40	a
Apaseo	460.85 ± 50.49	a	317.99 ± 63.54	a
Tecozautla	95.99 ± 5.17	b	286.01 ± 29.34	a

The a and b values are similarities between medias.

The uniform behavior in the physiology of the fruits of the 2018 harvest is due to the fact that on this occasion there was a greater control in the collection so that, as what was sought was the verification of optimal points calculated with the 2017 harvest for extraction of polyphenols, said physiological variation did not have an effect on the concentration and activity of the metabolites of interest. Moreover, was observed that at the time of drying the peel, a longer time was taken because there was a greater area through which the water had to be transferred in addition to containing a greater mesocarp amount of the segments causing a greater work in the drying since this vegetable part acts as moisture reserves reducing the loss of water, so they must be removed (Mercado et al. 2011). According to the thikness (table II), for the 2018 harvest in comparison with 2017 harvest where the thickness was smaller and the drying was carried out best for storage at room temperature, free of moisture and protected from light.

Talking about the color, the calculation of the hue angle of each of the tones presented by the fruit peel was made where for the *Wonderful* variety there was an angle of 31.48° and 26.11° for the years 2017 and 2018 respectively belonging to the range of the reddish colors and where the decrease in angle indicates that there was a greater synthesis of metabolites in the peel which serve the fruit as a defense; These metabolites are mostly polyphenols and may indicate a slight

Table II. Phisiology of mexican pomegranate fruit (*P. granatum*). The data analized have a significance value (α) of 0.05 and are expressed as the mean ± standard deviation, n=10.

Variety	Ecuatorial diameter (cm)		Parcial longitudinal diameter (cm)		Total longitudinal diameter (cm)	
	2017 harvest	2018 harvest	2017 harvest	2018 harvest	2017 harvest	2018 harvest
Wonderful	9.01 ± 0.2 a	8.31 ± 0.58 a	8.55 ± 0.16 a	7.63 ± 0.53 a	10.28 ± 0.48 a	8.7 ± 0.68 a
Apaseo	9.51 ± 0.48 a	8.61 ± 0.59 a	8.53 ± 0.33 a	7.56 ± 0.56 a	10.53 ± 0.91 a	8.92 ± 0.67 a
Tecozautla	7.95 ± 0.43 b	8.3 ± 0.43 a	6.91 ± 0.41 b	7.53 ± 0.43 a	8.77 ± 0.17 b	8.62 ± 0.45 a

The a and b values are similarities between medias.

increase in the ripening period. For the *Apaseo* variety there was an angle of 67.63° and 76.62° belonging to the range of green-yellow colors; and for the *Tecozautla* variety, there was a 24.02° hue angle typical of the reddish-colored bed and 59.6° in the range of red-yellow colors for 2017 and 2018 respectively, where this increase is also explained in the phenomenon happened in the *Wonderful* variety. On the other hand, the characterization (table I and II) managed to corroborate the type of variety worked, since the *Wonderful* variety has as its main characteristic a reddish coloration in its shell, the *Apaseo* variety green-yellow coloration and the variety *Tecozautla* a yellow-reddish coloration once maturation is reached which occurs around 120 days after the flower of the pomegranate tree is fertilized and the development of embryos in the seeds begins.

For hardness could be observed that *Apaseo* variety in 2017 was the one with the highest resistance to deformation being 65.06±61.94 N followed by the *Wonderful* variety with 47.91±3.83 N and finally the *Tecozautla* variety with 39.45±11.35 N being the latter is smaller than the rest because during transport to Morelia it had a visible softening with the humidity of the medium. For the year 2018 the hardness were of; *Wonderful* 34.61±9.071 N, *Apaseo* 61.94±13.83 N and *Tecozautla* of 43.22±7.7 N, a decrease being appreciable which reaffirms what is observed in the color of the husks since a decrease in the deformation resistance of the fruits translates to a softening of the cell walls of the fruits by effect of the metabolic enzymes that are activated as a fruit matures.

Compared the 2017 and 2018 harvests with other works where the physical changes that the fruit of the pomegranate has during its ripening process were monitored once the “set fruit” or the mooring of the fruit it can be established that the fruits of the *Wonderful* and *Apaseo* variety of the 2017 harvest have a maturity greater than 140 days (4 and a half months) after the fruit is tied; however, the *Tecozautla* variety has an approximate maturity of 30 days; which is why that variety was the smallest 'Yasoubi et al. 2007. For the attributes of the 2018 harvest, a uniform development was observed in the three varieties, as well as a similar maturation time, having an approximate development of 140 days after the set fruit.

Talking about the hardness in table II a decrease being appreciable which reaffirms what is observed in the color of the husks since a decrease in the deformation resistance of the fruits translates to a softening of the cell walls of the fruits by effect of the metabolic enzymes that are activated as a fruit matures. Based on the literature it is possible to establish that, for Mexican varieties, as a parameter of maturity control, a resistance to deformation can be taken as a reference in a range of 40 to 50 N 'López-Rubira et al. 2017. In general, the softening

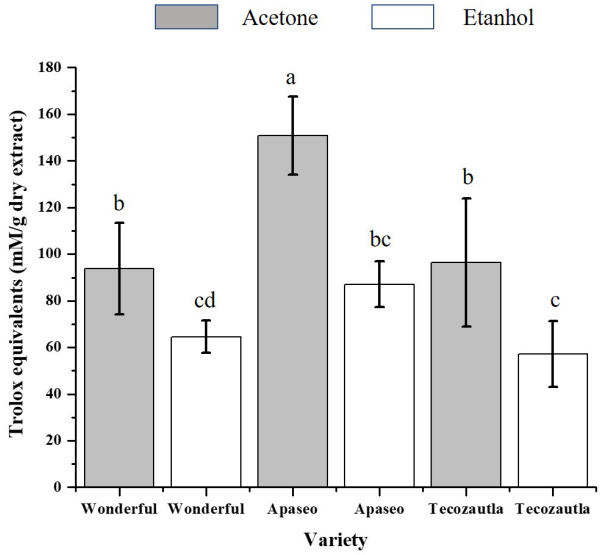

Figure 1. Antioxidant activity under optimal operational points of ketonic and ethanolic extracts of pomegranate peel (*P. granatum*). The labels about the letters are similarities between medias in the Tukey HSD analysis.

