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Determination of Sorbitol Concentration in Diet Chocolate by High-Performance Liquid Chromatography

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Summary

Confectionery products are among the ever increasing group of healthy foodstuffs. In the diet chocolate mass used in this study the sucrose was replaced with sorbitol and sweetener aspartame. A new method for determination of sorbitol in diet chocolate by high-performance liquid chromatography was developed, using the method of external standard on an ion–exchanger sugar column (Bio-Rad; Aminex HPX 42C, 300 × 7.8 mm). The repeatability of the method was confirmed on the identical diet chocolate sample injected six times. The statistical analysis of retention time of sorbitol peak, sorbitol peak area, sorbitol peak height and sorbitol concentration in the sample included the following parameters: mean value (Δx), standard deviation (S.D.), relative standard deviation (R.S.D.) and confidence interval (C.I.). The results indicate that this technique enables exact determination of sorbitol content in diet chocolate, as well as other polyols in diet chocolates enabling new products development.

Key words: diet chocolate, sorbitol, HPLC

Introduction

Sugar-free low-calorie products, enriched with healthy ingredients, are becoming more popular, because consumers demand healthy products in confectionery industry as a part of their general dietary attitudes. Diet foodstuffs are those that are intended for individuals with metabolic disorders, *i.e.* diabetics. Such foodstuffs must satisfy certain basic criteria: *(i)* fat fraction must not exceed the fat fraction in similar or related foodstuffs; *(ii)* glucose, glucose syrup, invert-sugar or disaccharides must not be added during production of these foodstuffs. Diet milk chocolate is one of the low-calorie products and may be consumed by diabetics (*1,2)*. In the cocoa products industry in Croatia the following sugar substitutes may be used: sorbitol, xylitol, mannitol, isomalt maltitol, maltitol syrup, lactitol and polydextrose (*3*).

The aim of the experiments was to establish a new inexpensive method for routine determination of sorbitol by HPLC in chocholate mass, when sucrose was substituted by sorbitol. Furthermore, other methods of sorbitol determination were compared.

There are several methods used for sorbitol determination: *(i)* polarimetric method based on the fact that different sorbitol concentrations in water differ in the rotation of the plane of polarized light *(4)*; *(ii)* enzymatic method based on the reaction of sorbitol-dehydrogenase that in the presence of NAD^+ oxidizes D-sorbitol into fructose *(5)* and the obtained NADH is determined

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spectrophotometrically; *(iii)* periodic acid method which enables determination of sorbitol concentration on the basis of the used periodic acid *(6)*; *(iv)* gas-chromatographic (GC) method, carried out on the capillary column using the internal standard; and *(v)* high performance liquid-chromatographic (HPLC) method, in which external and internal standards are separately used *(7)*.

Materials and Methods

Chemical

For determination of sorbitol the following chemicals were used: redistilled water, *Kraš;* enzymatic test--combination for sorbitol/xylitol, *Boehringer* Mainnheim; Sorbitex P, *Krefeld, Germany*; Carrez-I-solution (potassium hexacyanoferrate $K_4[Fe(CN)_6] \cdot 3\ H_2O$), Carrez-II--solution (zinc sulfate, Zn $SO_4 \cdot 7\ H_2O$), *Kemika, Croatia;* NaOH, p.a. *Kemika, Croatia.*

Chocolate sample

The main ingredientes of the diet chocolate are: milk powder, sugar substitute sorbitol, cocoa butter, cocoa mass, vegetable fat, hazelnuts, aspartame and vanilin *(8)*.

Tehnological process of diet chocolate production includes: *(i) mixing* chocolate mass with sorbitol, powder milk, hazelnuts and other additions; *(ii) rolling*: fragmentation of chocolate mass into finely structure; and *(iii) conching*: producing the necessary aroma of chocolate mass *(9,10)*.

Sugar determination with enzyme test combinations was prenormed: 1 g of the diet chocolate sample was put into a 100 mL volumetric flask, 60 mL of redistilled water were added, and the mixture was incubated for 15 min at 70 °C. Proteins were precipitated with 5 mL Carrez-I, 5 mL of Carrez-II solutions, and 10 mL NaOH. The volumetric flask was filled up with redestilled water. The sample was left in refrigerator (4 °C) for 20 min and filtered. The pH of the solution was 6.3.

