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Original paper

The analysis of sodium butyrate content in various dietary supplements available on the Polish market

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ABSTRACT

Introduction: Natrium butyrate is a chemical compound which seems to have several beneficial properties, which alleviate signs and symptoms of gastrointestinal diseases. As a result, it has become a frequent component of therapeutic regimens. However, all of the oral products containing natrium butyrate available on the Polish market are registered as dietary supplements and none of them is approved for use as a medication, which might raise concerns about their quality.

Material and methods: Natrium butyrate from eight different dietary supplements available on the Polish market in April 2024 was extracted with diethyl ether. Subsequently, diethyl ether was evaporated and remnants were dissolved in methanol in order to perform high performance liquid chromatography (HPLC). Obtained solutions were passed through a chromatography column to assess exact content of natrium butyrate in each sample. Finally, obtained peaks were compared with standard samples and the content of natrium butyrate in each sample was calculated.

Results: Sodium butyrate content was detected in each of the analyzed samples. However, the percentage ratio of the amount detected to that declared by the producers varied from 45.51% to 106.15%.

Conclusions: In the majority of cases, analyzed dietary supplements contained natrium butyrate in quantities similar to those declared by manufacturers; however, compliance with the manufacturer's declaration varied between specific products, highlighting the need for further research in this area.

KEYWORDS

HPLC, high performance liquid chromatography, butyric acid, dietary supplement, natrium butyrate

INTRODUCTION

Short chain fatty acids are substances produced in a process of fermentation of indigestible carbohydrates performed by commensal bacteria inhabiting the human digestive tract. One of these substances is butyric acid, which is known for its variety of functions in vivo, starting from being a source of energy for colonocytes to the ability of activation of G-protein coupled receptors, and thus exerting metabolic effects [1,2]. Its derivative, natrium butyrate, is a common component of dietary supplements, and these products are thought to be beneficial in patients with some gastrointestinal conditions such as irritable bowel syndrome [3,4] and inflammatory bowel diseases [5,6]. Moreover, growing evidence confirms correlation between gut dysbiosis and metabolic disorders such as obesity and type 2 diabetes mellitus, which may suggest the advantageous effects of natrium butyrate supplementation in the course of these illnesses [7,8].

The following terms "medication" and "dietary supplements" are defined by pharmaceutical law, in this particular case by the Polish one. As far as medications are concerned, these are the substances used to treat or prevent specific diseases. Before introducing them to the market they must be

registered and approved by proper institutions, and after that they are subjected to constant monitoring. On the other hand, dietary supplements are designed to enrich and complete the diet, and institutional control over them is not as strict as in the case of medications [9,10]. Taking into account increasing popularity of dietary supplements among patients, it does raise concerns about quality, safety and legitimacy of their usage [11,12]. The analysis of the composition of specific dietary supplements, including those containing sodium butyrate, conducted by independent scientific institutions is gaining increasing attention as evidence continues to emerge, showing that many such products exhibit discrepancies between their declared and actual content. Regarding products containing sodium butyrate, to the authors' knowledge, this is one of the first analyses of its kind worldwide.

In April 2024 all of the oral products containing natrium butyrate, that were available on the Polish market, were registered as dietary supplements. In order to compare actual content of natrium butyrate within them with the amounts declared by manufacturers the authors performed appropriate high performance liquid chromatography (HPLC) analytical tests, utilising modified methods for quantitative analysis of short chain acid in stool that had been previously described by De Baere et al. [13] and Eberhart et al. [14].

MATERIAL AND METHODS

Reagents and laboratory equipment

During the experiment the following reagents were used: methanol, diethyl ether, trifluoroacetic acid (TFA) [POCh, Gliwice, Poland] and standard solution of propionic acid, as well as butyric acid [Sigma-Aldrich, Germany, Cat. No. 81910].

