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Evolution of sugars and organic acid content in three pomegranate cultivars (*Punica granatum* L.)

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SUMMARY – The aim of the present work has been to study evolution of sugars and organic acids during development and ripening of pomegranate. The fruits analysed belong to the cultivars: ME5, ME17 and MO6. The investigation was carried out in 1997. Fruits were previously labelled in the same phenological stage and in the four tree orientations. Then they were analysed by HPLC fortnightly. pH and soluble solid changes were also tested. As a result of the study we can conclude that the predominant sugars are fructose and glucose with predominance of the latter. Saccharose and maltose contents are practically negligible. In relation to organic acids, malic acid is present in greater quantity than citric acid in some cultivars and vice versa in others. pH stabilises early in the development of the pomegranate. When approaching the ripening period a positive increase in the amount of soluble solids can be observed.

Key words: Pomegranate tree, sugars, organic acids, ripening.

RESUME – *"Evolution de la teneur en sucres et en acides organiques chez 3 cultivars de grenadier (*Punica granatum *L.)". Le propos du présent travail a été d'étudier l'évolution des sucres et des acides organiques pendant le développement et le mûrissement des grenades. Les fruits analysés appartiennent aux cultivars : ME5, ME17 et MO6. La recherche a été menée en 1997. Les fruits étaient étiquetés au préalable à même stade phénologique et dans les quatre orientations de l'arbre. Ils étaient ensuite analysés par HPLC tous les quinze jours. Le pH et les changements en solides solubles étaient également testés. Comme résultat de l'étude nous pouvons conclure que les sucres prédominants sont le fructose et le glucose avec une prédominance du dernier. Les teneurs en saccharose et maltose sont pratiquement négligeables. En ce qui concerne les acides organiques, l'acide malique est présent en plus grande quantité que l'acide citrique chez certains cultivars et vice versa chez d'autres. Le pH se stabilise tôt dans le développement de la grenade. Lorsque la période de maturité approche, on peut observer une augmentation positive de la quantité de solides solubles.*

Mots-clés : *Grenadier, sucres, acides organiques, mûrissement.*

Introduction

The pomegranate is a non-climacteric fruit, which is why it should be harvested when it is ripe to taste (Ben Arie *et al*., 1984; Kader *et al*., 1984; Melgarejo, 1993).

In order to determine the optimum time for harvest, indices can be used such as external colour, soluble solid content (SS), assessable acidity (A) and ripeness or maturity index (IM) (Ben Arie *et al.*, 1984; Chace *et al.*, 1984; El-Sese, 1988; Celdrán, 1988; Sharma and Sharma, 1990; Artés, 1992; Paños, 1992, cited in Melgarejo, 1993; Melgarejo, 1997). For some varieties such as the Wonderful, both SS content (Israel) and acidity A (USA) have been used as a ripeness index. In Spain, the external colour has been traditionally used to decide upon the best harvest time, and more recently the ripeness index.

The parameters mentioned previously are determined by the sugar content and the organic acid in the seeds that make up the edible part of this fruit.

Due to the reasons put forward, the objective of this work is to study the evolution of the indices determining the optimum harvest time.

Materials and methods

In order to study the previously mentioned parameters, fruit from the pomegranate clones ME5, ME17 and MO6, was picked weekly and kept separate according to its orientation on the tree. These clones had been grown in homogeneous conditions in the experimental plot of the Polytechnical School of Orihuela (University Miguel Hernández). The fruit of these varieties were characterized as sweet and presented good organoleptic characteristics, being classified as recommendable and very recommendable (Melgarejo, 1993).

Once harvested, the kernels were removed from the pomegranates and the seed mixture homogenized. A sample was then taken and juice was extracted using a Moulinex liquefier.

Determination of sugars and organic acids

The sugar and organic acid content was determined by centrifuging the juice for 20 minutes at 14,000 rpm using a Selecta centrifuge, model 540.

The analysis was carried out by using a Hewlett Packard high-resolution liquid chromatography apparatus (HPLC), model 1,100 using a C18 column (12.5×0.4 cm; with a particle size of 5 µm).

