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Short communication

Application of capillary isotachophoresis for fruit juice authentication

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Abstract

A method using capillary isotachophoresis (ITP) was developed and applied for the determination of the anionic profile of orange juices with the aim to obtain some useful information on the authenticity or adulteration of imported and native beverage products. An EA 100 electrophoretic analyser (Villa-LABECO, Slovak Republic) was used for capillary isotachophoretic determination of anions in tested samples. More systems of leading and terminating electrolytes were used. Detection conductivity and UV detection at 254 nm were used. Sample injection volume was 30 μl. These systems allow one to determinate inorganic anions, organic acids and some additives – adulterants in anionic forms in orange juices. By capillary isotachophoretic determination the lengths or areas of characteristic zones were established and compared to authentic orange juices of different species and origin and with RSK reference values (Code of Practice). Special emphasis was placed on D-isocitric acid ITP determination as a reliable fruit juice authentication marker. The presented multi-component analysis of orange juice authenticity according to ITP anionic profiles obtained by capillary isotachophoresis presents an alternative information source necessary for deciding about authenticity of the products.

Keywords: Fruit juices; Food analysis; Isotachophoresis; Organic acids; Phosphates; Inorganic anions

1. Introduction

The main authenticity issues are those that arise from substitution of the authentic named material with cheaper alternatives. In the area of fruit juices, the named material can be bulked with water, sugar, fruit-derived extenders such as pulpwash or cheaper alternatives. Fruit juices and nectars are an important and fast growing sector of the food industry. Fruit juices and nectars are defined in a common regulation by Council Directive 93/77/EEC of 21 September 1993 [1]. Fruit juice is obtained from fruit by mechanical processes or from concentrated fruit juices by restoration. Addition of sugars to some fruit juices is authorised in order to correct their acidity and for the purpose of sweetening. This addition may be prohibited in certain Member States and in all cases is subject to appropriate labelling. Nectar is obtained by the addition of water and sugars to fruit juice, the minimum fruit content is specified in Council Directive 93/77/EEC. For the production of fruit juices and nectars certain enzymatic treatment and some quantities of permitted additives are authorised. Artificial sweeteners are permitted in nectars sold for dietetic purposes. Some differences exist between European Union (EU)

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regulations and the Codex Alimentarius definition of orange juice, which permits the addition of up to 10% of mandarin juice. The fruit juice industry Code of Practice published by the AIJN (Association of the Industry of Juices and Nectars from Fruits and Vegetables) provides guidelines for general fruit juice authenticity and quality criteria [2].

Our contribution aimed to compare the quality of some commercial ''100%'' orange juices with the quality of fresh made orange juice and to some RSK authenticity reference values (Code of Practice [2]). Orange juice quality was tested by capillary isotachophoretic profile of some inorganic anions, organic acids and for the presence of some artificial sweeteners (acesulfame K, saccharin and cyclamate). Special attention paid to D-isocitric acid determination as an authenticity marker for the authenticity of fruit juices.

2. Materials and methods

For capillary isotachophoretic orange juice authentication systems of leading (LE) and terminating (TE) electrolytes were used:

(1) For determination of chlorides and sulfates: LE: 10 m*M* HCl+β-alanine, pH 2.9 and TE: 10 m*M* citric acid. The current in the preseparation column was 250 μA, and in the analytical column 25 μA.

(2) For the majority organic acids and phosphates: LE: 10 m*M* HCl+histidine (His), pH 6.0 and TE: 10 m*M* caproic acid. The current in the preseparation column was 250 μA, and in the analytical column 30 μA.