As far as equipment is concerned, the following devices were used: high performance liquid chromatograph Merck/Hitachi D-7000 HPLC System along with Hitachi/Merck L-7350 Column oven and LaChrom L-7420 UV-Vis Detector. Liquid chromatography analysis was performed on a C-18 chromatographic column [XBridge C18 5µm, Waters Corporation, Milford, MA, USA]. A linear gradient separation was performed using a mobile phase consisting of 0.1% (v/v) TFA in water (A) and 0.1% (v/v) TFA in acetonitrile (B) running from 10% B to 20% B in 8 minutes (1.25% per minute). The injection volume was 30 µL and the column-oven temperature was set to 30°C. Flow rate was 1.5 mL/min. Monitoring of all fatty acids was done at $\lambda = 210$ nm.

Analytical procedure

Eight different dietary supplements containing natrium butyrate were tested. The products were selected based on its availability in three different pharmacies in the Silesian Voivodeship. One of them was in the form of tablets and the other ones were capsules. Each supplement was randomly assigned a number from 1 to 8. Next, the following procedure was repeated for each of the tested products. The tablet or capsule content was put in a mortar and ground into fine powder. The obtained samples were placed in the tubes and then 100 mg of propionic acid and 2 mL of diethyl

ether were added. The propionic acid was used as an internal standard. Then the mixture was vortexed and centrifuged. After that the supernatant was decanted from the sediment. In order to evaporate diethyl ether the vaporizer was used. Afterwards, remnants were dissolved in 1.8 mL of methanol using the ultrasonic bath. Subsequently, solutions were passed through a syringe filter and put in vials appropriate for autosampler. Finally, HPLC was conducted with the following parameters – temperature: 30°C; time: 8 minutes; flow: 1.5 mL per minute. For the detection the UV-VIS detector was set for 210 nm wavelength. For each sample chromatography was performed three times and the results were averaged. Based on the chromatograms the area under the curve (AUC) of the peaks corresponding to propionic acid and butyric acid was read.

Calibration curves for butyric and propionic acids were prepared in parallel, using standard solutions at six different concentrations (Figure 1).

Using the calibration curves based on the area under the corresponding peaks, the contents of propionic acid and butyric acid were determined in 30 µL of the analyzed samples. Then, using the proportions, the contents of the mentioned acids in the whole sample were calculated. Assuming that the extraction of both propionic and butyric acids was equally efficient and knowing the initial content of propionic acid, the initial content of butyric acid was quantified. Finally, having the knowledge that the evaluated butyrate residues came from the sodium salt of this acid and using the stoichiometric formula, the masses of sodium butyrate corresponding to the previously calculated masses of butyric acid were measured.

RESULTS

Based on the analysis of the obtained chromatograms (Figure 2) and the subsequent calculations, it was proven that all of the discussed products contained natrium butyrate in the following amounts: 1) 235.11 mg, 2) 117.41 mg, 3) 159.22 mg, 4) 141.96 mg, 5) 136.53 mg, 6) 142.98 mg, 7) 186.52 mg, 8) 101.15 mg. Whereas the ratios of the determined content to the content declared by the producers expressed as a percentage were 47.02%, 78.27%, 106.15%, 83.51%, 45.51%, 95.32%, 62.17% and 67.43%, respectively. These results are summarized in Table I and Figure 3.

DISCUSSION

In order to compare results with potential previous similar studies the base PubMed was searched with the following terms “natrium butyrate quality”, “natrium butyrate dietary supplements”, “natrium butyrate products”, and no matching results were found.

This study has confirmed that analysed products contained natrium butyrate, although the percentage of declared content varied among different natrium butyrate products. It seems that formulations with a higher declared content in a single dose (300-500 mg) in fact had relatively lower content of sodium butyrate (45-62%) than those with lower declared amount (150-170 mg) of active ingredient. It is worth noting that at the time of the experiments in April 2024, many more supplements containing butyric acid derivatives were available on the Polish market than the

analysed eight. Moreover, the same products potentially might vary between different series or even between tablets or capsules within one series. Given these considerations, further studies are needed to draw general conclusions about the quality of these types of dietary supplements.