The mobile phase used for the determination of sugars was water and acetonitrile (18:82). The determinations were carried out with an injection flow of 1.5 ml/min and a pressure between 100-103 bars. The refraction index was measured with a Hewlett Packard 1047A RI Detector.

The mobile phase used for the determination of organic acids was a solution of KH_2PO_4 0.01 M taken to pH 2.25 with H_3PO_4. The injection flow was 1 ml/min with a wavelength of 206 nm.

Determination of the pH and soluble solids

The evolution of the pH was measured by taking part of the liquefied juice using a Crison pH metre; model micro pH 2001 fitted with a temperature-compensating electrode. The total of soluble solids was measured using an Atago N1 refractometer, expressing the result in °Brix at 20°C.

Results and discussion

Evolution of the sugar and organic acid content

In the sugar content analysis, distinction has been made between fructose, glucose, saccharose and maltose which have been identified as the main sugars in pomegranate juice (Melgarejo, 1993). Table 1 shows the quantities in average percentages corresponding to each sugar throughout the period of the experiment. It can be observed how glucose is a more predominant sugar than fructose and in some cases by a significative difference. The quantities of saccharose and maltose are negligible.

The results of the fruit analysis are not shown according to the different orientations, as significative differences have not been obtained.

In previous studies carried out by different authors on the sugar content of pomegranate juice, these are not analysed separately but are grouped together according to whether they are reducing or non-reducing (Khodade *et al.*, 1990; Sharma and Sharma, 1990; El-Nemr *et al.*, 1992). However, the results agree that the non-reducing sugars appear as traces whereas the reducing sugars are quantitatively more important.

As regards the average content of organic acids in pomegranate juice, the results are shown in Table 2. This indicates how in the three clones studied, neither tartaric acid nor fumaric acid have been observed. Malic acid is the most predominant, followed by citric acid.

According to Tamaro (1984), the predominant acid of this fruit is malic acid. In studies carried out with the Mollar variety (Melgarejo, 1993), it is shown that many clones studied present similar quantities of malic and citric acid. The content of malic acid ranged between 0.143% and 0.249% and the citric acid between 0.147% and 0.4%, results which agree with those obtained in this experiment.

Table 1. Average sugar content in pomegranates

		Fructose	Glucose	Saccharose	Maltose	Total
ME5	15/09/97	5.994	7.643	0	0	13.637
	29/09/97	6.736	8.446	0	0	15.182
	13/10/97	6.282	7.657	0	0	13.939
	27/10/97	7.013	8.665	0	0	15.678
	10/11/97	5.701	7.725	0.045	0	13.471
ME17	15/09/97	6.577	8.479	0	0	15.056
	29/09/97	6.254	8.093	0	0	14.347
	13/10/97	5.885	7.517	0	0	13.402
	27/10/97	7.322	7.746	0.047	0.045	15.16
	10/11/97	7.064	7.44	0.425	0.172	15.101
MO6	15/09/97	6.766	8.418	0	0	15.184
	29/09/97	6.297	8.073	0.028	0	14.398
	13/10/97	6.753	8.951	0.03	0	15.733
	27/10/97	6.869	8.95	0.022	0	15.841
	10/11/97	6.895	9.298	0.022	0	16.215

Table 2. Average organic acid content according to orientation (%)

		Oxalic	Tartaric	Malic	Ascorbic	Acetic	Citric	Fumaric	Total
ME5	North	0.021	0.000	0.257	0.008	0.031	0.214	0.000	0.532
	South	0.017	0.000	0.244	0.010	0.016	0.215	0.000	0.502
	East	0.017	0.000	0.240	0.007	0.072	0.181	0.000	0.516
	West	0.016	0.000	0.194	0.009	0.013	0.152	0.000	0.384
ME17	North	0.023	0.000	0.210	0.004	0.019	0.110	0.000	0.367
	South	0.017	0.000	0.216	0.008	0.006	0.107	0.000	0.355
	East	0.016	0.000	0.219	0.008	0.076	0.133	0.000	0.452
	West	0.013	0.000	0.181	0.005	0.057	0.191	0.000	0.447
MO6	North	0.027	0.000	0.277	0.008	0.024	0.157	0.000	0.493
	South	0.017	0.000	0.224	0.007	0.013	0.126	0.000	0.387
	East	0.021	0.000	0.215	0.007	0.019	0.307	0.001	0.570
	West	0.019	0.000	0.288	0.012	0.035	0.120	0.002	0.476

The graphs in Fig. 1 show the distribution of the different organic acids according to orientations. It can be deduced that both the contents of the different acids and the total acid content present significant differences according to each clone. Thus, for example, the total organic acid content for ME5 presents decreases as low as 27.82%.

