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Determination Of Biologically Active Substances And Natural Antioxidants In The Juice Of Two Local Pomegranate Fruits

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Abstract: ***This study was conducted in the laboratories of Tikrit University / College of Agriculture / Department of Food Sciences, and it aimed to study the effect of some chemical and physical characteristics, activity, concentrations and stability of antioxidants present in sweet and sweet-sour pomegranate juice, as well as determining the proportions of phenolic compounds, sugars, vitamins and ascorbic acid concentration. The results showed a higher moisture content for sweet pomegranate juice, and a lower percentage of total soluble solids (TSS) for sweet pomegranate juice compared to sweet-sour pomegranate juice. Sweet pomegranate juice also recorded a significant increase in total sugars content and PH. sweet-sour had the highest value in citric acid, while sweet-sour pomegranate juice recorded the highest value in electrical conductivity (EC) which amounted to 3481, as well as a high level in density (1.101) g/ml. Whereas sweet-sour pomegranate juice gave the lowest viscosity value, which was 1.65 centipoise, and there was a discrepancy in the vitamin content, where vitamin B9 recorded the highest content in sweet pomegranate juice with a value of 23.35 mg/100 ml, followed by vitamin B2, then vitamin B6, and finally vitamin B12 with a value of 5.30 mg /100 ml, and sweet pomegranate juice gave the highest value in anthocyanin pigment content, as well as the highest content in the total phenolic compounds, reaching 30.65 mg/100 ml and 54.20 mg/100 ml respectively. and when cold storage for a period of 6 weeks, no significant differences were observed in the values of TSS and PH values for both juices in the first and second weeks, while the differences appeared clear at the beginning of the third week, and sweet-sour pomegranate juice recorded a noticeable decrease in the value of PH at the fourth and fifth weeks, and this decrease was most severe at the fifth week for both juices, especially in sweet-sour pomegranate juice.***

Keywords: Biologically active substances, Antioxidants, Pomegranate juice, Anthocyanins.

1. INTRODUCTION

The pomegranate is a summer fruit of the Myrtales order, and it belongs to the Punicaceae family and its scientific name is *L. Punica granatum*, its cultivation spread to the Levant, the Arabian Peninsula and India, then moved to other countries such as Spain and California. Natural pomegranate juices are considered one of the most popular types of juices in the world, especially the Middle East, where they are considered an important source of vitamins, including vitamin C, group B vitamins and phenolic compounds that are considered One of the most important natural antioxidants (Lansky, 2007), The fruits of pomegranate and orange differ in the proportions of their components, the active substances and the antioxidants in their composition, and they are considered an ideal healthy and protective

food prepared for the body to benefit directly from it, being of high nutritional value, as it is a food and a medicine at the same time. Smail et al., (2009) mentioned that pomegranate juice is gaining importance due to the potent activities of antioxidants, and this was confirmed by (Bassis, 2014). For this reason, the research aimed to study the active substances and natural antioxidants present in two types of pomegranates, sweet and sour pomegranate, and to study some of their chemical and physical properties.

2. MATERIALS AND METHODS

Materials:

The pomegranate fruits were obtained from the local market, and they were ripe and suitable for juice production. and pure chemicals (Chemical grade) were used, produced by the English company BDH.

Working Methods:

Production of natural pomegranate drink:

The fruits of the pomegranate sourced from the local markets were used, and they were cleaned of dust and suspended matter, then washed with water, and the damaged and immature fruits were isolated from them. Then it was prepared and squeezed separately in a manual fruit juice machine, after that the resulting juice was collected and packaged in airtight glass bottles (pre-sterilized with their lids), pasteurized at 74ºC for 20 seconds, then cooled quickly and kept by refrigeration at 5ºC.

Vitamin's determination

Vitamins were estimated using HPLC model (SYKAM) Germany, according to what was mentioned in (A. O. A C., 2004).

Determination of the total polyphenols content

The concentrations of the total phenol content of functional beverage samples were estimated using the Folin Ciocalteu reagent according to what was mentioned in the method of (Singleton and Slinkard, 1997).

Estimation of anthocyanins:

The anthocyanins were estimated in samples of natural pomegranate juices according to what was mentioned in (A. O. A C., 2004).