Another factor that potentially might have influenced the final results was the fact that analysed products varied in terms of concipients used to prepare them. The exact list of excipients declared by the manufacturers in each of the tested products is presented in Table II. Due to the unique nature of the method used, data on the potential impact of these substances on the extraction efficiency and chromatography result are limited. As a limitation of the study, it should be stressed that one batch of each dietary supplement was evaluated. Furthermore, the method used to detect butyric acid was primarily designed for stool testing, and designing new and more selective methods would possibly control the influence of concipients on the results.

Additionally, it is worth considering that presented amounts of natrium butyrate were detected in vitro, thus there is no information concerning both bioavailability of these products and their exact amounts reaching colon as the site of action.

Finally, regarding the popularity of dietary supplements and their potential unfavourable impact on the regular treatment administered by physicians, more studies on the quality of dietary supplements may contribute to increase the safety of their use. To provide context on supplement quality, illustrative evidence can be drawn from studies examining other dietary supplements available on the Polish market. For instance, Maćkiewicz et al. [15], in a study published in 2025, analyzed the composition of 24 dietary supplements containing iron. Among the products assessed, only two contained iron amounts consistent with the manufacturers' declarations, two contained lower amounts, and as many as 20 contained higher amounts than declared. Notably, one product contained only 0.062 mg of iron instead of the declared 27 mg, whereas another contained 214% of the declared iron content. Another study conducted in 2019 examined the caffeine content in dietary supplements available on the Polish market intended to support weight loss. Five dietary supplements and one medicinal product were assessed. The labeled caffeine content in the medicinal product and one dietary supplement matched the information on the packaging. Three products contained less caffeine than expected, and one contained more. The ratio of labeled caffeine to declared caffeine in the supplements tested ranged from 58.16% to 103.27% [16]. In addition, a Polish study assessing the magnesium content of 116 dietary supplements demonstrated that only 41.3% contained the declared dose or an acceptably similar amount (defined as up to 20% less or up to 45% more than declared). By contrast, 39.7% of the products contained less magnesium than declared, and 19.0% contained higher amounts [17]. Data from scientific studies showing the scale of discrepancies between the declared composition of dietary supplements and the actual one emphasize the importance of conducting further extensive research in this area and

also require vigilance on the part of consumers, as well as doctors and pharmacists who may recommend them.

CONCLUSIONS

Our results show that each of the analysed dietary supplements contained sodium butyrate. The measured content, as a percentage of the amount declared by manufacturers, ranged from 45% to 106% in the tested samples. This range indicates that, for some products, the discrepancies between the declared and actual sodium butyrate content in dietary supplements are significant, while other supplements contain an amount of sodium butyrate very close to the declared amount. Further research in this area is warranted to enhance the quality control and safety of dietary supplements.

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Conflict of interest

The authors declare that they have no conflict of interest.

Table I. Declared and empirically determined content of natrium butyrate in analysed samples

Product identification	Declared content of natrium butyrate [mg]	Actual content of natrium butyrate [mg]	Percentage of declared content of natrium butyrate [%]
1.	500	235.11 ± 9.75	47.02
2.	150	117.41 ± 3.34	78.27
3.	150	159.22 ± 9.79	106.15
4.	170	141.96 ± 9.79	83.51
5.	300	136.53 ± 5.06	45.51
6.	150	142.98 ± 6.11	95.32
7.	300	186.52 ± 8.95	62.17
8.	150	101.15 ± 5.28	67.43

Table II. Excipients declared by producers in tested products

Product identification	Excipients
1.	hydroxypropylmethylcellulose, dicalcium phosphate, anionic methacrylate copolymer, polysorbate 80, mono- and diglycerides of fatty acids, triethyl citrate, magnesium salts of fatty acids, colloidal silica
2.	fully hydrogenated triglycerides of fatty acids from palm oil, hydroxypropyl methylcellulose
3.	microcrystalline cellulose, maltodextrin, magnesium salts of fatty acids, hydroxypropyl methylcellulose, titanium dioxide
4.	hydroxypropylmethylcellulose, cellulose, maltodextrin, corn starch, ethylcellulose
5.	palm oil, hydroxypropylmethylcellulose, magnesium salts of fatty acids
6.	microcrystalline cellulose, hydroxypropyl methylcellulose, palm oil, magnesium salts of fatty acids
7.	maltodextrin, hydroxypropylmethylcellulose, gellan gum, magnesium salts of fatty acids, silicon dioxide
8.	fully hydrogenated palm oil, hydroxypropyl methylcellulose, sodium alginate, calcium carbonate, zinc oxide, starch, maltodextrin, gum arabic, mono- and diglycerides of fatty acids, carrageenan, potassium chloride

