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JARMILA LEHKOŽIVOVÁ, JOLANA KAROVIČOVÁ, ZLATICA KOHAJDOVÁ, MILAN SUHAJ, IVANA ŠIMONOVÁ

ISOTACHOPHORETIC ANALYSIS OF THE ARTIFICIAL SWEETENERS AND TIME-INTENSITY SWEETNESS EVALUATION OF SOFT DRINKS

S u m m a r y

The method of capillary isotachophoresis was used for determination of acesulfame K, saccharin and cyclamates in soft drinks. All samples complied with requirements of Decree of Slovak Republic for acesulfame K and saccharin content. The content of cyclamates was exceeded in some samples above 3.21 mg/l to 114.77 mg/l. Monitoring of time-intensity curves of sweet taste was found that the samples containing the artificial sweeteners had longer progress of sweet taste ceasing and higher maximum intensity. The total duration ranged from 18 s to 36 s in these samples and maximum intensity of sweet taste from 28.3% to 40.5%. In samples containing natural sweeteners the total duration was from 15 s to 21 s and maximum intensity of sweet taste from 20.0% to 36.6 %.

Key words: capillary isotachophoresis, artificial sweeteners, time-intensity method, sweetness

Introduction

Soft drinks beverages are common everyday products, which are produced regionally [1]. Sucrose is the important raw material used in soft drink manufacture. Glucose, starch syrup, inverted syrup and fructose are less frequent. In addition to these saccharides, the artificial sweeteners have importance for production of dietetic drinks [2]. The artificial sweeteners are additional substances, which are used to sweet taste addition to food products or as table sweeteners. Particularly polyalcoholic saccharides, acesulfame K, aspartame, cyclamic acid and its sodium and calcium salts, neohesperidine DC, saccharin and its potassium, sodium and calcium salt and

Ing. J. Lehkoživová, Doc. Ing. J. Karovičová, PhD., Ing. Z. Kohajdová, PhD., Ing. M. Suhaj, CSc., Ing. I. Šimonová, Institute of Biotechnology and Food Science, Department of Food Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37, Bratislava, Slovak Republic

thaumatine belong to this group. The artificial sweeteners can be used separately or in combination with other only in that amount which can achieved standard sweet taste. The highest allowable amounts of the individual sweeteners are needed to reduce proportionally in their combination [3].

Sucrose – sugar fulfils not only function of sweetener but influences the sense of taste fullness, harmonizes other taste components of drinks, accentuates aromatic, mainly fruity parts [2].

Saccharin is salt of anhydride of sulfaminobenzoic acid. It is the oldest and the most common used sweetener in the world. The sweetness of saccharine is 400–550 times higher than sucrose but it has long-time bitter metallic aftertaste. It is used in the form of Na or Ca salts, well soluble in the water. Technologically it is good stable towards the increased temperature [2, 4, 5].

Cyclamates are used in the form of sodium or calcium salt of cyclohexylsulfamic acid. The sweetness of cyclamates is 30–60 times higher than sucrose. They are well soluble in water, stable in acid medium and resistant to higher temperature. Their sweet taste is pure without secondary tastes [2, 6].

Acesulfame K is potassium salt of 6-methyl-1,2,3-oxathiazinone-2,2-dioxide. In comparison with 3–5% solution of sucrose it is 150–200 times sweeter, whereupon threshold of sensitivity for sweetness is 0.08–0.12 mmol/l, for bitterness 3–7 mmol/l. The sweetener is not metabolized by the human body and thus contributes no energy to the diet. Acesulfame K contains two amide and one ester bond that they can hydrolyze at higher temperature mainly in acid conditions. In these conditions acetoacetic acid is formed. In the next ketonic breakdown, acetoacetic acid is decomposed into acetone, carbon dioxide and ammonium sulfate [7, 8].

