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Determination of some organic acids in sugar factory products

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Abstract

This work deals with the identification and quantitative evaluation of the following acids: citric, malic, lactic, formic, acetic, propionic, butyric, valeric, and pyroglutamic acid. The determination has been carried out using a Dionex ion chromatograph, and compared with standard high-performance liquid chromatographic methods and enzymatic analysis. Differences in products from sugar beet factories have been analyzed with the aim to control the fermentation process used. Satisfactory results have been obtained, despite the presence of the particularly complex matrix. With the exception of a few cases, it has been possible in this way to standardize an analytical method for the whole production process.

1. Introduction

For some time now the Laboratorio Chimico Centrale ERIDANIA, Ferrara, Italy, has been trying to determine which organic acids are present in various sugar industry products. This study has three main aims:

(1) To control undesired fermentation in sugar syrups, particularly the development of malic, acetic and lactic acids, the presence of which lowers the pH of the juices, thus facilitating sucrose inversion which leads to the formation of glucose and fructose with consequent sugar losses. These monosaccharides provide the substrate for the so-called Maillard reactions which take place between the monosaccharide C = O carbonyl groups and the NH_2 groups of amino acid compounds. These reactions lead to an undesired increase in juice colour.

(2) To control the type of fermentation occurring in the sugar beet pulp used as animal feed, because such use depends on the type of fermentation which takes place during storage in silos. Determination of the organic acid content of the pressed pulp is of particular importance for the sugar industry since this product is used as cattle feed. At the time of production, only traces of organic acids (lactic and acetic acids) are present in the pulp. However, during storage, uncontrolled fermentation increases the acid content to ca. 2–3% of the product. Of the greatest importance is the type of acid formed (i.e. lactic, acetic, iso, *n*-butyric, valeric) [1]. In the case of lactic fermentation, a good quality product is obtained with organoleptic properties making it suitable for use as cattle feed. On the other hand, the formation of acids such as propionic, butyric and valeric acids make the feed unpleasant; not only is it less appetizing, but it also causes environmental problems due to the intense, repelling odour.

(3) To control the formation of pyroglutamic acid, which is an indication of the presence of glutamine. As is shown in Fig. 1, the loss of

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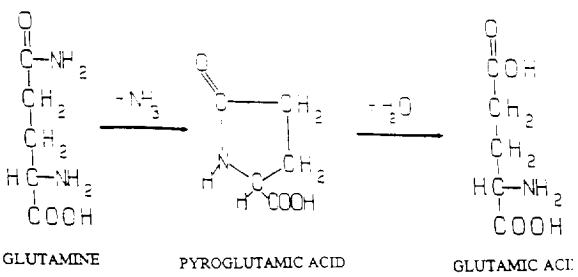

Fig. 1. Formation and hydrolysis of pyroglutamic acid.

ammonia during the calcocarbonic purification of glutamine leads to the formation of pyroglutamic acid which is partially hydrolyzed into glutamic acid. Given the fact that a poor sugar beet quality is also associated with a higher glutamine content and that the loss of NH_3 by the latter leads to increased air pollution and environmental damage, it can be inferred that the determination of pyroglutamic acid is of great importance.

2. Experimental

The analyses were performed using three methods: ion chromatography, high-performance liquid chromatography (HPLC) and enzymatic analysis.

2.1. Ion chromatography

The separation technique used in high-performance ion exclusion chromatography (HPIEC) is based on the Donnan effect. On the basis of this principle, the ionized compounds are more rapidly eluted than non-ionic ones which are held back by electrostatic partition forces such as the Van der Waals forces or the like. Weakly ionic compounds are then eluted more or less quickly, depending on their pK value and hydrophobicity.

The analyses were performed using a Dionex 4000i ion chromatograph with a 50-μl loop equipped with a conductometric detector of greater sensitivity than the UV detectors normally used in HPLC. As can be seen in the chromatogram of a standard mixture, peaks of a certain entity can be easily seen at 100 ppm (Fig. 2).

