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DETERMINATION OF ASPARTAME AND ITS METABOLITES IN SAMPLES OF CARBONATED SOFT DRINKS AND BEVERAGES WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Key words: α-aspartame, diketopiperazine, L-phenylalanine, drinks

Various beverages were determined for the levels of: α-aspartame, diketopiperazine, and L-phenylalanine. The main material was: dietary cola, dietary orangeade, dietary soft drinks and dietary energizing drinks with sweeteners: aspartame and acesulfame K. All analysed drinks were of good quality for they contained a low level of aspartame metabolites and a permissible level of aspartame, under 600 mg/L.

INTRODUCTION

Aspartame (N-L-α-aspartyl-L-phenylalanine methyl ester) is a low-calorie sweetener commonly used in carbonated soft drinks and beverages. Products sweetened with aspartame are usually of low calories contents. Using products with aspartame can help controlling weight. Aspartame is also recommended for people with diabetes. Aspartame has limited stability in aqueous solutions. Temperature and pH of the system are important factors for the stability of aspartame during storage. In many countries all products containing this sweetener must be labeled for phenylalanine because a small group of people who suffers from a hereditary disease phenylketonuria is sensitive to phenylalanine – one of the metabolites of aspartame. There are hypotheses that the diketopiperazine might promote seizures. Because α-aspartame can lose its sweetness under this conditions determination of aspartame and its breakdown products is extremely important for dietary food quality. The acceptable daily intake (ADI) values for aspartame, determined by the Joint FAO/WHO Expert Committee on Food Additives, is 0-40 mg/kg body mass [Deminralay *et al*., 2004; Gibbs *et al*., 1996; Kim *et al*., 1997; Zhu *et al*., 2005]. The objective of this study was to evaluate carbonated soft drinks and beverages, produced in Poland, for the contents of aspartame and its metabolites (diketopiperazine, L-phenylalanine).

MATERIALS AND METHODS

The samples for measurements were 4 groups of beverages: dietary soft drinks, dietary cola, dietary orangeade, dietary energizing drinks. Each sample was measured with duplicate injections into the chromatographic column. All beverages used for analysis were bought in a local supermarket.

The α-aspartame and its metabolites contents of the samples were determined with a liquid chromatographic method using a Varian ProStar system equipped with Rheodyne injector with a 20 μL loop and a ProStar 330 series Photodiode Array Detector. Chromatograms were recorded at 210 nm (Figure 1), with spectra (200-400 nm) taken continuously throughout the elution for confirmation. Varian Star Workstation for LC software was used for data processing. The analytical column (150 mm x 4 mm i.d.) obtained from Varian Chrompack was made of stainless steel, packed with Omnispher C18 stationary phase (5 μm) and operated at ambient temperature. The mobile phase (flow rate of 1.0 mL/min.) was aqueous 0.0125 mol/L monopotassium phosphate buffer (adjusted to pH 3.5 with phosphoric acid) and acetonitrile 88:12 (v/v). It was filtered through a 0.45-μm membrane filter and degassed ultrasonically just before analysis by means of high-performance liquid chromatography (HPLC). Water used in all experiments was generated by Milli-Q gradient A10 system from Millipore.

The samples of drinks were degassed and filtered through a 0.45-μm membrane filter before injection into the column.

RESULTS AND DISCUSSION

The analyses have indicated a wide range of aspartame level in the group of dietary cola (35-523 mg/L). Nevertheless a variety of the contents of aspartame has been proved in various drink producers. The group of dietary cola was characterised by an average higher mean level contents of aspartame than the other researched beverages (Table 1). In the group of orangeade statistically insignificant differences have only been observed among various researched series and different producers. In contrast, in dietary soft drinks the differences between particular researched series and various drinks producers have been significant (Table 2).