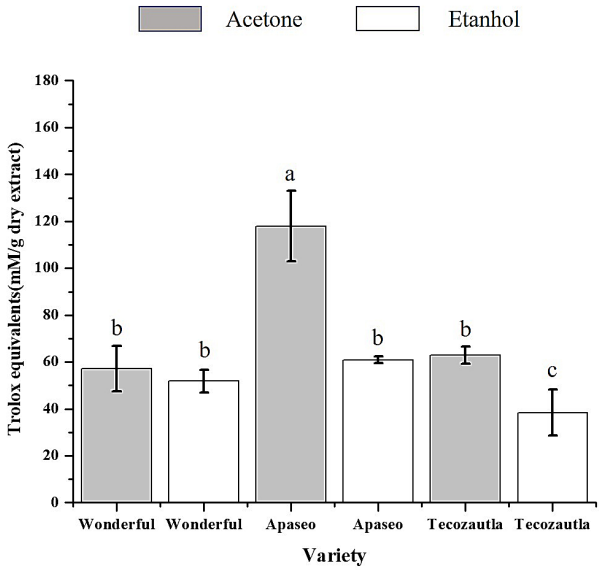

Figure 2. Stabilization of the DPPH˙ radical by pomegranate peel extracts (*P. granatum*) obtained by Soxhlet method under optimal extraction conditions. The labels about the letters are similarities between medias in the Tukey HSD analysis.

of the fruit is a process of modifications in the structure of the cell wall where depolymerization of glycans and solubilization of pectin, and where enzymes such as polygalacturonase (PG), pectinmethylesterase (PME), endo-1,4- are involved β-glucanase, α-arabinosidase and β-galactosidase among others. The activity of these enzymes during maturation is related to a change in cell wall polysaccharides; The time and moment of activity varies between enzymes [Brummell et al. 2004]. Similarly, it has been observed that the action of pectinmethylesterase catalyzes the desterification of the carboxyl group of polygalacturonic acid and increases the susceptibility of pectins to polygalacturonase [Sanchez et al. 1996].

Chemical parameters

Regarding the pH in 2017 harvest, the *Wonderful* variety had a value of 1.17±0.23, *Apaseo* was 1.37±0.08 and *Tecozautla* was 3.37±0.06, in the case of acidic fruits which had a concentration of 0.19±0.05 mg/ml of citric acid, 0.04±0.002 mg/ml of citric acid and 0.31±0.05 mg/ml of citric acid respectively which explains the decrease in the pH of the first two varieties. For the 2018 harvest the pH was; *Wonderful* 3.18±0.5, *Apaseo* of 3.05±0.6 and *Tecozautla* 3.7±0.2 which show a greater uniformity with a concentration of citric acid for *Wonderful* of 0.13±0.05 mg/ml, *Apaseo* of 0.04±0.005 mg/ml and *Tecozautla* of 0.05±0.04 mg/ml when compared with other mature varieties of pomegranate are close by what can be deferred that the fruits if they reached the state of maturation.

Regarding the total soluble solids in the 2017 harvest, the concentration was; *Wonderful* 16.8±0.14 °Brix, *Apaseo* 14.8±0.5 °Brix and *Tecozautla* was 14.8±0.33 °Brix where compared with other cultivars the parameter is close. For 2018 harvest the concentration was; *Wonderful* 17.6±0.92 °Brix, *Apaseo* of 13.6±1.4 °Brix and *Tecozautla* was 17±0.62 °Brix.

The observations on pH in the juice fruit were differents where a decrease in pH is seen, may be due to the increase of free organic acids

in the fruit 'Laguado et al. 1999. On the other hand, when there is an increase in the pH in a fruit but with a difference in ripening time, what happens is that during the filling of fruits a large part of the accumulation activity is given by symport, where the H^+ ions perform an important paper; these are part of the formation of substrates such as sucrose and glucose, and make their concentration at the vacuolar level decrease during the last stages of maturation, so the pH is slightly increased 'Álvarez-Herrera et al. 2009.

Regardind the total soluble solids has been observed that the concentration of total soluble solids in the fruit juice increases as the fruit develops, which could be verified since the *Tecozautla* variety was preliminary analyzed in a green state and was the one with the least amount. The existence or synthesis of a greater amount of soluble solids in fruit juices can be explained since in mature varieties there is a hydrolysis of starches that are transformed into sugars, as well as a decrease in tannins and other products causes astringent taste due to the decrease in acidity due to the degradation of organic acids. Therefore, the variation of soluble solids by the conversion of starch into sugars is attributed to an increase in the activity of starch hydrolase enzymes 'Álvarez-Herrera et al. 2009'. The stage of the fruits, in comparation with other were proxim, so they can be considered ripe fruits 'Mercado et al. 2011.

Drying peel

With the analysis of the response surface applied in the drying of the peel, an approximate drying time of 40 h around 55 °C could be estimated by measuring the antioxidant activity in response by the ABTS•+ radical method; however, for further analysis an analysis was made with the response surface contour diagram with which an operational time of 36 h at a temperature of 55 °C could be obtained concluding that the effect of time was greater on the response with Regarding the temperature applied. The above was corroborated when analyzing the dry samples with respect to the antioxidant activity but now with the determination of total phenols by the *Folin-Ciocalteu* method with which it was verified that the experimental run in which they were subjected to longer exposure time (72 h and 81.94 h) had the lowest concentration of total phenols.