Separation was performed with an HPIEC-AS1 sulfonic column ($-SO_3H$) with approximately 9% styrene-divinyl benzene as the support, isocratic elution being performed at room temperature (column pressure 65 bar). In addi-

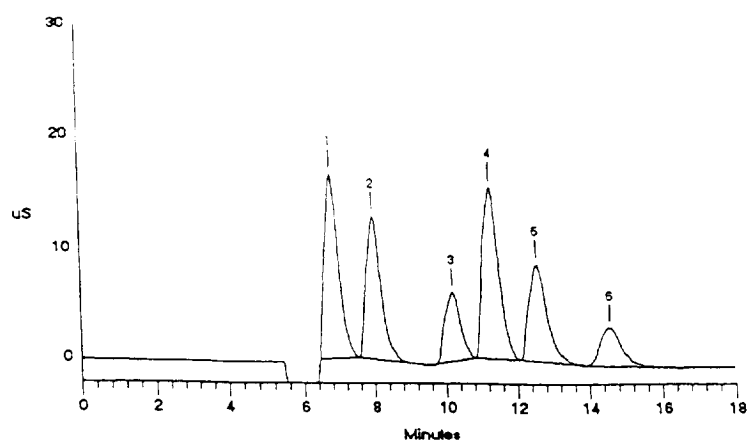

Fig. 2. Ion chromatogram. Column ICE AS1 Dionex. Eluent 2 mM HCl. Flow-rate 0.8 ml/min. Detector PED-conductivity. Standard solution of organic acids, 100 ppm. Peaks: 1 = citric; 2 = malic; 3 = lactic; 4 = formic; 5 = acetic; 6 = pyroglutamic.

tion, an AMMS-ICE membrane suppressor was set between the column and the detector to reduce the background conductivity and thus improve signal response [2]. A 2 m*M* HCl eluant was used at a flow-rate of 0.8 ml/min; regeneration of the cationic suppressor sites was achieved with 10 m*M* of the regenerant tetrabutyl ammonium hydroxide (TBAOH) at a flow-rate of 4 ml/min.

It must be pointed out that the method does not require any pretreatment of the sample solution. It was prepared dissolving the sample in the mobile phase at a concentration of about 1% of dry matter and subsequently filtrated on a 0.45-μm membrane, although any column contamination by proteins and the presence of interfering peaks caused by cations can be prevented by batch treatment with H^+ cation resins.

2.2. High-performance liquid chromatography

High-performance liquid chromatography was performed using a Varian 5000 chromatograph with a 10-μl loop equipped with a UV 100 detector. Separation was performed on a Biorad HPX-87H sulfonic column employing styrene-divinyl benzene as the support, isocratic elution taking place at 50°C (column pressure 50 bar). The eluant used was 5 m*M* H_2SO_4 at a flow-rate of 0.6 ml/min; detection was at a wavelength of