(3) For D-isocitric acid determinations two systems of electrolytes were tested: (I) LE_1: 10 m*M* His·Cl+His+0.2% methylhydroxypropylcellulose (MHPC), pH 6; LE_2: 6 m*M* His·Cl+6 m*M* His+2 m*M* $CaCl_2$+0.1% methylhydroxyethylcellulose (*m*-HEC), pH 6, TE: 10 m*M* citric acid. The current in the preseparation column was 200 μA and in the analytical column 30 μA. (II) $LE_{1,2}$: 6 m*M* His·Cl+6 m*M* His+2 m*M* $CaCl_2$+0.1% *m*-HEC, pH 6, TE: 10 m*M* citric acid. The current in the preseparation column was 250 μA and in the analytical column 30 μA.

For all ITP determinations conductivity and UV detection at 254 nm were used and the injection volume of the sample was 30 μl. All ITP systems allowed one to identify alternative sweeteners acesulfame K, saccharin and cyclamates.

(4) D-Isocitric acid enzymatically: decarboxylation by NADP in presence of the enzyme isocitrate dehydrogenase (test kit of Boehringer Mannheim, Germany).

Information about the methodology of fruit juice authentication was obtained from the literature [4–7].

3. Results and discussion

Results of the ITP anionic profile determinations of some commercial orange juices declared ''100% original'' products are presented in Tables 1–4. Fig. 1 illustrates an isotachophoreogram from the D-isocitric acid determination in commercial orange juice.

The results from chloride determinations in Table 1 correspond to the normal values in orange juices. In some samples sulfate content exceeded the limit value. The increased sulfate concentrations may indicate sulfur dioxide treatment of some fruit juices (permitted under EU legislation).

The results from Table 2 show a normal situation in content of phosphates, citric and malic acids, but not of lactic acid, where only two samples had required values. This is due to partial fermentation of some manufactured fruit juices.

Table 3 presents the results of citric (ITP) and D-isocitric acids (enzymatic) determinations being essential for the identification of a ratio of these acids, which can be also used to a certain extent for informative identification of fruit variety and provenance. In our case, we surprisingly found that many samples did not reach the minimum required value according to the Code of Practice [2]. We can suppose that the lower content of isocitric acid in tested orange juice samples may be due to particular dilution connected with the addition of some additive solutions or another cause. We also found lower values of isocitric acid in orange beverages made from orange syrups and powdered drinks (0–10 mg/l). These products contain only a small proportion of natural orange.

Because of the significant importance of D-isocitric acid as an authenticity marker, we additionally tested

Table 1
Results from ITP determination of chlorides and sulfates in orange juices ($n=3$)

Orange juices	Chlorides (mg/l)	Sulfates (mg/l)	μ_A[a] (mg/l)
RSK reference values	Max. 60	Max. 150	
Original fresh	69.46	162.31	3.26
Fragopolis	21.38	154.65	3.29
Yo	49.85	140.89	6.33
O'Pfanner	61.87	238.38	3.20
Top Joy	45.29	79.33	3.33
Gold Glocken	37.01	66.99	–
Hello	41.15	178.87	3.24
Cappy	4.17	269.85	3.25
Hohes C	–	73.58	3.68
Fructal	4.14	57.83	–
Triumph	5.98	229.17	3.11
Rajo	–	151.80	3.51

[a] μ_A: Uncertainty of measurement type A (standard deviation).

this acid by the ITP method. We applied a common method [3], which uses Ca^{2+} cations in the LE. Optical isomers of citric acid in this system are separated thanks to their different ability to create calcium chelates. Table 4 presents the results of D-isocitric acid determinations by the ITP method and these results are compared to enzymatic determination. We tested the ITP method with two electrolyte systems. In first one, Ca^{2+} cations were not present in the preseparation column, and in the second system this cation was used in both the preseparation and analytical columns. With these two systems relatively very different results were obtained (Table 4). In system I analysis time was short [recovery in Happy Day orange juice – 85%, limit of detection (LOD) 1.25 mg/kg and limit of quantitation (LOQ) 5.70 mg/kg]. The presence of the Ca^{2+} cation in the preseparation and analytical columns (system II) improved the parameters of this analysis (recovery in Glocken Gold orange juice – 103%,

Table 2
Results from organic acids determination in orange juices by the ITP method ($n=3$)