Chemical and physical tests:

Several important chemical and physical tests were carried out, including estimating the percentage of total dissolved solids using the Hand Refractometer, and the percentage of total solids determination using the electric drying oven method, in addition to measuring the percentage of total acidity using the titration method and calculating the volume pH Determination using a PH meter and electrical conductivity using an E.C. meter and total dissolved salts using a TDS meter. Estimation of Relative Viscosity Determination using the Ostwald device, Determination of Relative Density using a pycnometer bottle, and Moisture Determination using an electric drying oven, all of the above tests were estimated according to what was stated in (A. O. A C., 2004).

statistical analysis:

The experiments were carried out using the CRD design and according to the significant differences between the means at the probability level (0.05) according to the ready-made statistical program Spss and according to the correlation factors.

3. RESULTS AND DISCUSSION

Chemical tests:

Table (1) shows the results of the characteristics of moisture, total soluble solids, and total solids

treatment average	(T.S.) %		treatment average	(T.S.S) %		treatment average	Moisture %		Properties / Plant
	T2	T1		T2	T1		T2	T1	
19.5 b	20	19	**18.0 b**	19	17	**85.0 a**	87	83	**sweet pomegranate**
23.0 a	22	24	**21.0 a**	20	22	**81.0 b**	80	82	**sweet-sour pomegranate**
	21.0 a	**21.5 a**		**19.5 a**	**19.5 a**		**83.5 a**	**82.5 a**	**treatment average**

Table (1) shows the chemical tests in the juice of two types of pomegranates: sweet and sweet-sour, where the results showed significant differences at the probability level of 5% in the moisture content of both sweet and sweet-sour pomegranate juices. The tests also showed slight significant differences in the concentrations of Total Soluble Solids (TSS), while sweet-sour pomegranate juice recorded a significant increase in the content of Total Soluble Solids (TSS) and also water-insoluble Total Solids (TS).

Note that the expected moisture content in fresh pomegranate fruits, dry pomegranate fruits and peel powder ranges between (77-78.2%), (4.5-14.7%) and (18.23%), respectively, and this discrepancy in chemical properties is due to the use of different types of pomegranates (Blandinoet al., 2003), and (Kedia et al.,2007) indicated that there are several biochemical changes that lead to a decrease in the level of simple carbohydrates and indigestible polysaccharides according type.

Table (2) shows the total sugars concentration, pH and total acidity

treatment average	total acidity (acid **citric)**		treatment average	**pH**		treatment average	total sugars **%**		Properties / Plant
	T2	T1		T2	T1		T2	T1	
1.09 b	1.10	1.08	**3.56 a**	3.54	3.58	**18.3 a**	19.7	16.9	**sweet pomegranate**
2.26 a	2.24	2.28	**2.98 b**	2.97	2.99	**13.3 b**	13.1	13.5	**sweet-sour pomegranate**
	1.67 a	**1.68 a**		**3.26 a**	**3.29 a**		**16.4 a**	**15.2 a**	**treatment average**

The results of Table (2) for the juice of the studied pomegranate cultivars showed a discrepancy in the total sugar content of the studied pomegranate juices, where sweet pomegranate juice gave a higher concentration compared to sweet-sour pomegranate juice in order to increase the concentration of fructose sugar in the juice produced, and the pH values of the produced juices ranged between (2.99-3.58) and these values are an indication that

pomegranate juice falls within the degree of acidic medium, and this is consistent with what was reached by Fadavi and others (2005).
Total acidity comes in the second place in terms of importance after total soluble solids (TSS), which determines the specifications of any type of pomegranate, as its percentage ranged between (1.10-2.24), and in a study conducted by Lansk et al., (2007) I used pomegranate juice and its fruits It is widely and extensively used in therapeutic medicines in many countries, due to its importance to the fact that it is a medicinal plant that is limited by the data collected from modern science research.

Physical tests:

Table (3) shows the results of electrical conductivity characteristics, total dissolved salts, and viscosity characteristics.