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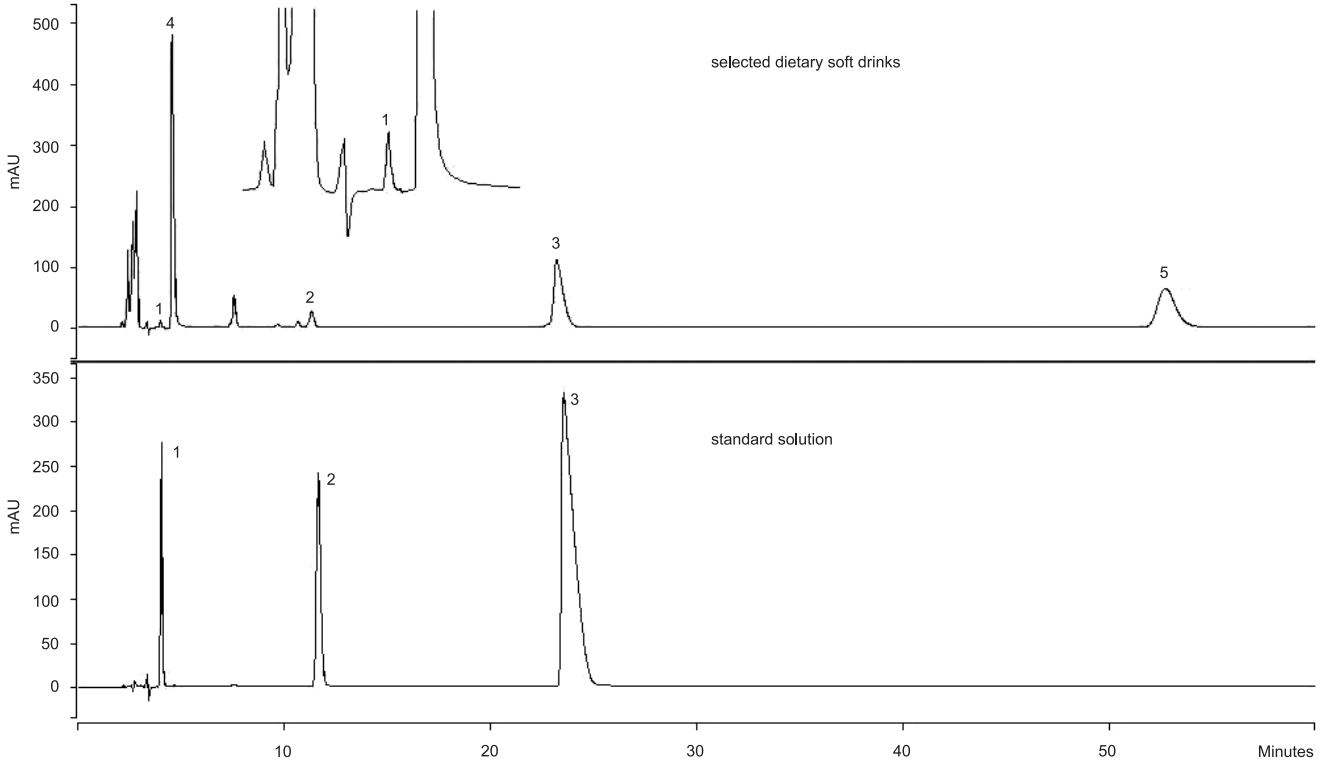

FIGURE 1. Chromatograms of standard solution of α-aspartame, diketopiperazine, L-phenylalanine and selected dietary soft drink (L-phenylalanine – 1, diketopiperazine – 2, α-aspartame – 3, acesulfame K – 4, benzoic acid – 5).

TABLE 1. The contents of α-aspartame in carbonated beverages.

Beverage	Number of samples	Aspartame – ranges (mg/L)	Aspartame – mean level (mg/L)
Dietary cola	24/*24	35-523	240
Dietary energizing drinks	12/*12	57-124	82
Dietary orangeade	12/*12	96-151	130
Dietary soft drinks	36/*36	81-343	162

*with detected level of α-aspartame

TABLE 2. The contents of α-aspartame in carbonated soft drinks from different producers.

Producers	Number of samples	Aspartame – ranges (mg/L)	Aspartame – mean level (mg/L)
Producer 1	4/*4	137-145	141
	4/*4	145-152	148
	4/*4	120-126	124
	4/*4	130-136	133
Producer 2	4/*4	100-106	103
	4/*4	91-94	93
	4/*4	81-82	81
Producer 3	4/*4	324-343	333
	4/*4	289-318	303

* with detected level of α-aspartame

TABLE 3. The contents of diketopiperazine in carbonated beverages.