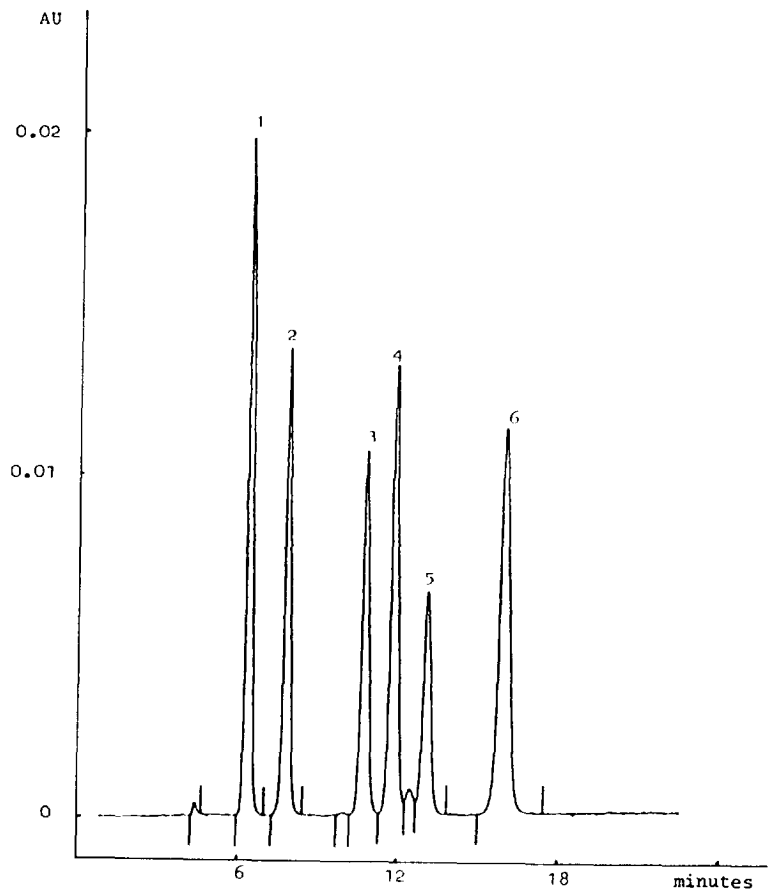

Fig. 3. High-performance liquid chromatogram. Column HPX-87H Biorad. Eluent 5 m*M* H_2SO_4. Flow-rate 0.6 ml/min. Detector UV 210 nm. Column temperature 50°C. Standard solution of organic acids, 500 ppm. Peaks: 1 = citric; 2 = malic; 3 = lactic; 4 = formic; 5 = acetic; 6 = pyroglutamic.

210 nm. The chromatogram in Fig. 3 shows the profile of a standard mixture.

2.3. Enzymatic analysis

For this technique the Boehringer Mannheim test was used containing predetermined quantities of selected, strictly controlled reagents. These were developed for the analysis of foodstuffs although they can be generally used for all types of matrices [3].

The NADH formed in the enzyme reaction is directly proportional to the amount of organic acid present and thus makes a precise, accurate determination possible. Tests were run at room temperature since the reactions caused by the enzymes proceed quite rapidly, even under these conditions [4].

Although this technique is not more difficult than the instrumental techniques described above, it gives greater accuracy since the enzyme only acts on the specific organic acid involved; in addition, it has the ability to distinguish between the various isomer forms. Thus, for example, for lactic acid we can determine both the L- and D-forms.

3. Results and discussion

Small amounts of some organic acids are present in sugar beets while others are present in beet processing products as a result of the breakdown of more complex organic structures or of transformation reactions taking place during production. In fact, when ion chromatograms of raw juice and molasses are compared (Figs. 4 and 5), one sees that marked amounts of lactic and pyroglutamic (PCA) acids are present in the latter. The former, derived from the chemical and biological destruction of sugar taking place during the production process, passes from an average of 0.2% of dry matter in raw juice to 2.5% in molasses, thus practically providing an index of sugar losses within the sugar factory. On the other hand, PCA is formed during the purification process as a result of a loss of ammonia by the glutamine (see Fig. 1).

Our methods of analysis do not allow the separation between propionic acid and PCA, because they are coeluted in the chromatogram; however, we have information from the literature on sugar beets that pyroglutamic acid (PCA) only is formed during the production process. Therefore, for the chromatograms con-

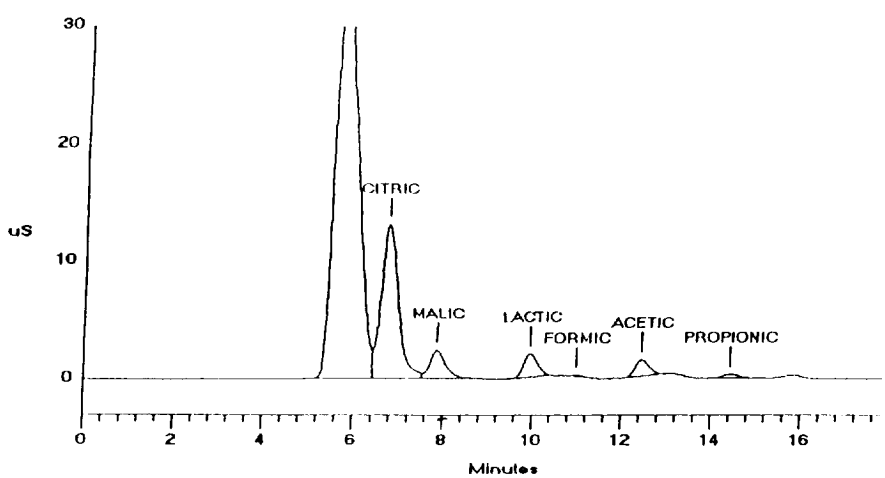

Fig. 4. Ion chromatogram. Sample solution of raw juice 10%.