Aspartame is L-aspartyl-L-phenylalanine methyl ester. Its sweetness is 180 times higher than sucrose. The taste of aspartame is similar to sucrose and it has the ability to strengthen some aromatic substances. Aspartame is unstable at higher temperatures as well as in aqueous medium. Eventually it is decomposed. The speed of decomposition and the type of those products depends on temperature, pH and moisture content. This decomposing process reduces sweetness because the sweet taste is linked with the structure of α-aspartame and it is not related to other decomposing products [2, 6, 9].

The taste perception has some rarities. It relatively takes long time because taste active substances have to dissolve at first, penetrate into taste buds and then through mucous layer to the taste cells in them. The ceasing of taste is long too because taste active substances have to drift out with saliva from taste cells and then from taste buds into the oral cavity. The temperature has influence upon the taste perception too [10, 11].

The time-intensity evaluation is dynamic sensory method. The change of sensory attributes is monitored in dependence on time. It is concerned about determination of time dependency of some taste components in dependence on time from putting into mouth and after swallowing mainly. These procedures are important for monitoring of ceasing of long-time taste, e.g. bitterness and astringency because the speed of their perception is relatively slower than other taste qualities. The time needed for ceasing of taste monitoring is different and it is dependent on stimulus, its type (quality) and quantity [12-18].

There is possible to find out four basic parameters from time-intensity curve, namely maximum intensity of the taste (IMAX), time to maximum intensity (TMAX), total duration (DUR) and area under the curve (AUC). These values can enter into other analyses (e.g. principal component analysis PCA), where is possible to find out differences among the samples or panelists [14, 19-23].

We used capillary isotachophoresis (ITP) for determination of the artificial sweeteners in some soft drinks. ITP is a form of steady-state electrophoresis in which a voltage gradient generated by using buffers of differing mobility at constant current is used. It is therefore a discontinuous system. Electrophoresis proceeds until all ionic analysis are migrating with the same (iso) speed (tacho). The theory is dependent on the Kohlrausch regulatory function. ITP has high sensitivity and precision [24]. In food analysis, ITP can be used for the determination of food additives, preservatives, organic acids, biogenic amines, nitrates, food colorants and *etc.* The advantages of ITP include its independence of derivatisation and deproteination of the sample and in comparison with HPLC simple sample preparation and short analysis time [25].

The aim of this study was using capillary isotachophoresis and testing of suggested electrolytic system for determination of the artificial sweeteners (acesulfame K, saccharin and cyclamates) in some soft drinks. In these drinks evaluate and obtain time-intensity curves of sweetness.

Materials and methods

Samples

20 samples of the soft drinks were purchased in a local food market. 14 of them contained the artificial sweeteners. The samples were from different producers. The soft drinks data are defined in Tab. 1. The volume of all drinks was 2 l.

Table 1

Description of soft drink.
Charakterystyka napojów bezalkoholowych.

Sample Próba	Soft drink name Nazwa napoju bezalkoholowego	Declarative artificial sweeteners Deklarowane dodatki słodzące	Producer Producent
1	Coca-Cola light	E 950, E 951, E 952	SR
2	Relax Green Apple	E 951, E 952, E 954	SR
3	Relax Pink Grepp	E 951, E 952, E 954	SR
4	Toma Slice Pomaranč (orange flavour)	E 950, E 951	SR
5	Relax Vínny (grapy flavour)	E 950, E 951, E 952	SR
6	Senza Línia Multivitamín	E 950, E 951, E 954	SR
7	Senza – Ľadový čaj citrón (lemon ice tea)	E 950, E 951, E 952,E 954	SR
8	Olé – Ľadový čaj citrón (lemon ice tea)	E 950, E 952, E 954	CR
9	Wild Raspberry	E 950, E 952, E 954	SR
10	Prameň Citrón (lemon flavour)	E 950, E 952, E 954	SR
11	Kolča (cola flavour)	E 952, E 954	SR
12	Ice Tea – Ľadový čaj citrón (lemon ice tea)	E 950, E 951, E 952,E 954	CR
13	Lift Jablko (apple flavour)	E 950, E 952, E 954	SR
14	Multivitamín (multivitamin soft drink)	E 950, E 952, E 954	SR
15	Coca-Cola	-	SR
16	Kofola Original	-	SR
17	Mirinda Pomarančová Explózia (orange flavour)	-	CR
18	Jupí Frupper jahoda, pomaranč, jablko (strawberry, orange, apple flavour)	-	SR
19	Figo Línia Multivitamín (multivitamin soft drink)	-	SR
20	Márka Červená	-	SR