Beverage	Number of samples	Diketopiperazine – ranges (mg/L/)	Diketopiperazine – mean level (mg/L)
Dietary cola	24/*24	2-45	18
Dietary energizing drinks	12/*12	12-41	30
Dietary orangeade	12/*12	2-4	3
Dietary soft drinks	36	0-9	4
	*22	3-9	6

*with detected level of diketopiperazine

The analyses have proved that the dietary beverages in Poland are of good quality considering the content of metabolites of aspartame as compared to a permissible level of aspartame – under 600 mg/L and a permissible level of diketopiperazine – under 100 mg/L.

Only in 28% of the measured drinks has L-phenylalanine been detected, whereas diketopiperazine has been detected in 83% samples of the drinks examined. The measured level of phenylalanine and diketopiperazine was low. The highest average value of aspartame metabolites (2.7 mg/L of L-phenylalanine and 30 mg/L of diketopiperazine) among the measured beverages was obtained in energizing drinks (Tables 3 and 4).

CONCLUSIONS

The measurements have indicated the difference of the contents of aspartame and its metabolites (diketopiperazine,

TABLE 4. The contents of L-phenylalanine in carbonated beverages.

Beverage	Number of samples	L-phenylalanine – ranges (mg/L)	L-phenylalanine – mean level (mg/L)
Dietary cola	24 *8	0-2.1 1.5-2.1	0.6 1.8
Dietary energizing drinks	12/*12	1.0-4.0	2.7
Dietary orangeade	12/*0	ND	ND
Dietary soft drinks	36 *4	0-0.4 0.3-04	0.04 0.3

ND - not detected, *with detected level of L-phenylalanine

phenylalanine) with regard to groups of dietary beverages. Dietary orangeade and soft drinks have been characterised by a lower average level of aspartame than dietary cola. The higher level of aspartame metabolites and a lower level of aspartame have been obtained in energizing drinks.

REFERENCES

1. Çubuk Deminralay E., Özkan G., Optimization strategy for isocratic separation of α-aspartame and its breakdown products by Reversed Phase Liquid Chromatography. Chromatographia, 2004, 60, 579-582.
2. Gibbs B.F., Alli I., Mulligan C.N., Simple and rapid high-performance liquid chromatographic method for determination of aspartame and its metabolites in foods. J. Chromatogr. A, 1996, 725, 372-377.
3. Kim S.K., Jung M.Y., Kim S.Y., Photodecomposition of aspartame in aqueous solutions. Food Chem., 1997, 59, 273-278.
4. Zhu Y., Guo Y., Ye M., James F.S., Separation and simultaneous determination of four artificial sweeteners in food and beverages by ion chromatography. J. Chromatogr. A, 2005, 1085, 143-146.

OZNACZANIE ZAWARTOŚCI ASPARTAMU I JEGO METABOLITÓW W GAZOWANYCH NAPOJACH BEZALKOHOLOWYCH Z ZASTOSOWANIEM TECHNIKI HPLC

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Substancje słodzące stosowane są w wielu produktach spożywczych, a produkty słodzone słodzikami charakteryzują się obniżoną wartością kaloryczną. Są cenione przez ludzi stosujących diety niskokaloryczne oraz przez diabetyków. Jednak spożywanie dużych ilości produktów z dodatkiem słodzików może powodować negatywne skutki zdrowotne.

Celem pracy było oznaczenie zawartości aspartamu i jego metabolitów: diketopiperazyny i L-fenyloalaniny w bezalkoholowych napojach gazowanych. Badania wykonano metodą wysokosprawnej chromatografii cieczowej (HPLC) po odgazowaniu i przesączeniu próbek napojów.

Przeprowadzone oznaczenia wykazały stosunkowo dobrą jakość badanych napojów, w aspekcie zawartości dopuszczalnych ilości aspartamu i obecności metabolitów tej substancji słodzącej. Oznaczone napoje typu cola charakteryzują się najwyższą średnią zawartością oraz największym zakresem zawartości aspartamu spośród badanych napojów. Najwyższe średnie poziomy metabolitów aspartamu (fenyloalaniny i diketopiperazyny) wykryto w grupie napojów energetyzujących.