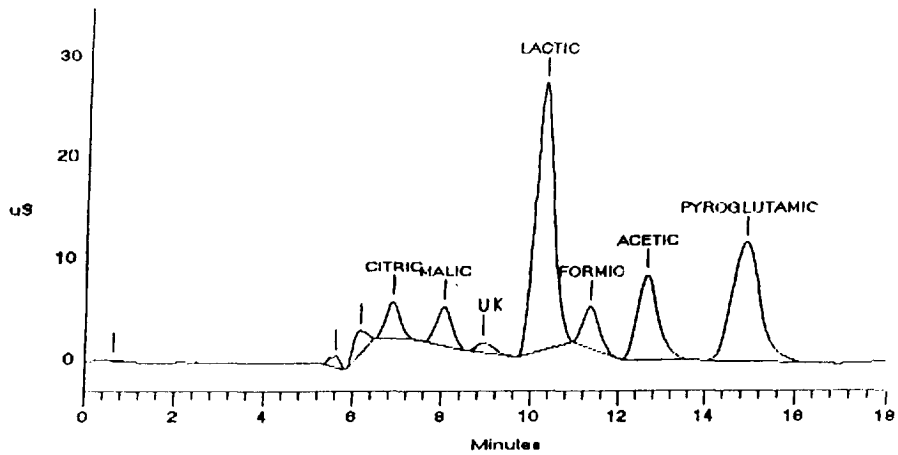

Fig. 5. Ion chromatogram. Sample solution of molasse 2%.

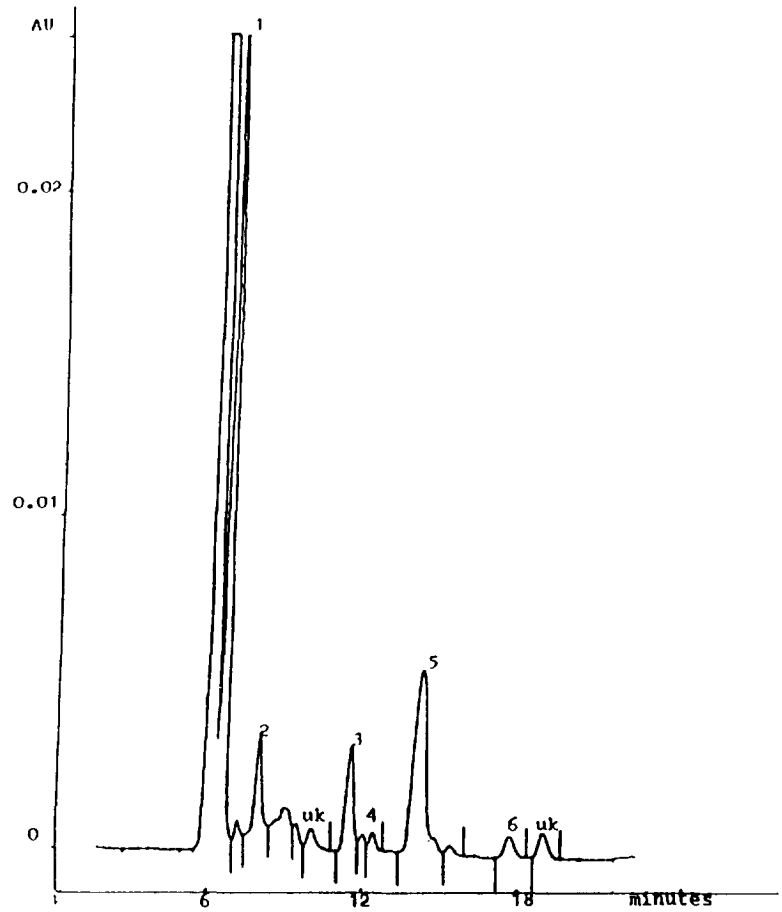

Fig. 6. High-performance liquid chromatogram. Sample solution of raw juice 10%. Peaks: 1 = citric; 2 = malic; 3 = lactic; 4 = formic; 5 = acetic; 6 = propionic; uk = unknown.

