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Use of ion chromatography for the measurement of organic acids in fruit juices

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Abstract

A gradient ion chromatographic method to separate and determine main organic acids in fruit juices was developed. The method allows the separation of organic anions on Dionex OMNI PAC PAX-500 column by NaOH gradient elution and conductometric detection. The main organic acids of fruit juices (citric, malic, tartaric) were separated together with other less abundant acids. More than 500 samples of fruit juices were analysed, mainly orange juices from Brasil, Italy, Florida, California, as well as apple, cherry, grape and blackcurrant juices of different origin. The method has shown a high sensitivity and a satisfactory accuracy. The method also allows the simultaneous separation of lactic and acetic acids, produced by microbiological spoilage of juices, and of some important inorganic anions such as chlorides and nitrates.

1. Introduction

The identification and dosing of the various organic acids present in a fruit juice are of considerable importance, since they provide at the same time useful information regarding not only the authenticity of the product under examination but also regarding any processes of microbiological alteration it may have undergone previously.

At present, organic acids are measured [1–7] using enzymatic methods or liquid chromatographic techniques. Enzymatic analyses, however, require specific kits for each individual organic acid, they are rather time-consuming and use large amounts of reagents. The traditional HPLC techniques with refraction index or UV

detection not always allow the separation of minor organic acids and often require preventive purification techniques to eliminate, for example, the interference of sugars or phenolic compounds, in particular the anthocyanines in red juices. This has a negative influence on the simplicity and rapidity of the method, especially evident in the case of routine quality control analysis. The introduction of conductivity detectors combined with ion chromatography with chemical suppression, has eliminated many of the above mentioned problems. Initially, this technique was applied above all to the analysis of inorganic ions [8–15], but recently methods suitable for the measurement of organic anions have also been developed [16,17].

The aim of the present work was thus to optimize the method proposed for wine samples by Kupina et al. [17] in order to develop a reliable and highly sensitive method allowing the

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measurement not only of the main organic acids present in fruit juices (citric, malic and tartaric) but also of the minor acid compounds (galacturonic, isocitric, lactic and acetic) without the need for previous purification procedures.

2. Experimental

2.1. Instrumentation

The chromatographic system consisted of three Model 306 pumps with titanium head, a Model 805 electronic dumper, a Model 811C dynamic mixer, a Model 231 automated sample-injector (all from Gilson Medical Electronics, France), and a Model 431 conductivity detector (Waters, Milford, MA, USA).

The following columns and accessories were from Dionex Corp (Sunnivale, CA, USA): OmniPac Pax-500 Guard Column (P/N 42153) 50 × 4 mm I.D., OmniPac Pax-500 Column (P/N 42152) 250 × 4 mm I.D., eluent purification column Ion pac ATC-1 (P/N 37151).

Chemical suppression was achieved by the Anion Micromembrane Suppressor AMMS-II (P/N 43074) with about 50 mM H₂SO₄ regenerant at a flow-rate of approximately 2 ml/min.

Acquisition and integration of chromatograms was performed with an AT 386 personal computer linked with a Gilson GSIOC 506 C system interface.

2.2. Chemicals

The eluents were prepared using redistilled water, anhydrous ethyl alcohol (J.T. Baker, Philipsburg, NJ, USA), methyl alcohol (HPLC grade, Carlo Erba, Milan, Italy) and 50% NaOH (J.T. Baker).

The samples were calibrated using solutions prepared with citric, malic, tartaric and acetic acids (Carlo Erba), D-isocitric, shikimic and lactic acids (Sigma, St. Louis, MO, USA), galac-

turonic acid (Merck, Darmstadt, Germany) and quinic acid (Fluka, Buchs, Switzerland).

The method under evaluation also allows the simultaneous separation of inorganic anions; for this purpose, standard mixtures were prepared containing chlorides (KCl), phosphates (NaH₂PO₄·H₂O), nitrates (NaNO₃) and sulfates (Na₂SO₄) (Carlo Erba).

For all anions, stock solutions of 1000 mg/l were prepared weekly in redistilled water and, from these, mixtures were made with a concentration suitable to construct calibration curves over a linear range, depending on the amounts naturally occurring in the juices examined.

