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DETERMINATION OF BENZOIC AND SORBIC ACIDS IN FOODS

OZNACZANIE ZAWARTOŚCI KWASU BENZOESOWEGO I SORBOWEGO W PRODUKTACH SPOŻYWCZYCH

Abstract: The aim of this work was the analysis of benzoic acid and sorbic acid content in selected beverages and processed fruit and vegetable products. The analyses of these preservatives in 34 different food products (ketchups, carbonated and non-carbonated beverages, beverage concentrates) were performed. In this purpose, the conditions of analyzed compounds extraction from the food samples were optimized and method of analysis by reversed-phase high performance liquid chromatography method (RP-HPLC) was developed. Very good separation of the analytes was achieved using methanol-water-acetate buffer pH 4.4 (40 : 40 : 20) as a mobile phase.

Keywords: food preservatives, benzoic acid, benzoates, sorbic acid, sorbates, beverages, fruit and vegetable products

Majority of food products available on the market contain different types of additives, among which preservatives are playing an important role. These additives, applied in order to maintain food quality and prolong storage time, often make consumers anxious about its safety. Very popular preservatives are benzoic acid and sorbic acid, used mostly in the form of the well soluble sodium, potassium or calcium salts. These compounds retard the growth of yeast and moulds and are effective against wide range of bacteria [1]. Analytical methods used for their determination are based mainly on UV spectrophotometry, *gas chromatography* (GC) and *high performance liquid chromatography* (HPLC) [2–6]. These preservatives very often are used together due to its complementary properties, so the simultaneous determination of these two acids is desirable. In many cases prior to the analysis sample preparation is required, in order to isolate the analytes from the complex food samples. The aim of this work was the analysis of benzoic and sorbic acid content in selected beverages and processed fruit and vegetable products. In this purpose, the conditions of analyzed compounds extraction from the food samples were optimized and method of analysis by *reversed--phase high performance liquid chromatography* method (RP-HPLC) was developed.

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Materials and methods

The analyses of benzoic and sorbic acid used as the preservatives in different food products available on the home market (ketchups, carbonated and non-carbonated beverages, beverage concentrates) were performed.

Analytes were extracted from the food samples by chloroform after previous salting out using natrium chloride solution. In order to optimize this step, the influence of different factors during sample preparation was studied (solvent volume, multiplication, pH, filtration). It was found that using chloroform as a extraction solvent, more than 95 % of analytes were obtained in the first three extractions; in the samples from fifth extraction analytes were not found. So it was stated that four extractions with 5 cm^3 of chloroform is proper. The time consumable filtration process of salted out sample which was used in preliminary works appeared to be not necessary. Instead of this centrifugation of final sample was performed.

Finally, 2.5 g of ketchup or 15 cm^3 of beverage was placed in a measuring flask, filled to 25 cm^3 volume with saturated natrium chloride solution and well mixed. Carbonated beverages were degassed previously by using water aspirator. Next, 20 cm^3 of obtained solution was placed in separatory funnel and extracted four times by using 5 cm^3 of chloroform. All extracts were connected and filled up to 25 cm^3 volume with methanol. Before the chromatographic analysis samples were centrifuged.

The analyses were performed by *high performance liquid chromatography* (HPLC) method. The reversed phase mode was applied, with *non-polar octadecylsilane* (C18) stationary phase and polar mobile phase with lowered pH. LC-5B liquid chromatograph equipped with UV-VIS detector and glass column (150 × 3.3 mm I.D.) filled with SEPARON SGX C-18 (7 μm) stationary phase was used. The monitoring wavelength was λ = 254 nm. The analyses were performed at room temperature with the mobile phase flow rate 0.5 cm^3/min.