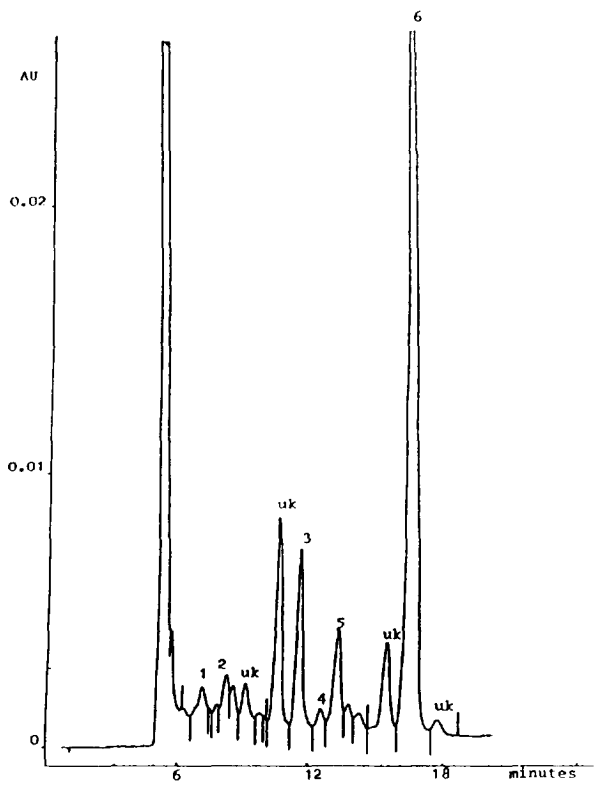

Fig. 7. High-performance liquid chromatogram. Sample solution of molasses 2%. Peaks: 1 = citric; 2 = malic; 3 = lactic; 4 = formic; 5 = acetic; 6 = pyroglutamic; uk = unknown.

cerning the raw juice (Figs. 4 and 6) the peak labelled 6 is identified as propionic acid, and for the chromatograms concerning the molasses (Figs. 5 and 7) the peak labelled 6 is identified as pyroglutamic acid.

In this regard it is worth noting that in the HPLC analysis the same samples showed different peaks, many of which are not identified (Figs. 6 and 7). This is due to the fact that, to be successful, HPLC analyses of organic acids in sugar production juices require isolation of the acid fraction through a difficult sample pretreatment process which is impractical for normal routine controls; therefore the samples for analysis were not submitted to any pretreatment [5–7].

With regard to the thick juices and factory-produced molasses, it has been found that L-lactic acid is predominant; in addition, the sum of the two isomers (D + L) is in good agreement with the total lactic acid determined by ion chromatography. This can be seen in Table 1, where the values are reported for a series of analyses performed on thick juices of eleven average samples from the 1993 sugar campaign.

The three methods were performed in parallel to determine the principal organic acids present. As an example, the results obtained in a series of molasse samples are given in Table 2. The values

Table 1
Comparison of enzymatic and ion-chromatography methods for lactic acid in thick juice

	Enzyme D-lactic (mg/g)	Enzyme L-lactic (mg/g)	Enzyme (D + L)-lactic (mg/g)	Total IC lactic (mg/g)
1	151	272	423	424
2	207	273	480	473
3	164	321	483	498
4	153	184	337	342
5	165	596	751	770
6	70	92	162	180
7	382	388	770	817
8	152	246	398	390
9	232	270	502	487
10	234	536	770	773
11	149	396	545	566

Table 2
Comparison of enzymatic analysis, HPLC and ion chromatography for organic acids in molasses