2.3. Mobile phases

Ternary gradient elution with a 1 ml/min flow-rate was used with the following mobile phases: Eluent A, 0.60 mM NaOH in water–ethanol–methanol (66.5:20:13.5, v/v); eluent B, 20 mM NaOH in water–ethanol (65:35, v/v); eluent C, 60 mM NaOH in water–ethanol (65:35, v/v). A linear gradient was used as shown in Table 1.

All solvents were filtered (0.45 μm) and degassed in an ultrasonic bath under vacuum for 5 min.

The operating conditions were: room temperature; injection volume, 20 μl; conductivity detector, 10 μS FS.

Table 1
Eluent gradient for ion chromatography

| Time (min) | Eluent A (%) | Eluent B (%) | Eluent C (%) |
|------------|--------------|--------------|--------------|
| 0 | 100 | 0 | 0 |
| 4 | 100 | 0 | 0 |
| 15 | 0 | 100 | 0 |
| 19 | 0 | 100 | 0 |
| 27 | 0 | 0 | 100 |
| 39 | 0 | 0 | 100 |
| 40 | 100 | 0 | 0 |

Equilibration time between each injection: 15 min.

2.4. Sample preparation

Around 500 samples of orange, grapefruit, apple, grape, cherry and blackcurrant juices were taken into consideration. Particular attention was given to orange juices (ca. 60% of the samples), subdivided with respect to provenance area and extraction technology (first and second pressing juices). All samples were diluted with redistilled water to obtain organic acid concentrations falling within the linear range of the calibration curves, filtered with 0.45- μm filters and frozen at -18°C up to the time of analysis.

3. Results and discussion

Reproducibility was tested by performing six analyses of a standard mixture containing the various organic acids. The data summarized in Table 2 show a good reproducibility.

Optimization of the analytical conditions resulted in the separation of a large number of organic and inorganic anions, as shown in Fig. 1; the concentrations of the various anions and their relative retention times are reported in Table 3 together with the determination limits.

The chromatograms presented in Figs. 2–4 show the profiles characteristic of the separation of organic and inorganic anions for orange, apple and grape juices; it can be seen that ion chromatography allows quantitation of the acids under study with acceptable sensitivity, despite the

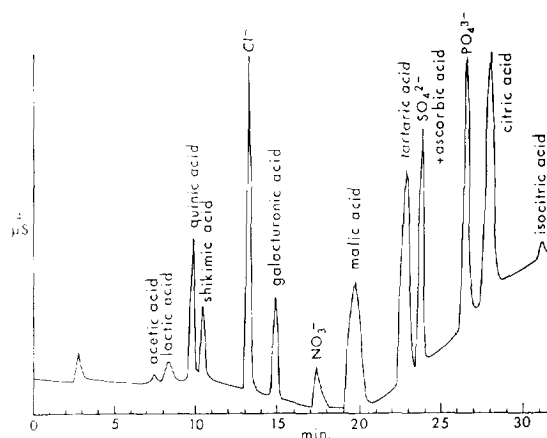


Fig. 1. Chromatogram of organic and inorganic anion standard solution.

complexity of the matrices and the considerable quantitative imbalances between the various anions.

The differences in composition for the organic acids can be clearly seen in Tables 4–8, each of which refers to an individual organic acid.

As to the characterization of the examined orange juices, particular attention was paid not only to the data regarding citric, malic and isocitric acids, but also to the citric acid/malic acid and citric acid/isocitric acid ratios; these data were subsequently related to provenance area and extraction technology.

Table 2
Reproducibility tested on standard solutions ($n = 6$)

| | Concentration (mg/l) | S.D. | R.S.D. (%). |
|----------------|-------------------------|------|----------------|
| Citric acid | 750 | 22 | 2.88 |
| Malic acid | 200 | 3 | 1.55 |
| Isocitric acid | 10.0 | 0.2 | 2.04 |
| Tartaric acid | 750 | 15 | 1.94 |

S.D. = standard deviation; R.S.D. = relative standard deviation.