The composition of eluent was optimized in order to achieve good separation of benzoic acid, sorbic acid and chloroform. In this purpose, different mobile phases were prepared using mixtures of methanol and water, with pH lowered by addition of acids or buffer. Due to the similarity of pK_a values of benzoic and sorbic acid (4.21 and 4.76, respectively) separation by reversed phase liquid chromatography is difficult. Using mobile phases with additions of acids, with pH 3.0 (0.1 % of phosphoric acid) or 3.6 (1 % of acetic acid), separation of analytes was not achieved. Good separation was enabled by using eluents of pH between pK_a values of analytes (with addition of acetic buffer pH 4.4). For all tested mobile phases, retention times of benzoic and sorbic acid and extraction solvent (chloroform) was measured and peak resolution (R_s) was calculated from the formula [7]:

$$R_s = 1.177\ d\ /\ (w_{0.5(1)} + w_{0.5(2)})$$

where: d – distance between peaks centers [mm],
$w_{0.5}$ – widths at half height of peaks [mm].

Among the tested eluents the best results provided mixture of methanol (40 %), water (40 %) and acetic buffer pH 4.4 (20 %) and it was finally used as a mobile phase. For

this eluent the obtained value of R_s factor for benzoic and sorbic acid was high above minimum criteria value 1.5 (complete resolution to the baseline) and the time of analysis was much shorter than using mobile phase with 30 % of methanol. The obtained data are presented in Table 1.

Table 1

Retention times and peak resolutions of benzoic and sorbic acids obtained for different mobile phase compositions

Mobile phase composition	Retention time [min]			Peak resolution R_s
	Benzoic acid	Sorbic acid	Chloroform	
50 % methanol 49.9 % water 0.1 % phosphoric acid	4.83	4.83	6.18	0
50 % methanol 49 % water 1 % acetic acid	5.30	5.30	6.25	0
50 % methanol 30 % water 20 % acetic buffer (pH 4.4)	3.67	4.17	6.25	1.36
40 % methanol 40 % water 20 % acetic buffer (pH 4.4)	**5.28**	**6.75**	**10.35**	**2.97**
30 % methanol 50 % water 20 % acetic buffer (pH 4.4)	8.37	11.33	16.67	3.52

Identification of preservatives in various foodstuffs was based on the retention time comparison of peaks obtained in analysis of the standard solutions and the samples. Quantification was performed by external standard method using calibration curves which were rectilinear in the range of method application (correlation coefficients: 0.9980 for benzoic acid and 0.9997 for sorbic acid). *The limits of detection* (LOD) were determined by decreasing the concentration of the prepared standard solutions to achieve the smallest detectable peaks, and multiplying these concentrations by 3. The estimated LOD values in proposed method were 4.0 mg/dm^3 for benzoic acid and 0.12 mg/dm^3 for sorbic acid. These values are much below the permitted limits detailed for the individual foods, which are in majority of cases between 150 mg/dm^3 – 1000 mg/dm^3 or mg/kg for benzoic acid and 200 mg/dm^3 – 2000 mg/dm^3 or mg/kg for sorbic acid [8].

Results and discussion

The performed analyses of 9 different types of *ketchups* (K) confirmed that in agreement with the producer's information, in two of them preservatives were not detectable. In the remaining 7 ketchups sodium salt of benzoic acid was applied. The

level of benzoic acid in one of them (1776 mg/kg) was found to be high above the maximum allowed limit for this group of products (1000 mg/kg). The content of benzoic acid in other samples ranged from 226 mg/kg to 1009 mg/kg (Table 2).

Table 2

Preservatives contents in *ketchups* (K)

Product	Benzoic acid [mg/kg]	Sorbic acid [mg/kg]	Summary content (benzoic + sorbic acid) [mg/kg]
K-1	416	n.d.	416
K-2	941	n.d.	941
K-3	226	n.d.	226
K-4	1776	n.d.	1776
K-5	660	n.d.	660
K-6	1009	n.d.	1009
K-7	695	n.d.	695
K-8	n.d.	n.d.	n.d.
K-9	n.d.	n.d.	n.d.

n.d. – not detected.

The results of the analyses of the *non-carbonated* (NCB) and *carbonated beverages* (CB) are collected in Tables 3 and 4, respectively. All analyzed non-carbonated beverages contained benzoic acid in the range of 73 mg/dm^3 – 174 mg/dm^3, and additionally in two samples sorbic acid (27 mg/dm^3 and 30 mg/dm^3) was found. Majority of the analysed carbonated beverages contained benzoic acid (16–406 mg/dm^3) or its mixture with sorbic acid (73–169 mg/dm^3), in one sample only sorbic acid was applied (169 mg/dm^3). The amount of benzoic acid in two samples was above the maximum allowed limit for beverages – 150 mg/dm^3. The limit for sorbic acid in beverages was set at 300 mg/dm^3 when it is used separately or 250 mg/dm^3 when it is applied together with benzoic acid. These values were not exceeded in none of the analysed samples of beverages.