Orange juices	Phosphates (mg/l)	μ_A[a] (mg/l)	Citric acid (g/l)	μ_A (g/l)	Malic acid (g/l)	μ_A (g/l)	Lactic acid (mg/l)	μ_A (mg/l)
RSK reference	353–645[b]		6.3–17		0.8–3		Max. 500	
Original fresh	281–410	2.79	9.8–11.9	0.06	0.77–2.83	0.018	323–618	6.62
Fragopolis	436.81	4.16	9.2	0.06	1.76	0.012	830.43	9.87
Yo	433.80	4.13	10.7	0.06	1.98	0.013	574.79	7.51
O'Pfanner	360.06	3.62	9.3	0.06	1.29	0.009	722.40	8.83
Top Joy	238.39	2.83	8.4	0.05	1.09	0.008	572.93	7.55
Dago	308.01	3.17	7.3	0.04	0.98	0.007	502.35	6.25
Gold Glocken	450.87	4.25	10.4	0.06	1.86	0.012	780.57	9.36
Hello	295.56	3.18	7.2	0.04	1.09	0.008	511.65	6.97
Cappy	376.81	3.72	9.1	0.06	1.95	0.012	575.33	7.51
Hohes C	379.24	3.73	10.2	0.06	2.02	0.013	689.96	8.54
Fructal	446.25	4.21	12.4	0.08	2.04	0.013	780.79	9.37
Triumph	417.73	3.99	10.8	0.07	1.78	0.011	915.58	10.66
Rajo	425.01	4.06	10.8	0.07	2.14	0.014	745.09	9.03

[a] μ_A: Uncertainty of measurement type A (standard deviation).
[b] Calculated from RSK value for phosphorus (P).

Table 3
Results from citric and D-isocitric acids determination in orange juices ($n=3$)

Orange juices	Citric acid (ITP determination)		D-Isocitric acid[b] (enzymatic determination) (mg/l)	Ratio citric/isocitric acid
	(g/l)	μ_A[a] (g/l)		
RSK reference values	6.3–17		65–200	Max. 130
Original fresh	9.8–11.9	0.06	89.3–140.2	84.9–109.7
Fragopolis	9.2	0.06	43.2	212.9
Yo	10.7	0.06	49.3	216.5
O'Pfanner	9.3	0.06	53.8	172.8
Top Joy	8.4	0.05	55.4	151.6
Dago	7.3	0.04	57.8	126.3
Gold Glocken	10.4	0.06	55.4	187.7
Hello	7.2	0.04	81.8	88.0
Cappy	9.1	0.06	64.7	140.4
Hohes C	10.2	0.06	75.3	135.3
Fructal	12.4	0.08	80.0	155.4
Triumph	10.8	0.07	75.5	142.9
Rajo	10.8	0.07	76.1	141.9

[a] μ_A: Uncertainty of measurement type A (standard deviation).
[b] D-Isocitric acid was determined enzymatically, average standard deviation 10%.

LOD 1.82 mg/kg and LOQ 7.07 mg/kg). In contrast to these results it is very surprising that the results from ITP system I corresponded better to the results from the enzymatic method (Table 4). Increased values of D-isocitric acid found by ITP system II may be due to some interferences, but the good recovery found encourages us to further study and optimise this method.

In all the presented ITP conditions and electrolyte systems the artificial sweeteners (acesulfame K, saccharin and cyclamates) can be identified. During the inspection of orange juice by ITP procedures we

Table 4
Results from ITP and enzymatic determination of D-isocitric acid in fruit juices[a]

Type of juices	ITP (system I) (mg/kg)	ITP (system II) (mg/kg)	Enzymatic determination (mg/kg)
Orange juices (RSK value 65–200 mg/kg)			
Original	39.5	281.8	41.2
Happy Day, 100%	30.5	125.9	56.4
Rajo, 100%	25.9	96.1	52.6
Hello, 100%	28.8	118.9	34.0
Glocken Gold, 100%	23.1	105.6	48.5
Rio, 50%	50.9	–	27.3
Dago, 20%	10.8	39.8	14.5
Yo, 50%	37.9	89.9	50.9
Others			
Hello, 100% grapefruit (RSK value 140–350 mg/kg)	28.9	272.5	142.3
Fruiko, 100% tomato	28.4	–	35.7

[a] $n=2$–3, average uncertainty of measurement (A type) at ITP 5%, at enzymatic determination 10%.