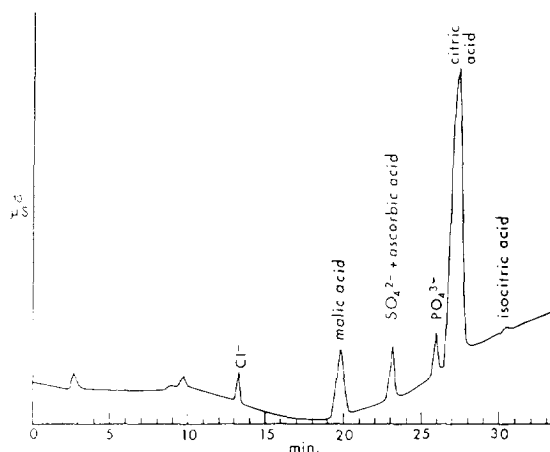


Fig. 2. Chromatogram of orange juice. Sample dilution 1:10.

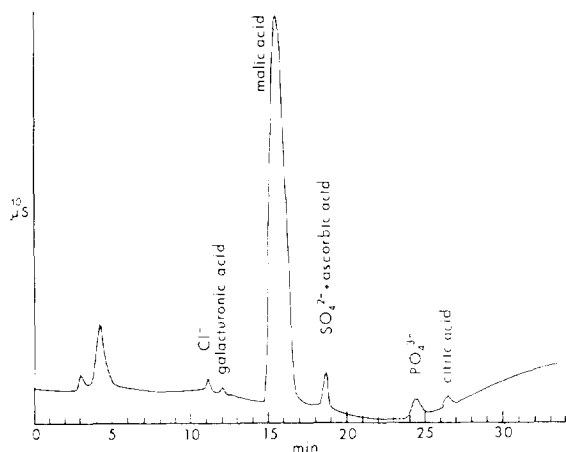


Fig. 3. Chromatogram of apple juice. Sample dilution 1:10.

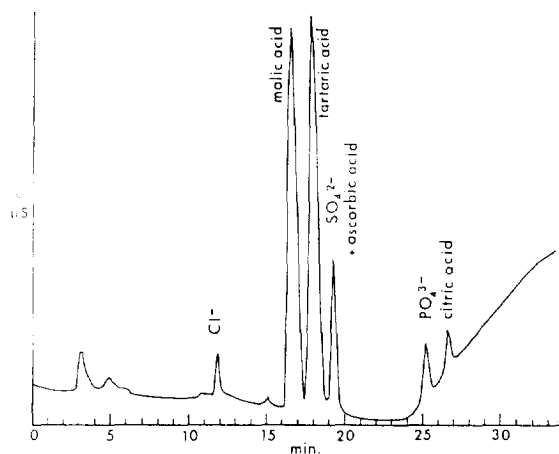


Fig. 4. Chromatogram of grape juice. Sample dilution 1:25.

The citric acid content (Table 4) shows considerable differences between samples based on provenance area, variety and extraction technology: juices coming from the Americas (Argentina, Brazil, Uruguay, Cuba, Florida and California) are characterized by a citric acid mean value substantially lower than that found in juices from the Mediterranean area (Morocco, Egypt, Israel) but above all in those of Italian provenance, which are characterized by high values. Extraction technology also influences the citric acid content, which is higher in first pressing juices; furthermore, juices of Italian origin

obtained from pigmented orange varieties show lower values than those obtained from oranges of the "Biondo comune" variety.

For isocitric acid (Table 5), an analogous behaviour to that described for citric acid was observed, while for malic acid (Table 6) no significant differences between the various samples were found.

The citric acid/malic acid ratio (Table 8 and Fig. 5) reveals all the more significant differences: in particular, it can be seen that this ratio is always lower in second pressing juices than in first pressing juices, and that pigmented Italian

Table 3

Retention times and concentrations of standards in Fig. 1. For each anion the determination limit is also reported

| | Retention time (min) | Concentration (mg/l) | Determination limit (mg/l) |
|-------------------------|-------------------------|-------------------------|-------------------------------|
| Acetic acid | 7.3 | 10 | 10 |
| Lactic acid | 8.3 | 10 | 10 |
| Quinic acid | 9.7 | 50 | 20 |
| Shikimic acid | 10.3 | 50 | 20 |
| Chloride | 13.0 | 10 | 2 |
| Galacturonic acid | 14.8 | 50 | 10 |
| Nitrate | 17.0 | 5 | 2 |
| Malic acid | 19.6 | 100 | 10 |
| Tartaric acid | 22.6 | 100 | 10 |
| Sulfate + ascorbic acid | 23.5 | 10 + 50 | - |
| Phosphate | 26.3 | 50 | 10 |
| Citric acid | 27.7 | 200 | 10 |
| Isocitric acid | 30.9 | 10 | 10 |