Standard solutions of sorbitol

Standard solutions were prepared in the same way as the sample and had the same pH=6.3. The concentration of sorbitol was: (I) $9 \cdot 10^4$ mol/L; (II) $1.5 \cdot 10^3$ mol/L; (III) $1.9 \cdot 10^3$ mol/L; (IV) $2.3 \cdot 10^3$ mol/L; (V) $2.9 \cdot 10^3$ mol/L; (VI) $3.2 \cdot 10^3$ mol/L; (VII) $3.9 \cdot 10^3$ mol/L.

HPLC-system

High-performance liquid chromatograph Hewlett--Packard 1100 series, U.S.A., consisting of binary pump, vacuum-degasser, manual injector (Rheodyne, U.S.A.), column heater (Jeatstream, Austria), signal converter and RI detector was used. Sorbitol concentration was determined from chromatographic runs performed on an ion-exchanger sugar column (Bio-Rad; Aminex 42C, 300 × 7.8 mm) and a precolumn (Bio-Rad; Micro-Guard, Carbo-C, 46 mm ID × 3 cm).

Chromatographic conditions for determination of sorbitol

Sorbitol concentration was determined at 80 °C using an RI detector. Redistilled water was used as the mobile phase, with an injection volumen of 20 μL and flow rate of 0.6 mL/min *(11)*.

Qantitative determination of sorbitol in diet chocolate was carried out by the method of external standard. Sorbitol standard was prepared from »Sorbidex P«, Krefeld, Germany which contains 98–100 % hexitol (pH=5.0–7.0).

From the prepared standard solutions detector response factors (RF) were determined and the calibration curve was estabished.

Results

Linearity of the method

A calibration curve for sorbitol was constructed by using different concentrations of standard solutions. The correlation coefficient was calculated and the linearity of the calibration curve was confirmed.

Calibration curve of sorbitol is shown in Fig. 1.

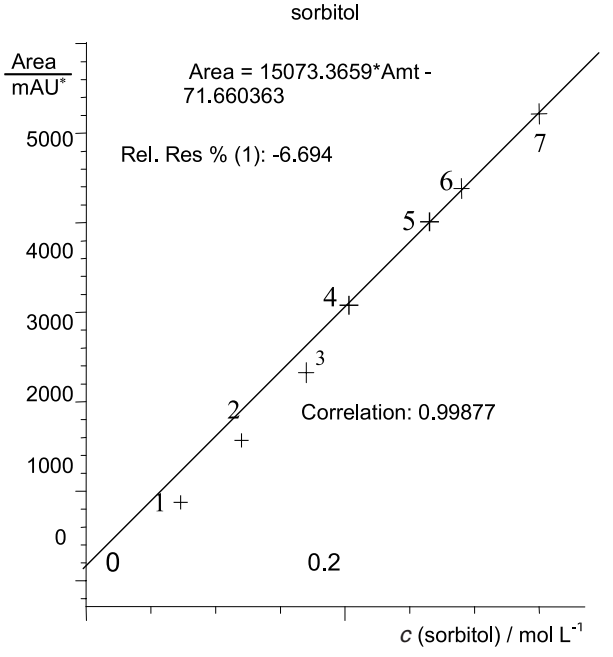

Fig. 1. Linearity of the method; calibration curve of sorbitol and the correlation coefficient for sorbitol * AU = Abundance Unit

Detection of 0.01 ppm of sorbitol standard was determined as the lowest detection limit.

Accuracy of the method (»Recovery test«)

Method accuracy was tested by injecting the known and precisely determined concentration of sorbitol standard solution ($1.4 \cdot 10^3$ mol/L). Sorbitol concentration was determined from the previously established calibration curve (Fig. 2).