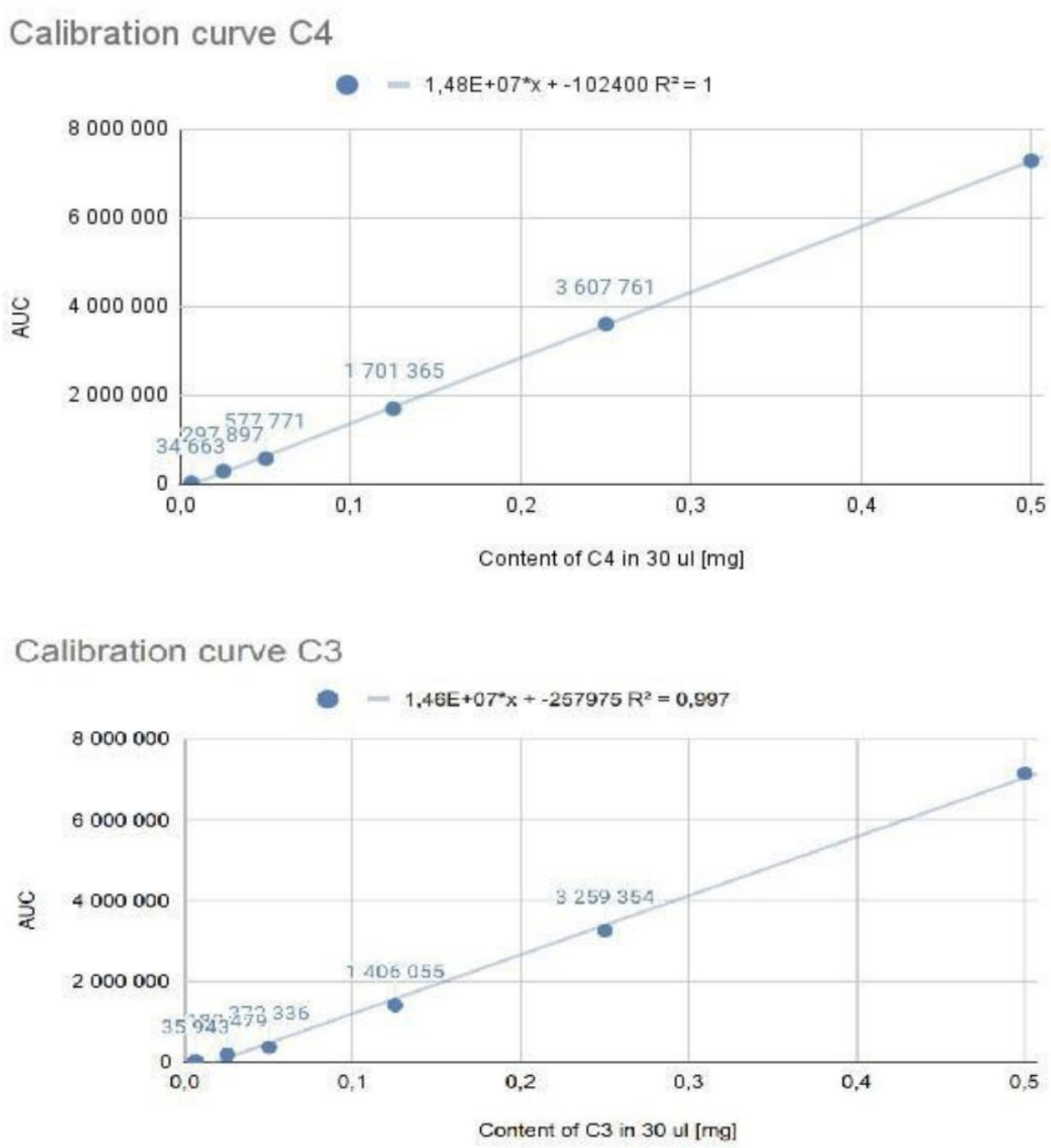

Fig. 1. Calibration curves for butyric (C4) and propionic (C3) acid

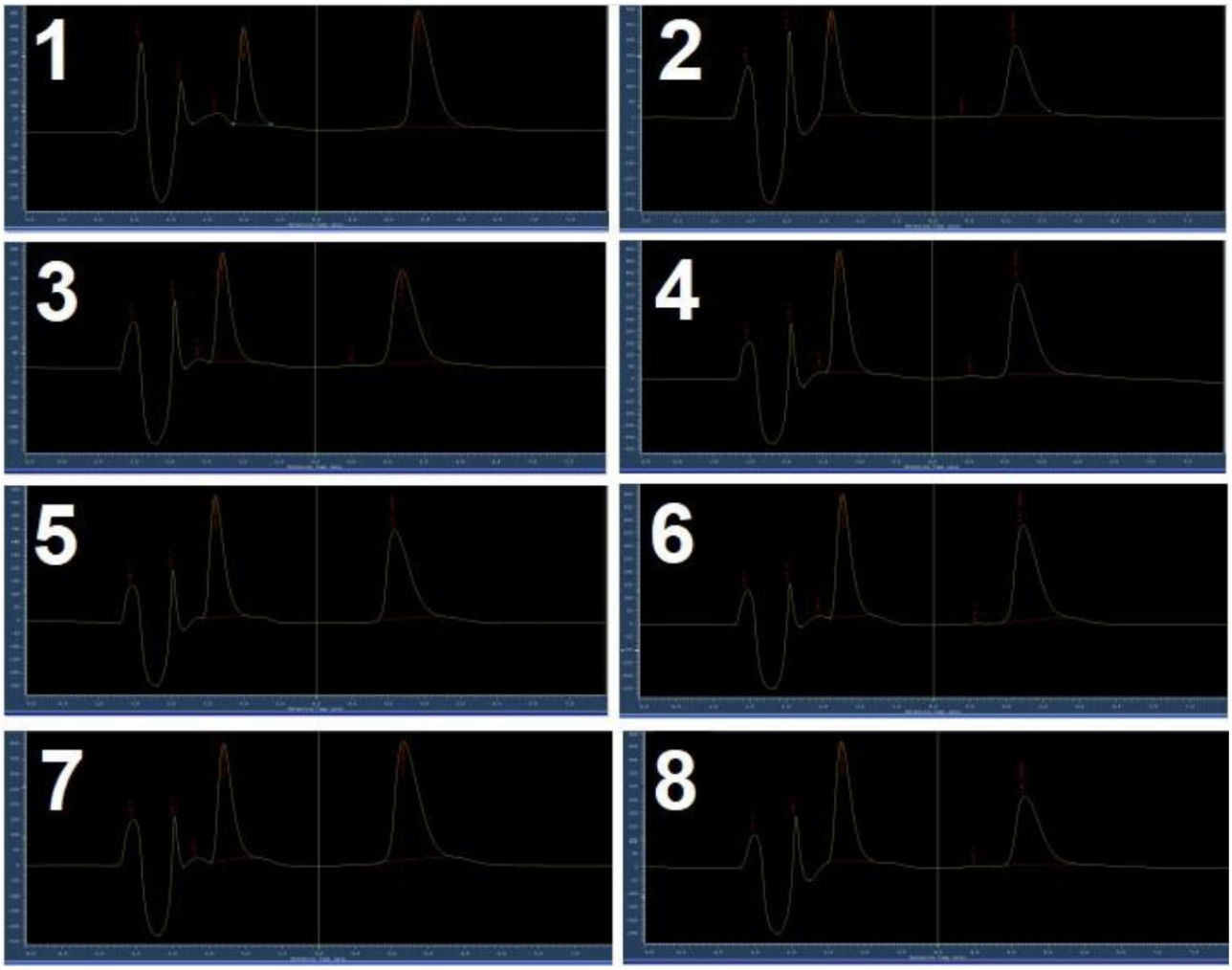