Table 4
Citric acid content (g/l) in different fruit juices

| | | Min. | Av. | Max. | n | S.D. |
|---|--------------------------------------|--------------|---------------|----------------|----------|--------------|
| Orange juices (data referred to 11.2°Bx) | Italy Blond I pressing | 10.42 | 20.05 | 26.62 | 15 | 4.68 |
| | Italy Red I pressing | 12.29 | 14.92 | 19.36 | 10 | 2.69 |
| | Italy Mix I pressing | 13.09 | 18.21 | 24.21 | 14 | 3.44 |
| | Italy Blond II pressing | 2.24 | 9.24 | 16.27 | 7 | 4.69 |
| | Italy Red II pressing | 5.12 | 8.28 | 12.99 | 7 | 2.59 |
| | Italy Mix II pressing | 8.04 | 10.01 | 14.03 | 7 | 1.98 |
| | Mediterranean Basin | 7.67 | 11.53 | 14.94 | 15 | 2.01 |
| | Brasil-I pressing | 6.32 | 9.01 | 17.24 | 58 | 1.80 |
| | Brasil-II pressing | 1.64 | 7.83 | 12.86 | 37 | 2.03 |
| | Others South America USA and CUBA | 4.64 3.57 | 11.44 8.36 | 17.99 19.40 | 20 34 | 3.20 3.70 |
| Grapefruit juices (data referred to 10.0°Bx) | 8.33 | 15.94 | 26.68 | 28 | 3.89 | |
| Apple juices (data referred to 11.2°Bx) | Germany | 0.03 | 0.08 | 0.14 | 42 | 0.02 |
| | Italy | 0.02 | 0.06 | 0.13 | 28 | 0.02 |
| | Others | 0.03 | 0.06 | 0.11 | 14 | 0.02 |
| Grape juices (data referred to 16.0°Bx) | France | 0.15 | 0.24 | 0.41 | 21 | 0.06 |
| | Italy | 0.17 | 0.39 | 0.85 | 31 | 0.15 |
| Cherry juices (data referred to 14.0°Bx) | | 0.05 | 0.23 | 0.62 | 40 | 0.14 |
| Blackcurrant juices (data referred to 12.5°Bx) | | 21.15 | 29.63 | 65.44 | 67 | 5.37 |

S.D. = standard deviation.

juices show values lower than those obtained from oranges of the "Biondo comune" variety. In the latter, the citric acid/malic acid ratio of first pressing juices shows mean values clearly higher than those measured in the samples of American provenance.

Knowledge of the full profile of organic acids in a fruit juice has the undoubted advantage of enabling the immediate identification of the presence of acids not characteristic of the juice under examination and allows detection of the addition of a juice of different origin (of commercially lower value) and of previously occurring fermentation.

Thus Fig. 6 shows that the addition of a low percentage of pear juice (which usually has a citric acid mean value of 2.9 g/l) is detected by a considerable increase in the citric acid content.

In cherry juice samples (Fig. 7) the addition of red beet juice is revealed by a rise in the chloride, nitrate and citric acid peaks. Fig. 8 shows the chromatogram of a red grape juice to which a low percentage of blackberry juice had been added, the addition being evident from the notable increase in isocitric acid, which is a characteristic of blackberry juice.

Examination of the acetic acid and lactic acid peaks leads to the detection of the onset of microbiological alteration the sample may have undergone; an example can be seen in the chromatogram in Fig. 9, referring to an altered orange juice sample.