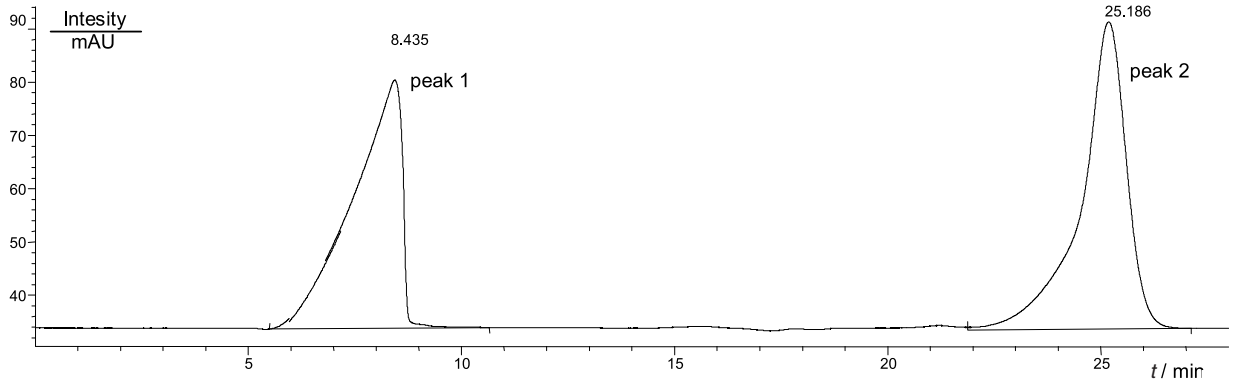

Fig. 2. Chromatogram of the »recovery test« (peak 1 = solvent peak; peak 2 = sorbitol peak; recovery 98 %); c(sorbitol) = $1.4 \cdot 10^3$ mol/L

Repeatibility of the method

For the purposes of statistical analysis, one sample of diet chocolate with sorbitol was injected consecutively several times. The following procedure was applied: *(i)* injecting standard solutions containing $9 \cdot 10^4$, $1.5 \cdot 10^3$ and $1.9 \cdot 10^3$ mol/L, respectively; *(ii)* establishing the calibration curve with external standard solutions; *(iii)* injecting the same sample of diet chocolate with sorbitol six times in a row (Fig. 3).

Losses in sorbitol concentration quantification

Standard sorbitol solution $c = 1.4 \cdot 10^3$ mol/L was prepared in the first experiment without treatment with Carrez I and II and NaOH, whereas in the second experiment the standard solution of equal concentration was treated with solvents Carrez I and II and NaOH. Comparison of chromatograms, *i.e.* of the areas of sorbitol peaks of samples prepared in the two described ways, reveals a difference in the obtained concentrations of sorbitol solutions. Taking into consideration that the mass of diet chocolate used in this study is 100 g, total loss can be calculated as 0.01 %.

Placebo

The simulated sample of diet chocolate contained all ingredients as the sorbitol-containing chocolate, except the sorbitol itself. The placebo samples were prepared in exactly the same way as the sample of the diet chocolate containing sorbitol, and 20 μL of prepared placebo samples were analyzed. The obtained chromatograms preformed no peaks at retention time in which the sorbitol peak would occur. This was convincing evidence that the placebo sample contained no sorbitol, and that the sorbitol peak in the chromatogram of the diet chocolate with sorbitol originated exclusively from sorbitol and no other ingredient (Fig. 4).

Statistics

After six consecutive injections of diet chocolate with sorbitol the sample was analyzed for *(i)* retention time of sorbitol peak, *(ii)* sorbitol peak area, *(iii)* sorbitol

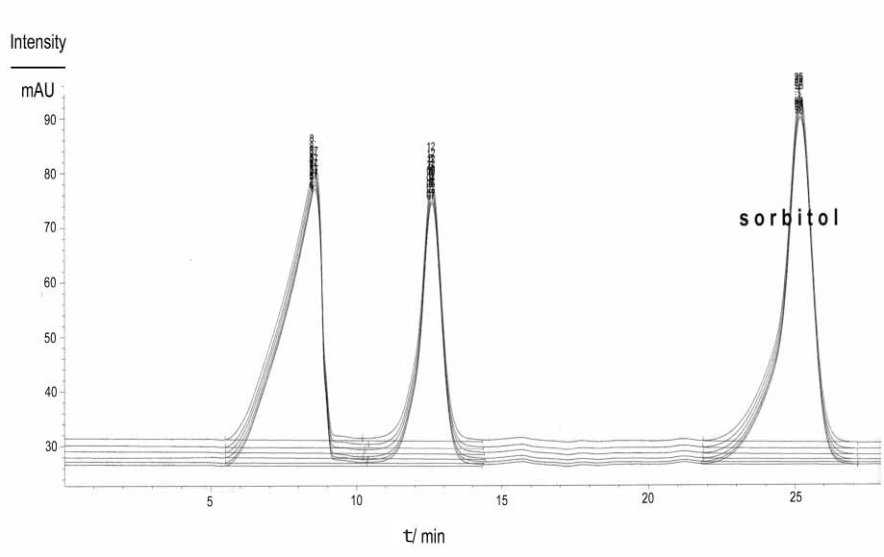

Fig. 3. Repeatibility of the method – overlap of six injections of the same sample of diet chocolate with sorbitol