Fig. 2. Chromatograms obtained from samples no. 1–8

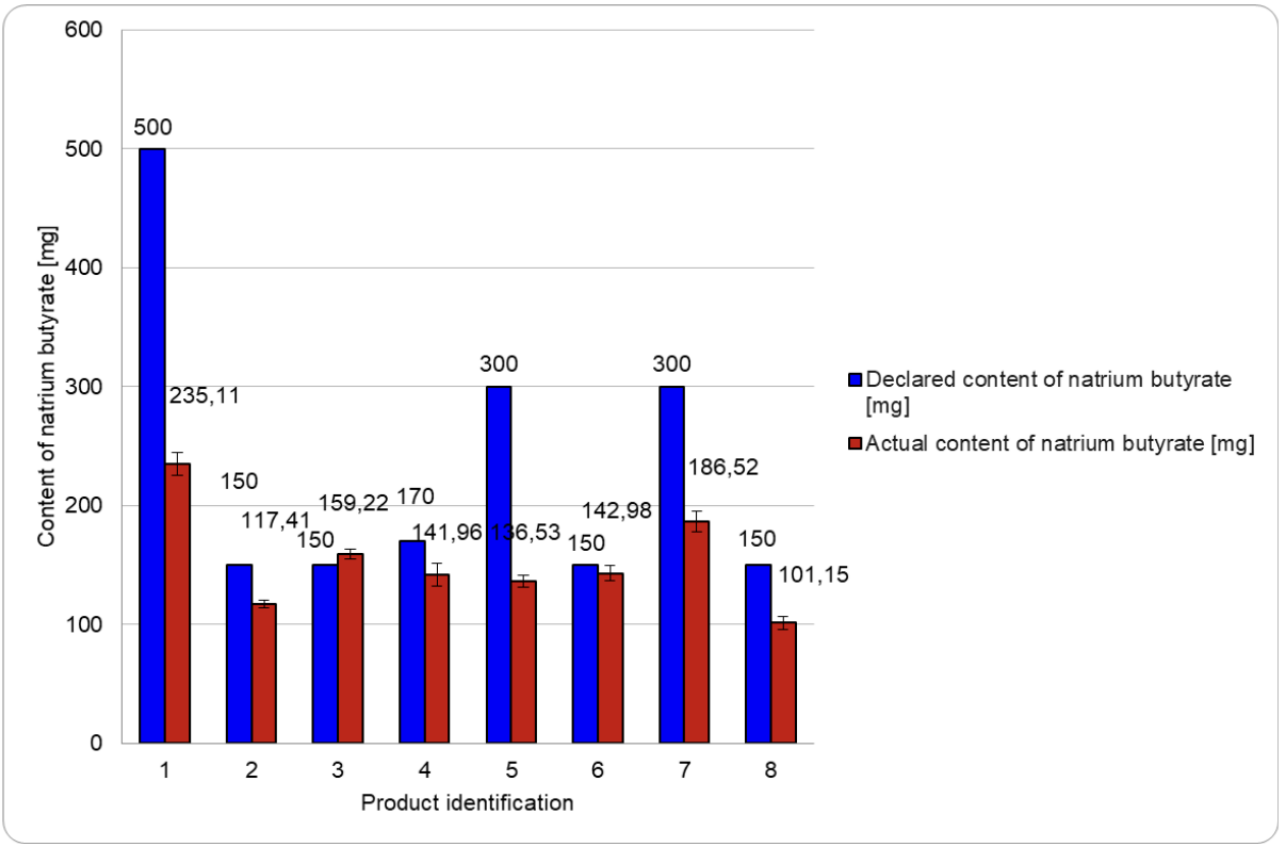

Fig. 3. Comparison of the actual and declared content of natrium butyrate in analysed products. Whisker plot represents standard deviation calculated based on three measurements

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