Table 3

Preservatives contents in *non-carbonated beverages* (NCB)

Product	Benzoic acid [mg/dm^3]	Sorbic acid [mg/dm^3]	Summary content (benzoic + sorbic acid) [mg/dm^3]
NCB-1	142	n.d.	142
NCB-2	73	27	100
NCB-3	73	30	103
NCB-4	141	n.d.	141
NCB-5	128	n.d.	128
NCB-6	174	n.d.	174
NCB-7	132	n.d.	132

n.d. – not detected.

Table 4

Preservatives contents in *carbonated beverages* (CB)

Product	Benzoic acid [mg/dm^3]	Sorbic acid [mg/dm^3]	Summary content (benzoic + sorbic acid) [mg/dm^3]
CB-1	149	84	233
CB-2	133	73	206
CB-3	136	n.d.	136
CB-4	92	n.d.	92
CB-5	117	n.d.	117
CB-6	138	n.d.	138
CB-7	78	n.d.	78
CB-8	132	n.d.	132
CB-9	123	n.d.	123
CB-10	129	n.d.	129
CB-11	n.d.	169	169
CB-12	93	88	181
CB-13	16	169	185
CB-14	406	n.d.	406

n.d. – not detected.

The analyses of selected *beverage concentrates* (BC) showed that calculated summary content of benzoic and sorbic acid in these products (after dilution according to the producer's recommendation) ranges between 76 mg/dm^3 and 189 mg/dm^3. These values, collected in Table 5, are below the maximum summary content limit of these two acids – 600 mg/dm^3.

Table 5

Preservatives contents in *beverage concentrates* (BC)

Product	Benzoic acid [mg/dm^3]	Sorbic acid [mg/dm^3]	Summary content (benzoic + sorbic acid) [mg/dm^3]	Recommended dilution of product (v:v)	Summary content (benzoic + sorbic acid) in diluted product [mg/dm^3]
BC-1	919	n.d.	919	1:10	92
BC-2	857	n.d.	857	1:10	86
BC-3	n.d.	304	304	1:4	76
BC-4	166	588	754	1:4	189

n.d. – not detected.

The obtained data indicate that the preservatives content in foods exceeded the permitted maximum levels only accidentally.

Conclusions

The analyses of benzoic and sorbic acid content in 34 different food products (ketchups, carbonated and non-carbonated beverages, beverage concentrates) were performed by high performance liquid chromatography. The proposed method enables the simultaneous and selective analysis of these popular preservatives in different types of products.

On the basis of the obtained results it was found that the content of the analyzed preservatives in most cases was below the permitted level. In one ketchup sample and two beverages samples, amount of benzoic acid significantly exceeded the allowed values.

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OZNACZANIE ZAWARTOŚCI KWASU BENZOESOWEGO I SORBOWEGO W PRODUKTACH SPOŻYWCZYCH

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Abstrakt: Przedmiotem badań była analiza zawartości kwasu benzoesowego oraz kwasu sorbowego w wybranych napojach i produktach owocowo-warzywnych. Oznaczono zawartość tych konserwantów w 34 artykułach żywnościowych: ketchupach, napojach gazowanych i niegazowanych oraz zaprawach do napojów. W tym celu dobrano warunki ekstrakcji oznaczanych związków z próbek artykułów spożywczych oraz opracowano metodę ich analizy za pomocą *wysokosprawnej chromatografii cieczowej w odwróconym układzie faz* (RP-HPLC). Bardzo dobry rozdział analitów uzyskano przy zastosowaniu jako fazy ruchomej mieszaniny metanol–woda–bufor octanowy pH 4,4 (40 : 40 : 20).

Słowa kluczowe: konserwanty, kwas benzoesowy, benzoesany, kwas sorbowy, sorbiniany, napoje, produkty owocowo-warzywne