Antioxidant activity by ABTS•+ and DPPH• radical method

In the 2017 harvest, 11 experimental runs were carried out with each of the solvents (acetone and ethanol) with the response surface methodology with which it was observed that in the extracts with ethanol there was the greatest activity in the *Wonderful* variety being 4.47 mM/ml because, at the time of removal of the solvent, the samples were the ones to which a greater purification was reached and therefore, that the extract was mostly concentrated. Once the effect of solvent concentration and extraction time was evaluated, an optimal extraction condition was estimated based on the inhibition of the ABTS•+ radical. For the varieties harvested in 2018, the estimated responses were evaluated and checked with the optimal operating points (Fig. 1) and it was until then that the effect of the solvent type would be evaluated since they could be standardized at a concentration of 50 mg/ml. Once the analysis was done, optimal conditions were estimated for each variety and each solvent used which are for the *Wonderful* variety with acetone as solvent a concentration of 94.1% (v/v) was necessary in a time of 2.28 h and with ethanol as solvents was with a concentration of 94.14% (v/v) in a time of 1.03 h. For the *Apaseo* variety with acetone as solvent, a concentration of 94.14% (v/v) was estimated with

an extraction time of 1.03 h and with ethanol as solvent a concentration of 80.16% (v/v) with a time of 2.57 h. Finally, for the *Tecozautla* variety, a concentration of 94.14% (v/v) of acetone was estimated with an extraction time of 3.41 h and with ethanol as solvent a concentration of 94.14% (v/v) with a time of 3.41 h. From all the previous observations it could be concluded that the *Apaseo* variety was in which it was possible to fully estimate an optimal extraction point since in the graphics it was possible to observe a proper curvature of said optimum points where an increase and decrease of the answers for the factors involved. In the case of the *Wonderful* and *Tecozautla* varieties, the graphs and analysis indicate that it is still possible to continue with explorations with which a curvature such as that obtained in the *Apaseo* variety can be achieved. The results of both methods show in the Fig. 1 and 2.

In the validation of the optimal points with the 2018 harvest (Fig. 1), and under a standardization of the volumes and concentrations of the extracts, it was observed that the *Apaseo* variety was the one with the highest extraction and presence of polyphenols at use acetone as a solvent because it was the solvent with which low temperatures were handled.

The concentrations obtained were; *Wonderful* 93.89 ET mM/g of dry extract and 64.6 ET mM/g of dry extract for acetone and ethanol, *Apaseo* was 150.78 ET mM/g of dry extract and 87.08 ET mM/g of dry extract for acetone and ethanol, and *Tecozautla* of 96.32 ET mM/g of dry extract and 57.24 ET mM/g of dry extract for acetone and ethanol (Fig. 1).

The extraction of polyphenols using different solvents of different polarities has already been analyzed and determined the content of polyphenols in the peel of Iranian pomegranate and also evaluated the antioxidant activity in the extracts obtained from this vegetable fraction. The polyphenol extraction method was using solvents by maceration method and by ultrasound extraction; where it was observed that the polyphenol extraction yield was higher where acetone was used as a solvent in both methods as observed in Fig. 1 [1]Yasoubi et al. 2007.

Subsequently, the highest yield was achieved with methanol, ethanol, water and finally ethyl acetate, being similar the observed in the present work. On the other hand, in that same year Chirinos et al. performed the optimization of the extraction conditions for phenolic compounds with antioxidant activity of an endemic plant in Peru called Mashua where it was evaluated; the type of solvent (water, ethanol, methanol, acetone and hexane), the pH of the solvent, the solvent mixed with portions of water, extraction time and acidification of the solvent with 0.1% HCl, observing that in the parameters measured as response variables, which were: antioxidant activity by ORAC method, quantification of total phenols, total flavonoids and total anthocyanins. The highest concentration and activity was obtained in the extracts where acetone and methanol were used as a solvent, which was attributed to the fact that the Mashua mostly contain secondary metabolites of moderate polarity which allowed the extracts to have that behavior. Similarly, it is reported that within the compounds belonging to the polyphenols that are easily extracted with these two solvents are those derived from hydroxycinnamic acid, flavonols, monomers of flavan-3-ol, flavones and flavonones as well as some anthocyanins. These same compounds have been identified in the pomegranate since there is a significant presence of hydroxycinnamic acid derivatives in the pomegranate peel as one of the main phenolic acids as well as the flavan-3-ol known as catechin, flavones and anthocyanins such as delfinidine, cyanidine and pelargonidine

'García-Viguera & Pérez 2004' so it can be said that similar behavior in pomegranate peel extracts where acetone was used as a solvent may be due to the fact that these specific compounds were extracted in greater quantity.

On the other hand, has been evaluated the antioxidant activity of tropical fruits of Yucatan, Mexico which were the purple star apple (*Chrysophyllum cainito* L.) and the yellow and red cashew (*Anacardium occidentale*) where the extracts were obtained from the fruit peels and presented a concentration around 3.05 in ET mM/g of dry sample and 3.322 ET mM/g of dry sample respectively, resulting in a lower concentration than that obtained (Fig. 1). Finally, it can be mentioned that in the same investigation in the shells which had a similarity in color to those in the grenade, detecting the presence of phenols as they are: ferulic acid, caffeic acid, cinnamic acid, gallic acid and ellagic acid. What would be interesting for future work, a characterization of metabolites by HPLC to the extracts worked in this thesis to know if there is a presence of them and if it can be concluded that the activity of inhibition of synthetic radicals is due to their presence 'Moo-Huchin et al. 2015.

To complement the analysis, a Pearson correlation was carried out between the inhibition of the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical (Fig. 3) obtaining a value of r^2 of 0.7542 so it can be said that there is a partial relationship in the activity both on the part of lipophilic compounds such as hydrophilic compounds, however, it is possible that there are other chemical compounds that were extracted and that can inhibit radicals, such as terpenic compounds.

Various investigations have been carried out where the antioxidant activity has been measured by ET through the $DPPH^{\cdot}$ method, such as that carried out where the antioxidant activities of guava fruit extracts obtained by maceration were compared with methanol and where the

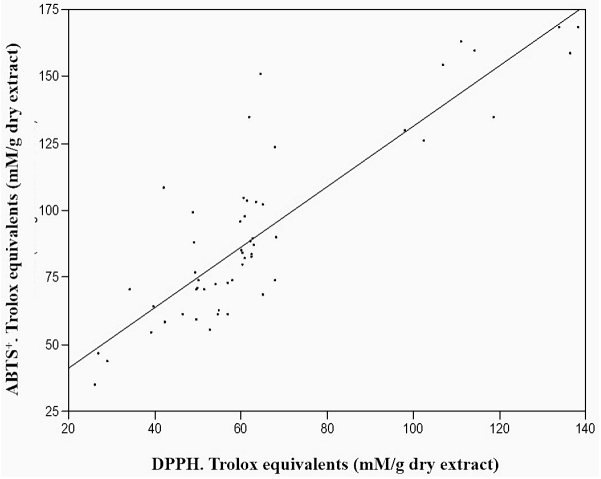

Figure 3. Pearson's correlation between the antioxidant activity evaluated with the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical method.