The detection and dosing of galacturonic acid is an important reference index for judging the quality of a juice: galacturonic acid is the monomer constituting the pectin chain and thus its

Table 5
Isocitric acid content (mg/l) in different fruit juices

| | | Min. | Av. | Max. | n | S.D. |
|---|--------------------------------------|--------|-----|------|----|------|
| Orange juices (data referred to 11.2°Bx) | Italy Blond I pressing | 154 | 201 | 232 | 11 | 25 |
| | Italy Red I pressing | 103 | 127 | 153 | 5 | 21 |
| | Italy Mix I pressing | 149 | 166 | 206 | 7 | 22 |
| | Italy Blond II pressing | 120 | 145 | 188 | 3 | 37 |
| | Italy Red II pressing | 83 | 97 | 114 | 4 | 13 |
| | Italy Mix II pressing | – | – | – | – | – |
| | Mediterranean Basin | 69 | 162 | 282 | 15 | 58 |
| | Brasil-I pressing | 51 | 83 | 185 | 58 | 21 |
| | Brasil-II pressing | 16 | 73 | 119 | 36 | 21 |
| | Others South America USA and CUBA | 51 | 112 | 178 | 20 | 35 |
| | | 28 | 94 | 228 | 31 | 42 |
| Grapefruit juices (data referred to 10.0°Bx) | | 121 | 227 | 389 | 27 | 69 |
| Cherry juices (data referred to 14.0°Bx) | | traces | 63 | 145 | 40 | 45 |
| Blackcurrant juices (data referred to 12.5°Bx) | | 128 | 246 | 424 | 40 | 73 |

S.D. = standard deviation.

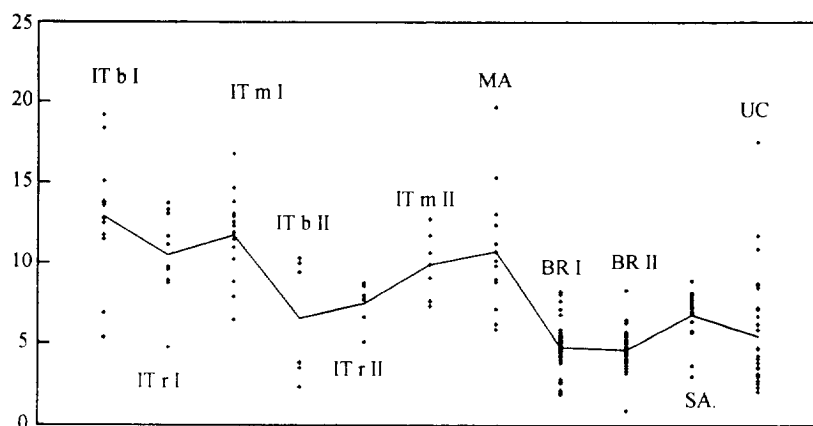


Fig. 5. Citric/malic ratio in different orange juices. IT bI = Italy Blond I pressing; IT rI = Italy Red I pressing; IT mI = Italy Mix I pressing; IT bII = Italy Blond II pressing; IT rII = Italy Red II pressing; IT mII = Italy Mix II pressing; MA = Mediterranean Basin; BR I = Brasil I pressing; BR II = Brasil II pressing; SA = other South America; UC = USA and Cuba.

Table 6
Malic acid content (g/l) in different fruit juices

| | | Min. | Av. | Max. | n | S.D. |
|---|--------------------------------------|-------|-------|-------|----|------|
| Orange juices (data referred to 11.2°Bx) | Italy Blond I pressing | 1.02 | 1.76 | 3.63 | 15 | 0.63 |
| | Italy Red I pressing | 0.92 | 1.53 | 2.70 | 10 | 0.51 |
| | Italy Mix I pressing | 1.07 | 1.61 | 2.12 | 14 | 0.36 |
| | Italy Blond II pressing | 0.94 | 1.36 | 1.98 | 7 | 0.34 |
| | Italy Red II pressing | 0.69 | 1.13 | 1.96 | 7 | 0.40 |
| | Italy Mix II pressing | 0.88 | 1.03 | 1.27 | 7 | 0.14 |
| | Mediterranean Basin | 0.19 | 1.16 | 2.12 | 15 | 0.48 |
| | Brasil-I pressing | 1.31 | 2.02 | 4.33 | 58 | 0.64 |
| | Brasil-II pressing | 1.11 | 1.72 | 2.53 | 37 | 0.34 |
| | Others South America USA and CUBA | 1.22 | 1.68 | 2.22 | 20 | 0.27 |
| 0.41 | 1.61 | 2.40 | 34 | 0.47 | | |
| Grapefruit juices (data referred to 10.0°Bx) | | 0.18 | 0.52 | 1.00 | 28 | 0.19 |
| Apple juices (data referred to 11.2°Bx) | Germany | 4.18 | 7.14 | 11.48 | 43 | 1.43 |
| | Italy | 2.64 | 4.04 | 5.35 | 28 | 0.66 |
| | Others | 3.45 | 5.17 | 8.44 | 14 | 1.48 |
| Grape juices (data referred to 16.0°Bx) | France | 1.49 | 3.62 | 9.67 | 21 | 1.81 |
| | Italy | 1.99 | 4.48 | 9.62 | 31 | 1.87 |
| Cherry juices (data referred to 14.0°Bx) | | 11.92 | 18.17 | 22.71 | 40 | 2.92 |
| Blackcurrant juices (data referred to 12.5°Bx) | | 0.90 | 1.89 | 3.07 | 67 | 0.58 |