Evolution of the pH and soluble solids

Figure 2 represents the average value of soluble solids throughout the duration of the trial, showing that they remain practically constant during the ripening process (from the end of September onwards).

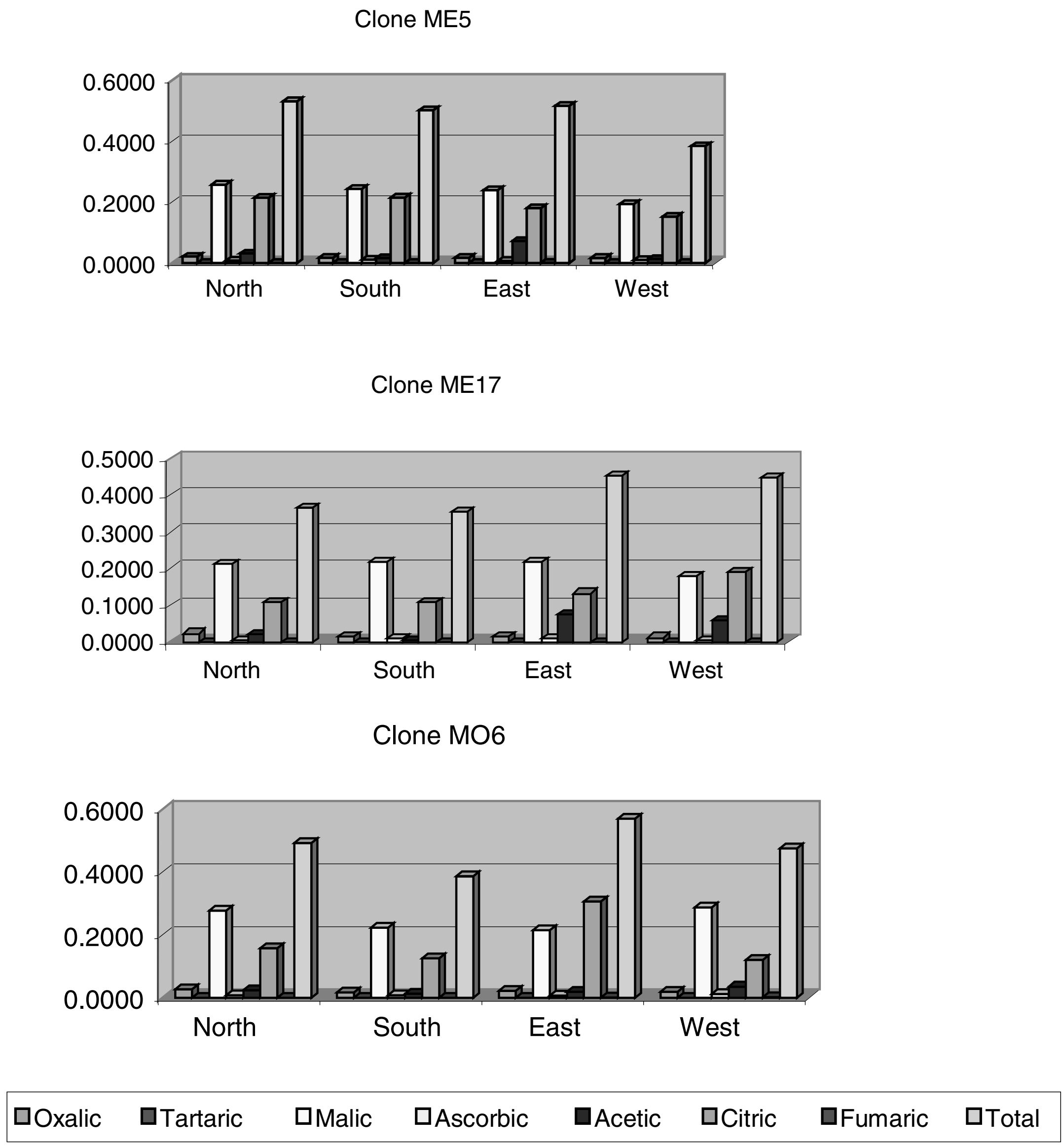

Fig. 1. Average organic acid content according to orientation (%).