$ABTS^{\cdot+}$, $DPPH^{\cdot}$, FRAP and ORAC techniques were applied, resulting in a concentration of 0.031 ET mM/g of fresh sample, 0.025 ET mM/g of fresh sample, 0.026 ET mM/g of fresh sample and 0.021 ET mM/g of fresh sample respectively. There is a greater presence of ET measured with the $ABTS^{\cdot+}$ don method compared to those measured by the $DPPH^{\cdot}$ method since with the first one, the hydrophilic and lipophilic antioxidant compounds are quantified compared to those quantified $DPPH^{\cdot}$ where only those of a lipid nature are quantified. On the other hand, it can be noted that the pomegranate peel represents an important source of these compounds with such activity since the ET concentrations are higher. In the same work the correlation of the activities measured as an observed response was evaluated, a r^2 of 0.85 which was greater than that obtained in the work (Fig. 3) 'Thaipong et al. 2006, because when using an extraction method with temperatures, such as Soxhlet, it is difficult to fully control the same temperature for all experimental runs, generating an increase in variability; However, this does not mean that it does not allow reproducibility in extractions, leaving the suggestion that for future research it is a question of controlling this factor as much

as possible. with the results of both correlations, it can be established how there is a clear relationship between methods since in most of the extractions the relationships of the activities have the same behavior and serve as validation to sustain the properties of phytochemical compounds (Fig. 3).

Total phenols determination

For the extraction of polyphenols in the 2017 harvest, it was observed that the solvent in which there was a higher concentration was ethanol for the three varieties, being 0.88 EAG mg / ml, 0.27 EAG mg / ml and 0.51 EAG mg / ml for *Wonderful, Apaseo* and *Tecozautla* respectively, with which an optimal extraction point could be established; However, in this first extraction only the effect of the temperature and the type of solvent was caused, so the extracts were not standardized to a specific concentration, and as the ethanol extracts were the ones that presented a greater removal of the solvent, for that reason they had a greater activity. Once the effect of temperature and the type of solvent had been evaluated, a second extraction was carried out with the 2018 harvest, but this time the extracts were standardized to the same concentration, which is why the following results were obtained, which are presented in Fig. 5.

As can be seen, the highest concentration was found in ketone extracts with a concentration of 5.35 EAG mg/g, 11.82 EAG mg/g and 6.69 EAG mg/g of dry extract by *Wonderful, Apaseo* and *Tecozautla* respectively. Regarding the concentration in the ethanol extracts were 3.05 EAG mg/g, 4.87 EAG mg/g and 2.9 EAG mg/g of dry extract by *Wonderful, Apaseo* and *Tecozautla*. Likewise, a Pearson correlation was carried out (Fig. 4) with which, with respect to the inhibition of the $ABTS^{\cdot+}$ radical, a value of r^2 of 0.64 was obtained and with respect to the inhibition of the $DPPH^{\cdot}$ radical, the value of r^2 was 0.8, so there

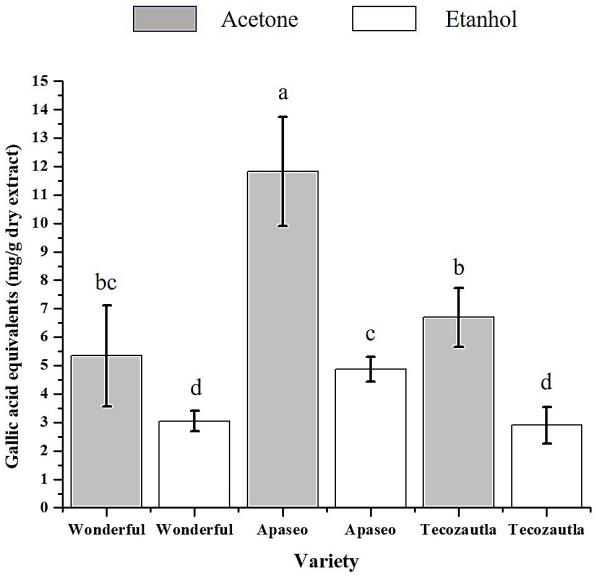

Figure 4. Total phenols concentration in pomegranate peel extracts (*P. granatum*) obtained by the Soxhlet method. The labels about the letters are similarities between medias in the Tukey HSD analysis.

is a greater presence of lipophilic secondary metabolites quantified by the $DPPH^{\cdot}$ radical that inhibits it than hydrophilic metabolites that inhibit the $ABTS^{\cdot+}$ radical.

As can be seen in figure 3 and 4, the presence of total phenols shows a relationship with the inhibition or stability of the radicals of $ABTS^{\cdot+}$ and $DPPH^{\cdot}$, so it is convenient to carry out in future research a characterization of the types of phenols that are present in the peel of the pomegranate; however, it has been reported that in the peel of the pomegranate the type of phenols that can be found belong to the family of flavonoids and specifically the type of flavones, flavonols and anthocyanidins; compounds such as catechin, epicatechin, epicalocatechin-3-gallate, quercetin, rutin, flavan-3-ol, kaemferols (kaempferol-3-O-glucoside and kaempferol-3-O-rhamnoglycoside), luteolin (luteolin-7-O-glucoside), narangina, pelargonidin The bright colors of the skin and of the juice are attributable to anthocyanidins, where the aglycones are: delphinidin, cyanidin and pelargonidin 'García-Viguera & Pérez 2004.