Explanatory notes: / Objaśnienia:
E 950 – acesulfame K / acesulfam K, E 951 – aspartame / aspartam, E 952 – cyklamate acid and its Na, Ca salts / kwas i cyklaminiany Na i Ca, E 954 – saccharin and its Na, K, Ca salts / sacharyna i jej sole Na, K, Ca,, SR – Slovak republic / Republika Słowacka, CR – Czech republic / Republika Czeska

Determination of the artificial sweeteners

The column coupling electrophoretic analyzer used was an EA 202 M (Villa Labeco, Spišská N. Ves, Slovak Republic). The artificial sweeteners were detected by a conductivity detector. The isotachopherograms were evaluated by a PC software package supplied with an analyzer. The electrolytic system of the following composition was applied: Leading electrolyte (LE): 0.5 mmol/l HCl + 0,1% MHEC,

pH was adjusted to 3.5 by ß-alanine. Terminating electrolyte (TE): 10 mmol/l citric acid. The driving current applied to the preseparation capillary was 200 μA and to the analytical capillary 20 μA.

Standards of sweeteners were: sodium cyclamate (99%, Merck, Germany), acesulfame K (99%, Fluka, Switzerland) and sodium salt of saccharin (99%, Aldrich, Germany).

Sample preparation for the determination of the artificial sweeteners

Samples that contained insoluble substances were filtered and samples that contained CO_2 were degassed in ultrasonic bath UC 005 AJ 1 (Tesla, ČSFR).

Time-intensity evaluation of sweetness

For time-intensity evaluation of sweetness model sweetener solutions of sucrose, aspartame and saccharine were prepared. Concentrations of sweetener solutions were followed: 9% sucrose, 0.05% aspartame and 0.9% saccharin.

Before evaluation panelists were tested for basic sensory tests: differentation of basic tastes, determination of threshold sensitivity, determination of threshold taste differences, determination of taste memory, determination of the rank according to aroma intensity, determination of the rank according to colour or turbidity intensity, determination of threshold aroma differences, determination of characteristic aroma substances.

The unstructured graphical scales were used for time-intensity evaluation. The result of its is time-intensity curve that showed how intensity of sense increases or decreases during time from tasting of sample.

The panelists were instructed to drink the sample, hold it in their mouths for 3 s and then swallow. They recorded extent of sweet taste intensity on the unstructured scale in 3 s (sense before swallowing of sample), 6 s (immediately after swallowing) and then every 3 s till total taste disappearing.

The arithmetic mean of sweet taste intensities were put into the graph in dependence on time and overlapped with regression curve.

Results and discussion

Determination of the artificial sweeteners

ITP and suggested electrolytic system was used for determination of the artificial sweeteners in 14 samples of soft drinks, in which was declared the presence of the artificial sweeteners: acesulfame K, saccharin and cyclamates. The results of this determination are shown in Tab. 2.

T a b l e 2

Amounts of acesulfame K, saccharin and cyclamates in soft drink samples determined by ITP method.
Zawartość acesulfamu K, sacharyny, i cyklaminianów w napojach, oznaczona za pomocą metody ITP