These results agree with those obtained by Shulman *et al*. (1984) with the varieties 'Mule´s Head' and 'Wonderful', where significant differences did not occur in the later stages of ripening. Likewise, Ben-Arie *et al.* (1984) observed an increase in soluble solids during the ripening of the 'Wonderful' variety, but this increase was not significative during the ripening period.

Regarding the pH values reached (Fig. 3), small variations can be observed that are possibly due to sampling errors, ranging from 3.60 to 4.15 during the development and ripening of the fruit.

Conclusions

(i) In the clones studied, the main sugars are glucose and fructose, with insignificant content of maltose and saccharose.

(ii) In the clones of the variety Mollar, neither tartaric nor fumaric acid is detected, malic acid being the most important organic acid followed by citric acid.

(iii) The soluble solid content remains stable after the end of September, reaching values near 15%.

(iv) The pH does not present great variations during the development and ripening of the fruit, ranging between 3.60 and 4.15.

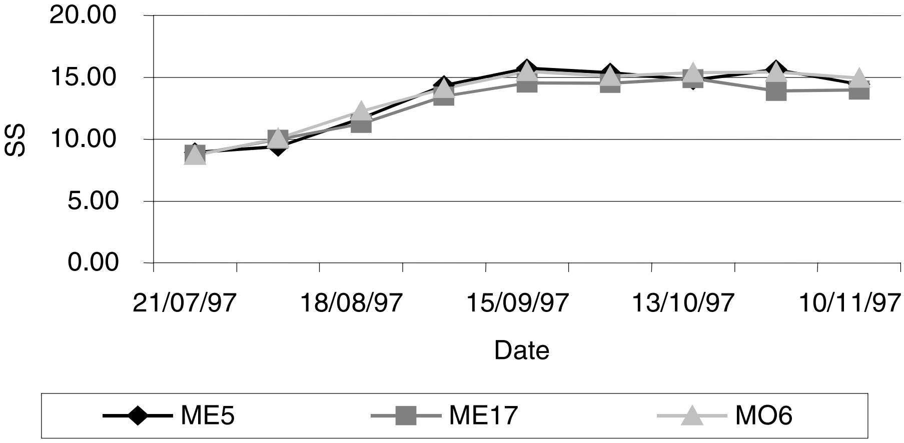

Fig. 2. Average evolution of SS in different clones (%).

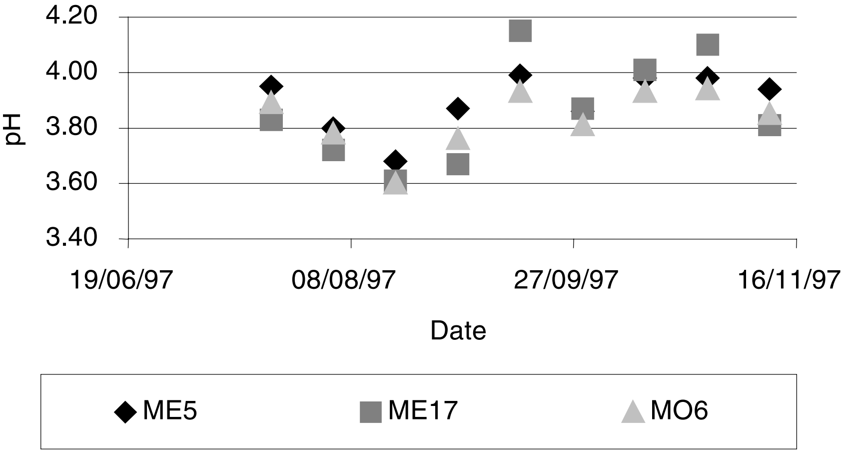

Fig. 3. Average evolution of pH in different clones.

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