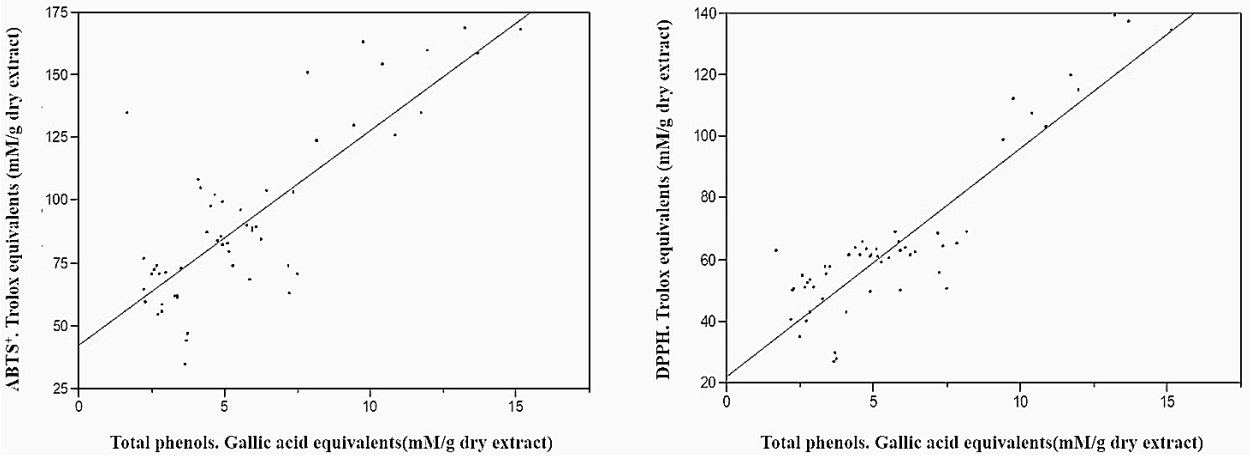

Figure 5. Pearson's correlation between the antioxidant activity evaluated with the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical method with respect to the quantification of total phenols.

In previous works where the pomegranate has been taken as a study model, has been posible evaluating the physicochemical changes of the pomegranate variety "Rabbab-e-Fars" during its ripening process and observed a concentration after 20, 80 and 140 days of the mooring of the fruit (fruit set) of 19.38 EAG mg/g of juice, 12.98 EAG mg/g of juice and 7.86 EAG mg/g of juice respectively, observing a decrease in concentration as the fruit matures 'Zarei et al. 2011'. This change is due to the activation of the polyphenoloxidase enzyme so this parameter allows to know and standardize the ripening time of the fruits. The results obtained and expressed in the same units with the varieties worked with acetone as a solvent were 5.35 EAG mg/g of dry extract, 11.81 EAG mg/g of dry extract and 6.69 EAG mg/g of dry extract for *Wonderful* varieties, *Apaseo* and *Tecozautla* respectively which indicates that growth conditions for pomegranate in Mexico are favorable for generating concentrations of polyphenols near or above, as in the case of the *Apaseo* variety, in pomegranate husks compared to the juice of this variety analyzed since, based on the physicochemical analysis, it was deduced that the ripening time of the fruit was around 140 days after the fruit was tied and a comparison could be made. On the other hand, the ethanolic extracts had a concentration of 3.05 EAG mg/g of dry extract, 4.87 EAG mg/g of dry extract and 2.9 EAG mg/g of dry extract for the *Wonderful, Apaseo* and *Tecozautla* varieties respectively, so, if working with this solvent, it would allow to obtain in the same way significant quantities of extractable polyphenols. on the other hand, it has been possible to analyze the secondary metabolites that the fruit of the pomegranate may contain, mentioning the polyphenols with significant presence, as well as a positive presence of tannins, flavonoids, mucilages, saponins, terpenes and glycosides, however, when making the determination of alkaloids, the response was negative, so it would be important to subsequently carry out a characterization of the secondary metabolites present in the ketonic and ethanolic extracts of the shells of Mexican pomegranates to know which of them are found in greater proportion and are directly responsible for the antioxidant or bactericidal activity, so based on Pearson's correlations there is a clear effect of the phenols on these activities 'Chebaibi et al. 2013.

Compared the extraction of polyphenols by ultrasound method and by maceration in the cocoa bean of Mexico City, determining the total

phenolic content in gallic acid equivalents. The maceration was carried out in two parts, first, the plant material was contacted for 2 h at room temperature (25 °C) using as a solvent a mixture of methanol-water at a concentration of 50% (v/v) to after filtering, subject the residue to a second maceration now with a mixture of acetone-water at a concentration of 70% (v/v) under the same conditions as in the first maceration. As for the ultrasonic extraction, the sample was subjected to the same solvents as in the maceration in a time of 30 min to concentrate the extracts obtained by the two methods first to rotavapor at 50 °C at 40-60rpm and then to dry at 30 °C for 24 h. As results it was observed that there was a greater extraction in the ultrasound method and that they were statistically different with a concentration of 135.92 EAG mg/g and 90.6 EAG mg/g for the methods with ultrasound and maceration respectively. The results were greater than those reported in the present work; However, it is important to mention that when working with pomegranate shells, an added value can be given to the waste generated.

The total phenols between three sectioned parts of the pomegranate peel were quantified; the outer part or skin, mesocarp and dividing membranes. The determined concentrations were 9.38 EAG mg/g of dry sample, 17.74 EAG mg/g and 21.18 EAG mg/g for the outer part or skin, mesocarp and dividing membranes, where it can be observed that there is a presence greater than those obtained in the work, however, is due to the fact that the extraction was carried out with ultrasound at 30 °C for 2 h, so there was no significant degradation of the metabolites, however it can be observed that in the skin or part outside the peel there was a lower presence than those reported in the thesis and that also did not work with the membranes since they were removed because they stored significant amounts of water that prevented proper drying of the peels. Finally, performed the optimization of the process of extraction of antioxidant substances from Mexican oregano using the response surface methodology in which the extraction was done by the maceration method evaluating the effect of three factors; Ethanol-water solvent concentration of 30%, 60% and 96%, extraction temperature of 25 °C, 55 °C and 75 °C as well as the particle size which were 0.423 mm, 1.018 mm and 1.808 mm. Extraction times were 60 min with 300 rpm stirring. As a result, it was obtained that the maximum concentration of polyphenols that could be had under these conditions was 101 EAG mg/g at the highest extraction temperature (75 °C), with the smallest particle size (0.423 mm) and with a concentration of solvent of 57.7% since the temperature allowed a greater rupture of the cell walls as well as diffusion of the solvent in the cell matrix to be in contact with the metabolites of interest, as for the particle size it was observed that this allowed a greater area of contact with the solvent and to diffuse more easily and the ratio of the solvent allowed both polarities of the solvents to extract a wide range of active compounds.