Table (3) shows the physical tests of sweet and sweet-sour pomegranate juices, where the

treatment average	Viscosity centipoise		**treatment average**	consistency g/ml		**treatment average**	TDS ppm		**treatment average**	E.C. µs		Properties / juice type
	T2	T1			T1		T2	T1		T2	T1	
1.85 a	1.8	1.9	1.087 a	1.086	1.088	206.0 b	205	207	3320 b	3318	3322	**sweet pomegranate**
1.65 b	1.6	1.7	1.101 a	1.100	1.101	274.5 a	273	276	3481 a	3480	3482	**sweet-sour pomegranate**
	1.7 a	1.8 a		1.093 A	1.095 a		239.0 a	241.5 a		3399 a	3402 a	**treatment average**

results of the tests showed significant differences between the results of electrical conductivity (EC) for both types, and the results also showed significant differences between the values of the total salt content -Total dissolved solids-(TDS), as given Sweet pomegranate juice had a higher percentage in the total salt content compared to sweet-sour juice, while no significant differences were recorded in the consistency content for both juices.
While sweet pomegranate juice recorded a higher percentage in viscosity compared to sweet-sour juice. The electrical conductivity test was successfully used in a study conducted by Al-Fahdawi, (2011) to produce functional drinks from some types of medicinal herbs. Significant differences were observed between drinks during the study period; The results also agreed with a study conducted by Hamad et al., (2018) when they tested several therapeutic drinks.

Table (4) shows the concentrations of vitamin B group and ascorbic acid (vitamin C).

treatment average	B6		**treatment average**	B2		**treatment average**	B1		Properties / juice type
	T2	T1		T2	T1		T2	T1	
14.85 a	14.8	14.9	18.05 A	18.0	18.1	0.0	0	0	**sweet pomegranate**
12.95 b	12.9	13.0	17.25 B	17.2	17.3	0.0	0	0	**sweet-sour pomegranate**
	13.85 a	13.95 a		17.6 a	17.7 a		0.0	0.0	**treatment average**

(4) Table

treatment average	Ascorbic acid mg/100 ml		**treatment average**	B12		**treatment average**	B9		Properties / juice type
	T2	T1		T2	T1		T2	T1	
17.75 a	17.7	17.8	7.05 a	7.0	7.1	23.35 a	23.3	23.4	**sweet pomegranate**
18.05 a	18.0	18.1	5.30 b	5.2	5.4	21.70 b	21.6	21.8	**sweet-sour pomegranate**
	17.85 a	17.95 A		6.10 a	6.25 a		22.5 a	22.6 a	**treatment average**

From Table (4) it is clear that the percentage of vitamin B1 for sweet and sweet-sour pomegranate juice is zero%, while vitamin B2 showed significant differences between the two juices, and the percentage was more high in sweet pomegranate juice, while the percentage of vitamin B6 decreased in sweet pomegranate juice -sour, significant differences were also recorded in the content of vitamin B9 for both juices, as well as B12, while the highest concentration of ascorbic acid appeared in sweet-sour pomegranate juice. Luzio and Nawawi, (2004) explained in a study conducted for some pomegranate cultivars that citric and malic acid are the predominant organic acids in the juice. Dykes and Rooney, (2007) confirmed that most of the phenolic substances are usually pigments and vitamins, and in a study to estimate some traits and biologically active substances In some types of natural juices, which ranged between (31.53-43.89) mg/100 ml, the result of estimating vitamin C was lower than what Khalid et al. (2013) indicated.

In another study for the estimation of vitamin C in some types of juice, the results of the study were somewhat consistent with what was indicated by Al-Bayati, (2011), which ranged between (44.69-53.77) mg / 100 ml, and between Afaq and others, (2005) that pomegranate juice is a product It is rich in sugars and phenolic compounds such as tannins and anthocyanins that have high antioxidant activity, and sweet pomegranate is rich in many important compounds, including ascorbic acid and the vitamin B group in addition to vitamin A. It is one of the fruits rich in important nutrients that the body needs (Khaled et al., 2013).