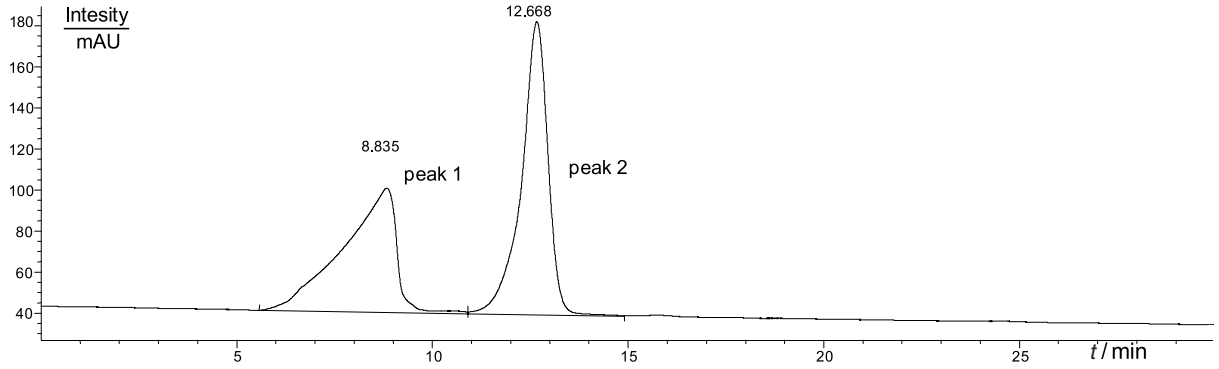

Fig. 4. Chromatogram of the placebo diet chocolate (peak 1=solvent peak; peak 2=lactose peak)

peak height, and (*iv*) sorbitol content in the sample, in terms of the following parameters: mean (Δx), standard deviation (S.D.), relative standard deviation (R.S.D.) and confidence interval (C.I.).

Table 1. Δx, S.D., R.S.D. and C.I. of retention time of peak, peak area, peak height and sorbitol concentration

Run # 6	Ret.time min	Run # 6	Peak area mAU*s
Mean	25.192	Mean	4672.250
S.D.	0.004	S.D.	8.301
R.S.D.	0.016	R.S.D.	0.177
C.I.	0.004	C.I.	8.700
Run # 6	Peak height mAU	Run # 6	*c*(sorbitol) mol/L
Mean	63.627	Mean	$1.6 \cdot 10^3$
S.D.	0.100	S.D.	0.0005
R.S.D.	0.178	R.S.D.	0.177
C.I.	0.119	C.I.	0.0005

Discussion

Until now polarimetric method for the determination of sorbitol concentration in diet chocolate has been used in »KRAŠ«. The method requires sample preparation that is more complicated than the described liquid chromatography method (HPLC) *(12)*. Besides, in the polarimetric method, a greater amount of reagent is needed and the results are less precise.

The six consecutive measurements of the amount of sorbitol in diet chocolates were done by polarimetric method. The amount of sorbitol and corresponding S.D. value were compared with the amount of sorbitol obtained by the chromatographic method.

Comparing the S.D. values of sorbitol obtained by the chromatographic, the polarimetric and the enzymatic method it was found that S.D. value of the amount of sorbitol was 0.0005 by the first method, 0.0108 by the second one, and 0.0003 by the third one. It can be concluded that the results obtained by liquid chromatography were more precise than those obtained by two other methods.

Moreover, only sorbitol as sugar alcohol in a mixture can be determined polarimetrically, whereas the proposed high-performance liquid chromatographic method enables the determination of some other sugar alcohols *(13,14)*. Mobile phase used in this method is redistilled water, ecologically acceptable and quite inexpensive solvent *(15,16)*.