Bactericide activity against *S. aureus*

In the 2017 harvest, maximum growth inhibition was obtained forming an inhibition halo in the *Wonderful* variety of 20.4 mm and 12.35 mm for acetone and ethanol whose extracts were standardized at a concentration of 50 mg / ml. For the *Apaseo* variety there was an inhibition halo of 15.8 mm and 14.15 mm for acetone and ethanol. Finally, for the truth, *Tecozautla* had an inhibition halo of 16.35 mm and 11.45 mm for acetone and ethanol, which proves that once standardized, acetone is the best extraction solvent, in addition to experimental runs with ethanol in which did not form inhibition halos and the bacteria could grow without problem.

For the validation of the experimental points it can be observed with Figure 5 that in 2018 in the *Wonderful* variety there were inhibition halos of 18.82 mm and 16.52 mm for acetone and ethanol respectively. For *Apaseo* variety there was a halo of 20.03 mm and 16.13 mm for acetone and ethanol and for *Tecozautla* variety there were halos of 18.92 mm and 17.07 mm for acetone and ethanol so it is again proven that acetone is a solvent recommended for extraction and that in *Apaseo* variety the highest results were achieved because it was the variety with which an optimal experimental point was obtained.

In compilations of flavonoids with antimicrobial activity, which included bacteria such as *E. coli* can be that the mechanism by which flavonoids affect the growth of bacteria is at the level of: inhibition of the synthesis of nucleic acids, proteins and lipids, inhibition of the functionality of the cytoplasmic membrane and in the inhibition of energy metabolism. In the inhibition of nucleic acid synthesis it is pointed out that, among other compounds, epigallocatechin can be deprotonated in the hydroxyl groups of ring B whose position is carbon 3, 4 and 5, which easily interact with the nucleic acid bases interfering in the synthesis of DNA and RNA. Reference is made to this type of flavonoid since it is one of those reported in the shells of pomegranates 'García-Viguera & Pérez 2004. Therefore, the effect observed in the bactericidal activity in the 2017 harvest may be due to this interaction of the flavonoids. On the other hand, quercetin, another flavonoid in the peel of the pomegranate 'García-Viguera & Pérez 2004' has an effect on the enzyme and ADNgirasa changing the structural conformation of the enzyme and, therefore, prevents a reduction in tension caused by the supercoiling of DNA that facilitates the replication of genetic material 'Plaper et al. 2003.

As for the inhibition in cytoplasmic membranes, naringinine acts on the transporter proteins, which decreases the selectivity of the membranes. Catechins are other compounds that have been observed as agents that damage cytoplasmic membranas has been reported to evaluate the effect of these flavonoids on gram-positive and gram-negative bacteria, observing that gram-positive bacteria are mostly sensitive than gram-negative bacteria, and it was demonstrated that catechin has a high bactericidal activity, being one of the more potent compared to other flavonoids. The mechanism proposed by the catechin is: a penetration occurs in the bilayers or the lipid layer and inactivate the enzymes responsible for building cell walls. The second mechanism that is proposed is that the catechins favor the fusion of the membranes causing leakage of the intercellular material and that it makes segregation of components. It is concluded in this work that gram-positive bacteria are more sensitive than gram-negative bacteria since they have lipopolysaccharides that act as a barrier preventing catechin from penetrating membranas 'Ikigai et al. 1993. Finally, in the inhibition of energy metabolism it has been observed that they interfere with the activation and functioning of ATPase, observed and evaluated mainly in *E. coli*, as well as in enzymes that for the selective transport of nutrients or biosynthesis of macromolecules where energy expenditure is required 'Cushnie & Lamb 2005.

On the other hand, has been evaluated the effect that kaempferol, quercetin and luteolin flavonoids have, among others, on bacterial growth where it was observed that, in the same order of kaempferol to luteolin, it is the interaction with bacterial membranes and their involvement. The previous behavior among these flavonoids is the theory that is due to hydroxyl groups in the C ring, mainly affecting the fluidity

of the membrane. These flavonoids penetrate the lipid bilayer and increase the order and dynamics inside causing a instability that affects the growth of the bacteria 'Wu et al. 2013.

Bactericide activity against *E. coli*

The activity against this bacterium was only determined in the 2018 harvest (Fig. 6) with which 12.58 mm and 9.28 mm inhibition halos for acetone and ethanol as solvent were formed for the *Wonderful* variety. For the *Apaseo* variety, 14.05 mm and 11.12 mm halos were formed for acetone and ethanol. Finally, for the *Tecozautla* variety, 11.17 mm and 9.57 mm halos were obtained for acetone and ethanol, so they are less sensitive to inhibition in all cases because gram-negative bacteria have mostly developed a dense mechanism against bactericide compounds. Next, Figure 7 shows the bactericidal effect obtained with different solvents of different polarities.

Comparing the results with others it can be observed that there is a similarity with that reported for *E. coli WT, E. coli BU40* and *E. coli FPL5014* since results of 12.2 mm, 12.6 mm and 11.3 mm respectively, being similar, and in some cases as in the *Apaseo* variety, superior, corroborating the majority sensitivity of the extracts in gram-positive bacteria than in gram-negative ones 'Naz et al. 2007.