Sample Próba	Acesulfame K [mg/l]	s	s_r [%]	Saccharin [mg/l]	s	s_r [%]	Cyclamates [mg/l]	s	s_r [%]
1	189.19	2.84	1.50	ND	-	-	286.68	1.45	0.50
2	ND	-	-	17.03	0.53	3.11	116.35	1.82	1.56
3	ND	-	-	13.47	0.64	4.75	113.96	2.23	1.96
4	86.21	0.29	0.34	ND	-	-	ND	-	-
5	85.41	0.34	0.40	ND	-	-	263.85	3.63	1.37
6	117.43	1.35	1.15	31.04	0.72	2.32	ND	-	-
7	28.70	0.14	0.49	73.13	0.13	0.18	364.77	0.82	0.22
8	22.02	0.15	0.68	53.31	1.48	2.78	267.28	2.74	1.02
9	34.65	0.39	1.12	70.73	0.29	0.41	161.24	1.40	0.87
10	14.27	0.29	2.03	61.31	0.41	0.67	189.91	0.93	0.49
11	1.65	0.05	3.03	4.86	0.16	3.29	253.21	1.96	0.77
12	20.91	0.16	0.76	25.45	0.16	0.63	139.68	1.85	1.32
13	118.75	0.65	0.55	48.98	1.82	3.71	247.43	0.40	0.16
14	35.65	1.18	3.31	71.92	2.41	3.35	203.15	3.54	1.74

Explanatory notres: / Objaśnienia:
s – standard deviation / odchylenie standardowe, s_r – relative standard deviation / względne odchylenie standardowe
ND – below the limit of determination / ponizej limitu oznaczenia

T a b l e 3

ITP characteristics method of artificial sweeteners determination.
Oznaczenie sztucznych substancji słodzących za pomocą metody ITP

Parameter Parametr	Value / Wartość		
	Acesulfame K	Saccharin	Cyklamates
Detection limit [mg/kg]	0.82	1.96	1.82
Quantification limit [mg/kg]	1.24	2.95	2.73
Linearity [mg/kg]	1.2-11.9	3.0 -11.1	2.7-12.1
Correlation coeffients (for calibration curves)	0.9996-0.9997	0.9992-0.9998	0.998-0.9998

Calibration curves were measured for standard solution of acesulfame K, saccharin and sodium cyclamate in concentration interval 1-25 mg/l. The method

characteristics, i.e. linearity, detection limit, quantification limit of the artificial sweetener determination are summarized in Tab. 3.

The required results were compared with requirements of Decree of Slovak Republic, which quotes the highest allowable amount of acesulfame K 350 mg/l, saccharin and its Na, K and Ca salts 80 mg/l, cyclamate acid and its Na, Ca salts 250 mg/l.

All samples complied with the requirement for content of acesulfame K and saccharin. The content of acesulfame K ranged in individual samples from 1.65 mg/l (Kolča), to 189.19 mg/l (Coca-Cola light) and the content of saccharin from 4.86 mg/l (Kolča) to 73.13 mg/l (Senza – Ice tea lemon).

The amount of cyclamates determined in soft drink samples was in the range from 113.96 mg/l (Relax Pink Grepp) to 364.77 mg/l (Senza – Ice tea lemon). In the samples of soft drinks labeled 1, 5, 7, 8 and 11 higher values of cyclamates were determined (from 1.3% to 45.9%) as maximal limit given in Decree of Slovak Republic.

Time-intensity evaluation of sweetness

Ceasing of sweet taste was monitoring in model sweetener solutions and in samples of soft drinks. In Tab. 4 average values of sweet taste intensities of the sample 15 (Coca-cola) are presented from 0 to 15 s and its statistical characteristics.

Table 4

Ceasing of sweet taste in sample 15 (Coca-cola)
Zanik smaku słodkiego w próbce nr 15 (Coca-cola)

Time / Czas [s]	Intensity / Intensywność [%]	s	s_r [%]	$L_{1,2}$
0	0.0	0.0	0.0	0.0
3	16.8	0.9	5.2	16.8 ± 0.5
6	23.2	1.7	7.3	23.2 ± 0,9
9	14.9	1.1	7.5	14.9 ± 0.6
12	8.1	0.4	5.3	8.1 ± 0.2
15	3.1	0.3	10.6	3.1 ± 0.2
18	0.0	0.0	0.0	0.0

The time-intensity dependence of model sweetener solutions of sucrose, aspartame and saccharin was increased at first, whereupon maximum intensity was reached after swallowing of sample (after 6 s). Then intensity of sweet taste was decreased. Decreasing shape of the curve has form of non-linear dependence (type $y = ax^b$), which limits to zero. So, the perception of sweet taste decreases with time till disappears at last. There were no significant differences in maximum intensity for the four model sweetener solutions, because the samples were equisweet to 9% sucrose. The maximum intensity of sweet taste was 27.6% for sucrose solution, 27.1% for aspartame solution and 29.9% for saccharin solution. The total duration of sweet taste was equivalent in all model solutions (15 s).