	Citric (mg/g)			Malic (mg/g)			Lactic (mg/g)			Formic (mg/g)			Acetic (mg/g)			PCA (mg/g)	
	Enz.	HPLC	IC	Enz.	HPLC	IC	Enz.	HPLC	IC	Enz.	HPLC	IC	Enz.	HPLC	IC	HPLC	IC
1	140	140	90	241	140	178	2111	1720	2256	277	225	264	594	1350	616	3320	2850
2	210	400	174	357	520	260	3058	2420	3254	305	240	268	411	375	520	2540	2194
3	205	180	138	255	60	190	2707	1920	2588	238	160	216	543	975	586	2740	2310
4	246	200	142	317	240	246	2076	1620	2324	345	180	324	647	612	670	3520	2954
5	163	400	80	305	540	244	2214	2720	2524	340	200	326	690	1488	732	3300	3014
6	98	480	36	163	80	118	1304	1000	1552	223	160	222	623	560	632	2420	2060
7	188	140	96	335	240	170	3444	3040	3980	309	180	282	673	1160	706	3320	3094
8	181	180	126	217	120	187	1601	1900	1931	264	220	266	642	1375	1374	3300	3055
9	159	140	105	217	80	168	2674	2320	3090	235	140	221	1078	1825	2228	2860	2753
10	244	380	53	364	700	284	2610	2620	2817	248	300	227	702	1450	1446	2840	2440
11	169	160	200	326	160	263	2220	1940	2686	307	180	299	434	788	910	2440	2321

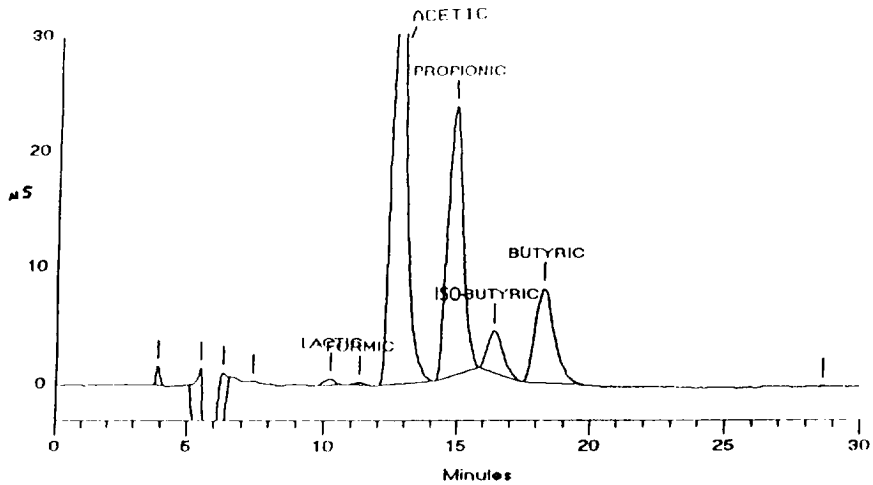

Fig. 8. Ion chromatogram. Sample solution of pressed pulps 5%.

clearly show that, except in a few isolated cases, the best agreement was always found between enzymatic analysis and ion chromatography [8]. Moreover, enzymatic analysis made it possible to show that, of the two isomers of malic acid (D and L), only L-malic acid is present in production juices.

The analyses of pressed pulp were performed in a similar manner as for the syrups and this study has shown that at the time the pressed pulp is placed in the silos the amount of organic acids present is quite low (about 5% of dry matter) and that controlled fermentation in an anoxic environment leads only to the formation of lactic and acetic acids. When fermentation takes place in an anoxic environment these acids are produced in such quantities as to lower the pH value to around 3.8–4.0. Since the low pH inhibits the onset of undesired fermentation, this makes it possible to maintain good organoleptic properties of the pulp, even during subsequent storage [9]. Hence, it can be noted that with decreasing anoxic conditions, the formation of undesired acids such as propionic and butyric acids will increase. The chromatogram (Fig. 8) of the analysis of a product of this type shows such a trend.

4. Conclusions

From the present study it can be concluded that a chromatographic analysis of the organic acids in our processed products does not allow a correct quantitative determination, and neither is it possible to distinguish between the D and L isomeric forms. In some cases enzymatic analysis was used to determine the amounts of D,L-lactic acid. The L-lactic acid content is an important parameter because it gives a measure of the fermentation activity in the different steps of the production process.

We also found that propionic acid and pyroglutamic acid cannot be separated by chromatography because they have the same retention time; enzymatic tests also do not allow their determination. Consequently we are now using a new ion chromatography column, IONPAC ICE AS6, with which their separation can be achieved if required.

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