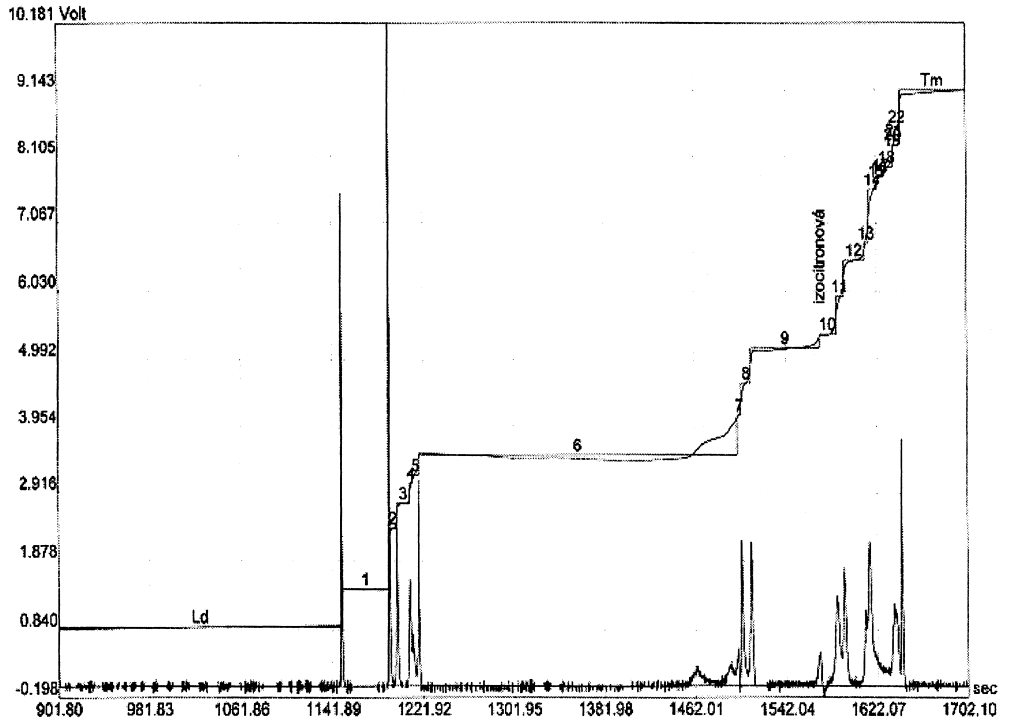

Fig. 1. Isotachophoretic determination of D-isocitric acid in Glocken Gold orange juice (dilution 10×, $LE_{1,2}$: 6 m*M* His·Cl+6 m*M* His+2 m*M* $CaCl_2$+0,1% *m*-HEC, pH 6 and TE: 10 m*M* citric acid). Note: izocitronová – isocitric acid.

have found none of these additives. The used methods can be useful for quality differentiation of nectars and 100% fruit juices.

4. Conclusions

It was found that ITP determination is a very useful method for authentication of fruit juices according to anionic profile. Results from some anion determinations (chlorides, sulfates, phosphates and citric, malic and lactic acids) correspond to the normal values in orange juice. Only a few samples exceeded the limit values. In the case of enzymatic determination of D-isocitric acid and its ratio to citric acid many samples do not meet the requirements according to the Code of Practice [2]. The ITP testing system I for D-isocitric acid content determination in orange juice shows similarities with enzymatic determination. The results from ITP testing in system II indicate further investigation in optimising of this method is needed.

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