Conclusions

On the basis of the experimental data, from the prepared samples of diet chocolate with sorbitol, obtained chromatograms, and statistical analysis of chromatographic parameters, the following conclusions may be drawn: *(i)* the method for determination of sorbitol content in diet chocolate by means of high-performance liquid chromatography, that uses external standards, is more accurate and precise than the polarometric method and is adequate for routine determination of sorbitol in diet chocolate in KRAŠ; *(ii)* the results are repeatable; *(iii)* sample preparation is simple and inexpensive chemicals are used (redistilled water is used as mobile phase); *(iv)* it is one of the least time-consuming methods for sorbitol content determination (sorbitol eluting in less than 30 min); *(v)* the method may be applied in determination of other polyols in diet chocolate (*i.e.* it offers possibilities of developing analytical procedures for new products).

References

1. T. Fuleki, E. Pelayo, R. B. Palabay, *J. Agr. Food Chem. 42* (1994) 1266–1275.
2. V. P. Hanko, J. S. Rohrer, *Anal. Biochem. 283* (2000) 192–199.
3. *Official Gazette, Rep. Croatia, 88* (1996) 3829.
4. T. H. Grenby: *Advances in Sweeteners*, London (1997) p. 72.
5. P. Ghahramani, M. S. Lennard, *J. Chromatogr. 685* (1996) 307–313.
6. M. S. Billaux, B. Flourie, C. Jacquemin, B. Messing, In: *Handbook of Sweeteners*, S. Marie, J. R. Piggott (Eds.), New York (1990) pp. 17–33.
7. A. G. Perez, R. Olias, J. Espada, J. M. Olias, C. Sanz, *J. Agr. Food Chem. 45* (1997) 3545–3549.

8. Y. Pameranz, C. E. Meloan: *Food Analysis; Theory and Practice*, Chapman & Hall, *3*, London (1994) pp. 324–350.

9. A. Pflugmacher: *Confectionery – School, Part I, Chocolate*, E. Stoffel (Ed.), Central College of German Confectionary Industry, Germany (1991) pp. 4.0–4.4.

10. P. S. Dimick, *The Manufacturing Confectioner*, *5* (1991) 109–114.

11. *Guide to Aminex HPLC Columns for Food and Beverage Analysis*, Bio-Rad, Chemical Division, Richmond, CA, USA.

12. M. Hudina, F. Stampar, *Acta Aliment. 29* (2000) 217–230.

13. D. James, In: *Sugar Confectionery Manufacture*, E. B. Jackson (Ed.), New York (1990) pp. 1–12.

14. P. Reschiglian, D. Melucci, G. Torsi, *J. Chromatogr. A, 740* (1995) 245–252.

15. M. S. Badiga, N. K. Jain, C. Casanova, C. S. Pitchumoni, *J. Am. Coll. Nutr. 9* (1990) 578–582.

16. M. J. Dennis, R. C. Massey, T. Bigwood, *Analyst*, *119* (1994) 2057–2060.

Određivanje koncentracije sorbitola u dijetnoj čokoladi pomoću HPLC

Sažetak

Konditorski proizvodi pripadaju u sve veću skupinu hrane koja je dobra za zdravlje ljudi. U čokoladnoj masi dijetne čokolade, upotrijebljene u ovom radu, saharoza je zamijenjena sorbitolom i zaslađivačem aspartamom. Razrađena je metoda određivanja sorbitola u dijetnoj čokoladi primjenom visokoučinkovite tekućinske kromatografije, metodom vanjskog standarda, na ionsko-izmjenjivačkoj koloni za šećere (Bio-Rad; Aminex HPX 42C, 300 $\times$7,8 mm). Ponovljivost metode utvrđena je u istom uzorku dijetne čokolade injektiranom šest puta. U statističkoj obradbi određeni su sljedeći parametri: srednja vrijednost (Δx), standardna devijacija (S.D.), relativna standardna devijacija (R.S.D.), interval pouzdanosti (C.I.), a odnose se na vrijeme retencije pika, površinu i visinu pika, te koncentraciju sorbitola u uzorku. Dobiveni rezultati pokazuju da se ovom tehnikom vrlo uspješno može odrediti koncentracija sorbitola u dijetnoj čokoladi, a na isti način i drugi polioli u dijetnim čokoladama što omogućava razvoj novih proizvoda.