Table (5) concentrations of anthocyanins, total phenolic compounds, and results of the test for DPPH %.

treatment average	DPPH %		**treatment average**	Total phenolic substances mg/100 ml)		**treatment average**	anthocyanins (mg/100 ml)		Properties / juice type
	T2	T1		T2	T1		T2	T1	
3.43 a	3.42	3.44	54.20 A	54.1	54.3	30.65 a	30.6	30.7	**sweet pomegranate**

2.95 b	2.89	3.00	47.95 B	47.9	48.0	22.45 b	22.4	22.5	**sweet-sour pomegranate**
	3.16 a	3.22 a		51.0 a	51.2 a		26.5 a	26.6 a	**treatment average**

Table (5) shows the concentrations of anthocyanin pigment and the antioxidant activity and activity for both types of juices. Significant differences were noted for the anthocyanin pigment content for both juices. The highest value of this pigment was recorded in sweet pomegranate juice, reaching 30.7, and significant differences were recorded in the phenolic content of both juices. The sweet-sour pomegranate juice recorded a decrease in the phenolic content, and the pomegranate fruit is a rich source of many phenolic compounds such as flavonoids, anthocyanins and others (Lansky, 2007), Afaq et al., (2005) also mentioned that pomegranate is a rich source of many phenolic compounds, while no significant differences were recorded when measuring the antioxidant activity in both juices. Yuha et al., (2008) stated that the extract of pomegranate fruit contains strong antioxidants and that most of them Of the phenolic substances, they usually represent pigments and vitamins (Dykes and Rooney, 2007), and the reason for this is due to the importance of the biological compounds that make up these products, and that most of the components of pomegranate fruits are used in the treatment of many infections.

Table (6) TSS and pH test results. The juices were stored by refrigeration for six weeks at 5ºC.

pH				TSS				Properties
sweet-sour pomegranate		sweet pomegranate		sweet-sour pomegranate		sweet pomegranate		Storage period
T2	T1	T2	T1	T2	T1	T2	T1	
2.99 bA	3.00 bA	3.55 aA	3.56 aA	19.30 aA	19.28 aA	18.20 bA	18.18 bA	First day
2.97 bA	2.98 bA	3.54 aA	3.55 aA	19.30 aA	19.25 aA	18.20 bA	18.19 bA	first week
2.93 bA	2.92 bA	3.51 aA	3.53 aA	19.30 aA	19.27 aA	18.18 bA	18.18 bA	second week
2.88 bA	2.87 bA	3.48 aA	3.50 aA	18.29 aB	18.30 aB	18.16 aA	18.16 aA	Third week
2.81 bAB	2.79 bAB	3.45 aA	3.47 aA	18.29 aB	18.31 aB	18.15 bA	18.14 bA	Fourth week
2.72 bB	2.70 bB	3.43 aA	3.45 aA	18.28 aB	18.27 aB	18.12 bA	18.11 bA	Fifth week
2.60 bB	2.58 bB	3.41 aA	3.42 aA	18.27 aB	18.28 aB	18.10 bA	18.09 bA	Sixth week

Small letters that are similar horizontally mean that there are no significant differences between them

Capital letters that are similar vertically mean that there are no significant differences between them

Table (6) shows the effect of cold storage for a period of six weeks at a temperature of 5 C on the total soluble solids in sweet and sweet-sour pomegranate juices. where it is noted that in the first week, no significant differences were recorded in the value of Total Soluble Solids (TSS) for both juices, as well as for the second week, while a significant decrease of these values was recorded starting from the third week for both juices, while sweet pomegranate juice witnessed a significant decrease in the fourth week more of sweet-sour pomegranate juice, as well as at the fifth week, and this decrease was most evident in the sixth week, with values of 18.09 and 18.25, respectively, for both juices.

From the same table, we note that no significant differences appeared at the first and second week in the PH value of both juices, while the differences appeared clear at the third week, while sweet-sour pomegranate juice recorded a significant decrease in the PH value at the fourth week as well as in the fifth week. This decrease was most severe at the fifth week for both juices, especially sweet-sour pomegranate juice.

4. CONCLUSIONS

1- The results showed significant differences in the moisture content of sweet and sour pomegranate juices, as well as the Total Soluble Solids (TSS) concentrations.
2- Significant differences in electrical conductivity appeared in the total salt content of both juices.
3- The percentage of vitamins varied in sweet and sweet-sour pomegranate juice, as the highest concentrations of ascorbic acid appeared in sweet-sour pomegranate juice.
4- Sweet pomegranate juice gave the highest value in terms of its anthocyanin content, while sweet-sour pomegranate juice recorded a decrease in phenol content.
5- When the juices were stored by refrigeration for a period of six weeks, there were no significant differences between the values of total soluble solids content during the first and second week, and the decrease occurred in the third and fourth week, as was the case for pH.

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