On the other hand, has been reported that pomegranate (*P. granatum*) contains phytochemical compounds with significant bactericidal activity, indicating that these compounds were extractable with high yields using solvent extraction methods and for which, the use methane, water ethanol and acetone proved favorable in the study. In the investigation it was possible to observe sensitivity against *S. aureus*, as well as gram-negative bacteria such as *E. coli*, so that for future experiments the evaluation of the bactericidal effect against this same bacterium is contemplated. With this same work it is possible to show that the amount of pigments in the shells of the shells does not have a correlation with the bactericidal activity since no significant correlation factor was found between

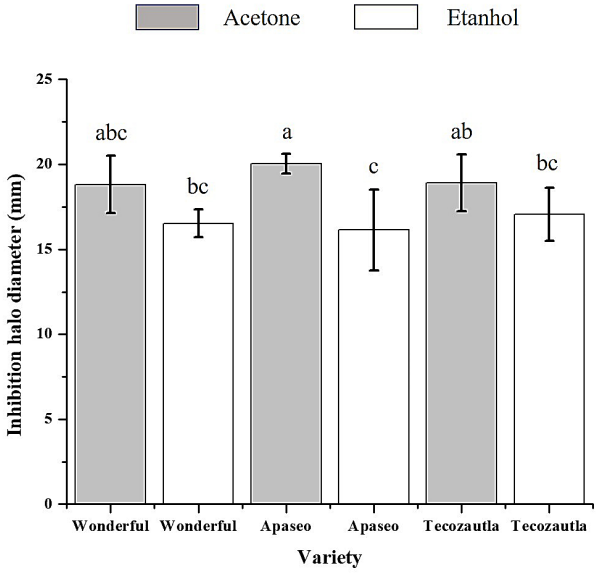

Figure 6. Bactericide activity due to disc sensibility against *S. aureus* with pomegranate peel extracts (*P. granatum*) obtained by the Soxhlet method. The labels about the letters are similarities between medias in the Tukey HSD analysis.

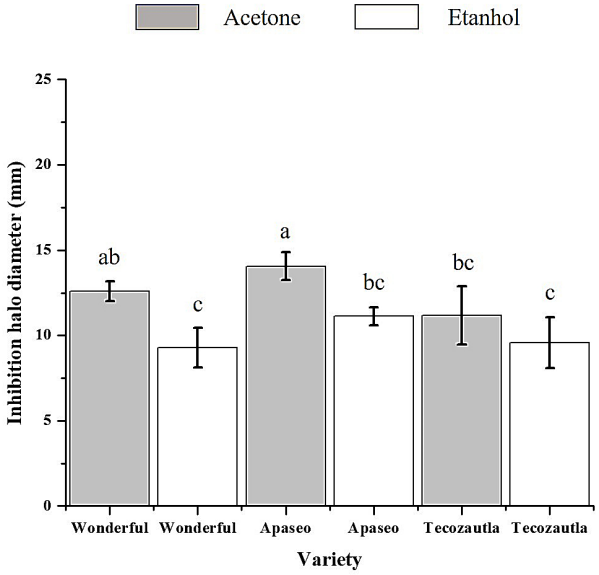

Figure 7. Bactericide activity by disc sensibility against *E. coli* with pomegranate peel extracts (*P. granatum*) obtained by Soxhlet method. The labels about the letters are similarities between medias in the Tukey HSD analysis.

the color parameter and the percentage of growth inhibition of the different bacteria analyzed, so it can be concluded that the coloration of the Mexican varieties does not directly influence the bactericidal effect on different types of bacteria 'Duman et al. 2009. It has been observed too that the bactericide activity of some extracts has direct effect with the concentration of simple phenolic compounds, such as gallic acid or ellagic acid as well as complexes (Tannins), however there is also a significant effect with the amount of tannins that can be extracted when using solid-liquid extraction techniques with solvents such as alcohols, acetone or water, and even greater extraction performance has been observed with mixtures of these solvents and to a lesser extent when pure ones are used. The effect that phenols generally have on microorganisms are the following: (I) Complexing of enzymes responsible for cell wall synthesis or lipid membranes, as well as being capable of complexing important substrates for growth (II) Tannins are toxic leg bacterial membranes destabilizing them and causing them to lose their selective character, (III) Complexing of metal ions (Akiyama et al. 2001).

Recently was evaluated the bactericide and antioxidant activity of pomegranate shell extracts of the "Gabsi" variety whose extracts were obtained by being in contact with different solvents which were methane and water at different ratios. of solvent volume and weight of the pomegranate peel powder whose ranges were from 15 to 30 v/w and where the remarkable thing is that different extraction temperatures were used which were 25 °C, 40 °C, 55 °C and 75 °C, once the extracts were obtained, they were standardized under concentrations of 61 mg/ml to 360 mg/ml and the effect of sensitivity to microorganisms such as *Sacharomices cerevisiae, Pseudomona putida* and *Penicillum digitatum* was evaluated obtaining inhibition halos for *S. cerevisiae* from 0.0 mm to 12.5 mm, for *P. putida* from 8.5 to 30.4 mm and for *P. digitatum* from 2 mm to 24.5 mm, where the greatest effect was obtained in the extracts where methanol was used as a solvent, and in some cases as in *S. cerevisiae* and *P. digitatum* there was no inhibition of the extracts where water was used as a solvent, so this background leaves the possibility to evaluate the effect of the extracts obtained in this work against different fungi and determine if the composition of the Mexican varieties is favorable for the inhibition of their growth 'Kharchoufi et al. 2018.

CONCLUSIONS

As a first conclusion, it can be mentioned that the drying carried out on the pomegranate peels did not have a significant effect with respect to the degradation of the polyphenols contained since there was a majority concentration and an antioxidant activity with respect to the $ABTS^{\cdot+}$ radical because the temperature, not being high, did not cause breakdown or oxidation of the metabolites, so it can be carried out in addition to the fact that the sample was not wet, allowing its conservation. Regarding the extraction of metabolites when using two solvents, it can be concluded that using acetone as a solvent is convenient for a greater extraction since, because the boiling point of acetone is lower than that of ethanol using the Soxhlet method extraction, causes less degradation of the metabolites during the extraction time and the cycles generated, thus allowing greater conservation of polyphenols, in addition to the fact that acetone, as it is a medium polarity solvent, allows it to be a variety of polyphenols extracted from the plant matrix, for which it is necessary to carry out a characterization in future works. Regarding the type of variety analyzed, it can be concluded that the *Apaseo* variety is the one that contains a greater amount of polyphenols which present a greater antioxidant and bactericidal activity with

respect to the *Wonderful* and *Tecozautla* varieties, which can be attributed to the cultivation condition as well as its responsiveness to stress, which makes it ideal to continue with the extraction of these metabolites. Finally, it can be concluded that in general the 3 Mexican varieties contain high concentrations of polyphenols in the peel compared to other fruits, which makes it a viable option to obtain this type of compound while giving added value to the fruit and this waste is used.

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REFERENCES

AKIYAMA H, FUJII K, YAMASAKI O, OONO T & IWATSUKI K. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother 48: 487-491.