Comparing the results of time-intensity sweetness evaluation in soft drinks was found out that samples containing the artificial sweeteners had higher maximum intensity and longer progress of ceasing too.

The maximum intensity of sweet taste was from 20.0% (Kofola Original) to 36.6% (Mirinda Pomarančová Explózia) in samples without the artificial sweeteners labeled 15, 16, 17, 18, 19 and 20 and total duration ranged from 15 s (Kofola Original) to 24 s (Figo Línia Multivitamín, Márka Červená). The samples with the artificial sweeteners had the maximum intensity of sweet taste from 28.3% (Toma Slice Pomaranč) to 40.5% (Lift Jablko) and the total duration was for these samples in interval from 18 s (Olé – Ľadový čaj citron, Prameň Citrón) to 36 s (Lift Jablko).

Conclusions

1. The method of capillary isotachophoresis and suggested electrolytic system is suitable for monitoring of the artificial sweeteners content. The advantage of applied method is simple sample preparation too. Determined amounts were compared with maximum allowed limits given in Decree of Slovak Republic. The samples of soft drinks (1, 5, 7, 8 and 11) exceeded maximum allowed amounts for cyclamates. They exceeded limit above 3.21 mg/l to 114.77 mg/l. All samples complied with requirements of Decree of Slovak Republic for acesulfame K and saccharin content.
2. In the next part of the study time-intensity evaluation of sweetness was monitored in the soft drink samples, which contained natural or artificial sweeteners. The time-intensity curves had similar shape with quick growth of sweet taste intensity and steeper decrease, whereupon the shortest progress of ceasing had samples containing the natural sweeteners (from 15 s to 21 s). The time of ceasing was longer at the samples containing the artificial sweeteners (18 s to 36 s). From this result, that the natural sweeteners are drifted out faster from taste buds and so ceasing of sweetness is shorter. Higher maximum intensity of sweet taste (28.3% to

40.5%) was in the samples containing the artificial sweeteners, while the samples without the artificial sweeteners had maximum intensity from 20.0% to 36.6%.

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IZOTACHOFORETYCZNE OZNACZANIE SZTUCZNYCH ŚRODKÓW SŁODZĄCYCH ORAZ OCENA ZMIAN INTENSYWNOŚCI SMAKU SŁODKIEGO W NAPOJACH BEZALKOHOLOWYCH

S t r e s z c z e n i e

W celu oznaczenia zawartości substancji słodzących: acesulfamu K, sacharyny oraz cyklaminianów w napojach bezalkoholowych zastosowano metodę izotachoforezy kapilarnej. Wszystkie badane próby spełniały wymagania Kodeksu Żywnościowego Republiki Słowackiej pod względem zawartości acesulfamu K oraz sacharyny. W kilku próbkach zawartość cyklaminianów przekraczała dopuszczalny poziom 3,21 mg/l, osiągając aż 114,77 mg/l. Przeprowadzono również sensoryczną ocenę zmian intensywności słodkiego smaku w napojach zależną od czasu, wykazując, że próby zawierające sztuczne środki słodzące wykazywały większą intensywność słodkiego smaku oraz dłuższy okres jego zanikania. Całkowity czas trwania smaku w tych próbkach wahał się w granicach od 18 do 36 s, a maksymalny poziom intensywności smaku słodkiego zawierał się w granicach od 28,3 do 40,5%. W próbach zawierających naturalne środki słodzące całkowity czas trwania wynosił od 15 do 21 s, a maksymalny poziom intensywności smaku od 20,0 do 36,6%.

Key words: izotachoforeza kapilarna, sztuczne środki słodzące, metoda time-intensity, smak słodki (słodycz)