S.D. = standard deviation.

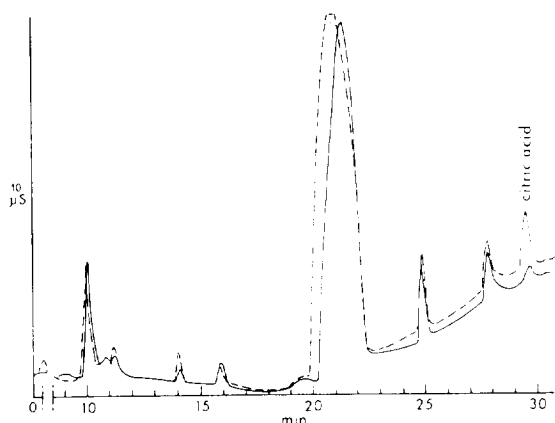


Fig. 6. Chromatograms of authentic apple juice (—) and apple juice with 10% of pear juice (---). Citric acid increase due to the addition of pear juice.

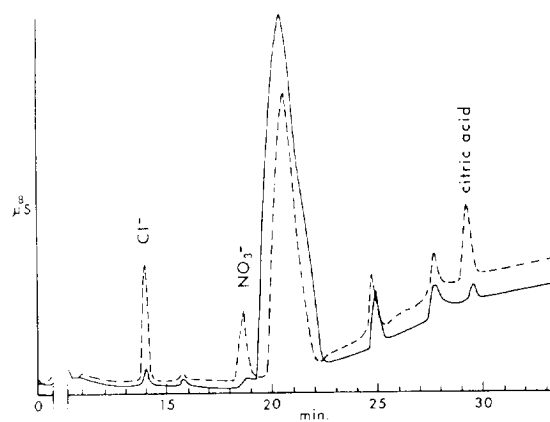


Fig. 7. Chromatograms of authentic cherry juice (—) and cherry juice with 10% of red beet juice (---). Chlorides, nitrates and citric acid increase due to the addition of red beet juice.

Table 7
Tartaric acid (g/l) content in grape juices

| | Min. | Av. | Max. | n | S.D. |
|--------|------|------|------|----|------|
| France | 1.60 | 3.58 | 5.42 | 21 | 0.97 |
| Italy | 1.85 | 3.80 | 5.82 | 31 | 1.04 |

S.D. = standard deviation. Data referred to 16.0°Bx.

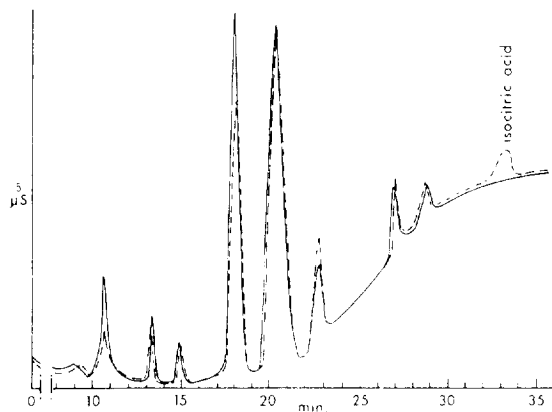


Fig. 8. Chromatograms of authentic red grape juice (—) and red grape juice with 5% of blackberry juice (---). Isocitric acid increase due to the addition of blackberry juice.