AL-MAIMAN S & AHMAD D. 2002. Changes in physical and chemical properties during pomegranate (*Punica granatum L.)* fruit maturation. Food Chem 76: 437-441.

ÁLVAREZ-HERREA J, GALVÍS J & BALANGUERA-LÓPEZ H. 2009. Determinación de cambios físicos y químicos durante la maduración de frutos de champa (*Campamonesia lineatifolia* R.& P.). Agron 27: 253-259.

BRUMMELL D, DAL CIN V, CRISOSTO C & LABAVITCH J. 2004. Cell wall metabolism during maturation, ripening and senescence of peach fruit. J Exp Bot 55: 2029-2039.

CHEBAIBI A & RHAZI F. 2013. Bactericidal activity and phytochemical screening of Moroccan pomegranate (*Punica granatum Linn*.) peel aqueous extracts. J Med Plant Res 7: 887-891.

CHIRINOS R, ROGEZ H, CAMPOS D, PEDRESCHI R & LARONDELLE Y. 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. Sep Purif Technol 55(2): 217-225.

CUSHNIE T & LAMB A. 2005. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26: 343-356.

DÍAZ L. 2009. Interacciones moleculares entre plantas y microorganismos: saponinas como defensas químicas de las plantas y su tolerancia a los microorganismos. RET 1: 32-54.

DUMAN A, OZGEN M, DAYISOYLU K, ERBIL N & DURGAC C. 2009. Antimicrobial activity of six pomegranate (*Punica granatum L.*) varieties and their relation to some of their pomological and phytonutrient characteristics. MDPI 14: 1808-1817.

GARCÍA-VIGUERA C & PÉREZ V. 2004. La granada. Alimento rico en polifenoles antioxidantes y bajo en calorías. Alimentación, Nutrición y Salud 11: 113-120.

GAUTAM M, GANGWAR M, NATH G, RAO C & GOEL R. 2012. In–vitro antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata Linn*. leaves. Asian Pac J Trop Biomed 2(3): S1660-S1663.

IKIGAI H, NAKAE T, HARA Y & SHIMAMURA T. 1993. Bactericidal catechins damage the lipid bilayer. BBA 132-136.

KABERA J, SEMANA E, MUSSA A & HE X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. J Pharm Pharmaco 2: 377-392.

KHARCHOUFI S, LICCIARDELLO F, SIRACUSA L, MURATOTE G, HAMDI M & RESTUCCIA C. 2018. Antimicrobial and antioxidant features of 'Gabsi' pomegranate peel extracts. Ind Crops Prod 111: 345-352.

LAGUADO N, PÉREZ E, ALVARADO C & MARÍN M. 1999. Physicalchemical and physiologycal characteristics of guava fruits of Criolla Roja and San Miguel types from two comercial plantations. Rev de la Fac de Agron 16: 382-397.

LÓPEZ-RUBIRA V, ARTÉS-HERNÁNDEZ F & ARTÉS F. 2017. Repositorio Digital de la Universidad Politécnica de Cartagena. Obtenido de Repositorio Digital de la Universidad Politécnica de Cartagena.

MERCADO E, MONDRAGÓN C, ROCHA L & ÁLVAREZ B. 2011. Effects of fruit condition and storage temperature on pomegranate quality. REMEXCA 2: 449-459.

MOO-HUCHIN V, MOO-HUCHIN M, ESTRADA-LEÓN R, CUEVAS-GLORY L, ESTRADA-MOTA I, ORTIZ-VÁZQUEZ E & SAURI-DUCH E. 2015. Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem 166: 17-22.

NAZ S, SIDDIQI R, AHMAD S, RASOOL S & SAYEED S. 2007. Antibacterial activity directed isolation of compounds from *Punica granatum*. J Food Sci 72: 341-345.

PLAPER A, GOLOB M, HAFNER I, OBLAK M, SOLMAJER T & JERALA R. 2003. Characterization of quercetin binding site on DNA gyrase. BBRC 306: 530-536.

SAI C & PRAKASH I. 2011. Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel-A Review. IJRCE 1: 1-18.

SANCHEZ M, PENA M, REVILLA G & ZARRA I. 1996. Changes in dehydrodiferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pinus pinaster hypocotyl*. Plant Physiol 111: 941-946.

SARKHOSH A, ZAMANI Z, FATAHUI R & EBADI A. 2006. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum L.*) genotypes. Sci Hortic 111: 24-29.

SEPÚLVEDA-JIMÉNEZ G. 2003. La participación de los metabolitos secundarios en la defensa de las plantas. Rev Mex Fitopatol 21: 355-362.

SYED D, AFAQ F & MUKHTAR H. 2007. Pomegranate derived products for cancer chemoprevention. Semin Cancer Biol 17: 377-385.

TEZCAN F, GÜLTEKIN-ÖZGÜVEN M, DIKEN T, ÖZÇELIK B & ERIM B. 2009. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chem 115: 873-877.

THAIPONG K, BOONPRAKOB U, CROSBY K, CISNEROS-ZEVALLOS L & HAWKINS D. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos 19: 669-675.

VILELA A, GONZÁLEZ-PALEO L & RAVETTA D. 2011. Metabolismo secundario de plantas leñosas de zonas áridas: mecanismos de producción, funciones y posibilidades de aprovechamiento. Ecol Austral 21: 317-327.

VIUDA-MARTOS M, FERNÁNDEZ-LÓPEZ J & PÉREZ-ÁLVAREZ J. 2010. Pomegranate and its many functional components as related to human health: A Review. Comprehensive Reviews in Rev Food Sci Food Saf 9: 635-654.

WU T, HE M, ZANG X, ZHOU Y, QIU T, PAN S & XU X. 2013. A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828: 2751-2756.

YASOUBI P, BARZEGAR M, SAHARI M & AZIZI M. 2007. Total phenolic contents and antioxidant activity of pomegranate (*Punica granatum L.*) peel extracts. J Agric Sci Technol 9: 35-42.

ZAREI M, AZIZI M & BASHIR-SADR Z. 2011. Evaluation of physicochemical characteristics of pomegranate (*Punica granatum L.*) fruit during ripening. Fruits 66: 121-129.

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