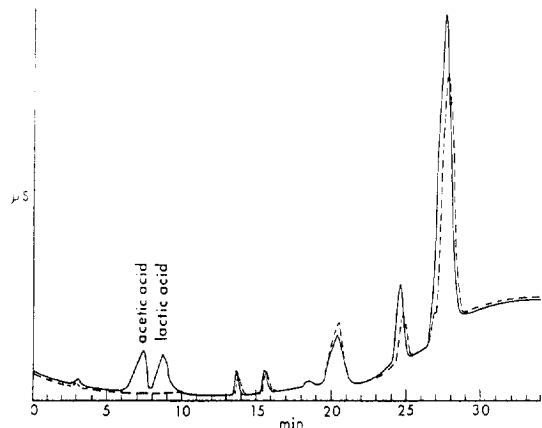


Fig. 9. Chromatograms of orange juices. The increase of acetic acid and lactic acid is indicative of microbial alteration.

presence in high quantities in a juice can be attributed to the spontaneous or induced enzymatic degradation of the pectins.

Therefore, while it is natural to find it in high quantities in clarified juices (Fig. 10), a high concentration of this compound in cloudy juices could indicate insufficient enzymatic inactivation.

Table 8
Citric acid/malic acid ratio in citrus juices

| | Min. | Av. | Max. | n | S.D. | |
|-------------------|--------------------------------------|-------|-------|-------|-------|-------|
| Orange juices | Italy Blond I pressing | 4.81 | 12.40 | 19.18 | 15 | 4.09 |
| | Italy Red I pressing | 4.79 | 10.46 | 13.74 | 10 | 2.73 |
| | Italy Mix I pressing | 6.50 | 11.72 | 16.78 | 14 | 2.73 |
| | Italy Blond II pressing | 2.37 | 6.85 | 10.28 | 7 | 3.43 |
| | Italy Red II pressing | 5.11 | 7.49 | 8.76 | 7 | 1.26 |
| | Italy Mix II pressing | 7.27 | 9.86 | 12.76 | 7 | 2.04 |
| | Mediterranean Basin | 5.88 | 13.47 | 56.70 | 15 | 12.53 |
| | Brasil-I pressing | 1.90 | 4.76 | 8.19 | 58 | 1.30 |
| | Brasil-II pressing | 0.95 | 4.63 | 8.27 | 37 | 1.16 |
| | Others South America USA and CUBA | 3.02 | 6.80 | 8.87 | 20 | 1.46 |
| | 2.14 | 5.92 | 17.52 | 34 | 3.65 | |
| Grapefruit juices | 15.62 | 35.11 | 95.67 | 28 | 17.79 | |

S.D. = standard deviation.

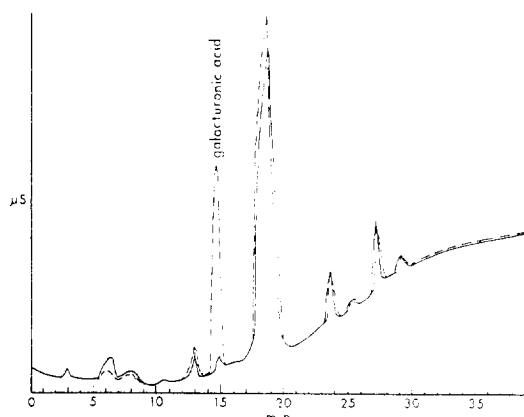


Fig. 10. Chromatograms of cloud apple juice (---) and clarified apple juice (—). Galacturonic acid increase due to the enzymatic degradation of pectins.

4. Conclusions

The analytical method proposed showed a high sensitivity and reproducibility and has the advantage of allowing quantitation of the main organic acids with a single analysis giving the complete profile of all the organic acids, including the minor ones, which characterize the juice.

Knowledge of the full organic acid profile is also very important in the control of the quality and genuineness of a juice.

Furthermore, ion chromatography also enabled the simultaneous separation of various inorganic anions of particular interest, such as chlorides and nitrates.

Finally, as for the characterization of orange juices, this study has shown the possibility of distinguishing Italian juices from those from other countries based on the organic acid content and their ratios; the extraction technique (first and second pressing juices) has also been found to have significant effects on the organic acid content.

Acknowledgement

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