Application of chromogenic dye in biogenic amines determination using spectrophotometry

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Abstract
Biogenic amines content can be the source of information in the field of freshness and suitability of certain foods for consumption. However, most analytical methods for their determination require complicated sample preparation and expert knowledge. Because of that, a novel approach based on chromogenic dye application was proposed. After the S_N1 reaction of an amine with S 0378 dye, the absorbance of the solution was measured using spectrophotometry. With the proposed methodology, it was possible to determine total biogenic amines content in the range of 0.5–100 $\mu g\,L^{-1}$ with a determination coefficient exceeding 0.98.

1. Introduction

The quality and safety evaluation is one of the most important aspects of food analysis. In order to ensure consumers' satisfaction, the quality of products is assessed both at the industrial and retail level. However, while typical levels of contaminants such as pesticides or pharmaceuticals are well-known and regulated, data concerning e.g. biogenic amines content is scarce. Biogenic amines are low molecular weight organic compounds formed as a result of metabolic processes occurring in animal, plant, or bacterial cells [1]. Since they are precursors of various compounds, they can be found in many protein-rich food matrices [2]. Because the concentrations of individual biogenic amines are significantly influenced by numerous external factors, such as microbial activity or time and storage conditions, biogenic amines content can be used in the assessment of freshness and suitability of certain foods for consumption [9–10]. In addition, since the high content of certain amines in food products can be the result of poor quality raw materials, inadequate food processing, and contamination [5], the determination of biogenic amines can prove to be useful information on spoilage and overall sanitary quality of food products [8]. However, the determination of biogenic amines in food is important primarily due

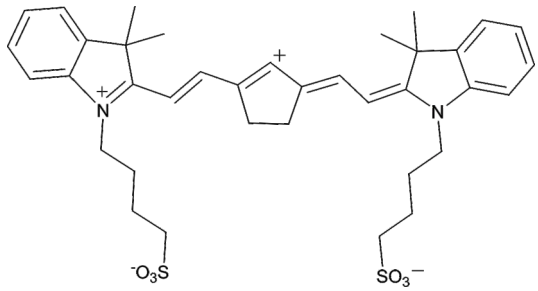

Fig. 1 Chemical structure of S 0378 dye.

to the negative impact that their excessive consumption may have on the health of the consumer. Based on the literature data, it can be concluded that these compounds are potential substrates for the formation of carcinogenic *N*-nitroso compounds. Primary amines react with nitrosating agents to form alkylating compounds that can react with other ingredients present in food [6]. In addition, in foods containing a large amount of fat, the formation of *N*-nitrosopyrrolidine from putrescine or spermidine is possible [1, 4, 7].

There are numerous methods of biogenic amines determination with the use of gas or liquid chromatography, however, their application is often associated with time-consuming and complicated sample preparation, as well as highly trained staff. An alternative approach would be to determine biogenic amines using spectrophotometric methods, which are relatively fast and easy to apply. What is more, most spectrophotometers are rather inexpensive and portable, which means that they can be used by producers, retailers, and consumers. While biogenic amines are weak absorbers of visible light, which makes their optical detection challenging [3], labelling them with a chromophore enables their spectrophotometric determination. S 0378 dye (Fig. 1) is a water-soluble cyanine dye, which reacts with primary amines by means of a unimolecular nucleophilic substitution mechanism (S_N1). During this reaction, the conjugate is formed, which results in a colour change from green to blue. The aim of this study is to evaluate whether S 0378 dye can be applied in the spectrophotometric determination of biogenic amines.

2. Experimental

2.1 Reagents and chemicals

Standard of putrescine was obtained from Merck. High-purity grade methanol was supplied by Avantor Performance Materials Poland. S 0378 dye was obtained from FEW Chemicals.

2.2 Instrumentation

Spectrophotometric measurements were performed with the use of the DR 3900 Hach Lange spectrophotometer. Absorbance was measured at 650 nm.

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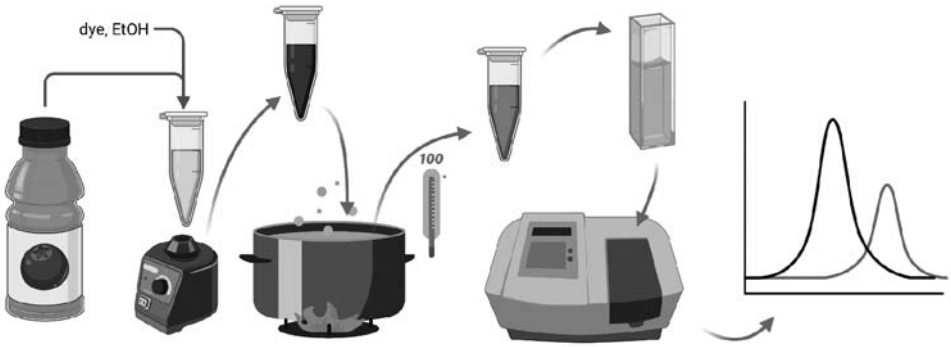

Fig. 2 Proposed procedure for biogenic amines determination.

3. Results and discussion

During the course of an experiment, it was evaluated whether biogenic amines can be determined using spectrophotometric methods. Because of that, the response of chromogenic dye to biogenic amines was tested using the procedure depicted in Fig. 2. First, a series of putrescine solution at a concentration ranging from 0.5 to 100 μg L^{-1} was prepared. Each of them was mixed with ethanol and green S 0378 dye. The mixtures were vortexed and then placed in a water bath for 2 h, during which dye reacted with putrescine forming blue conjugate. Solutions were then analysed using a spectrophotometer.

As can be seen in Fig. 3, it was possible to obtain a linear response in the range of 0.5–100 μg L^{-1}. What, is more, the determination coefficient exceeded 0.98, which suggests that this methodology might be applied for the determination of biogenic amines in real samples.

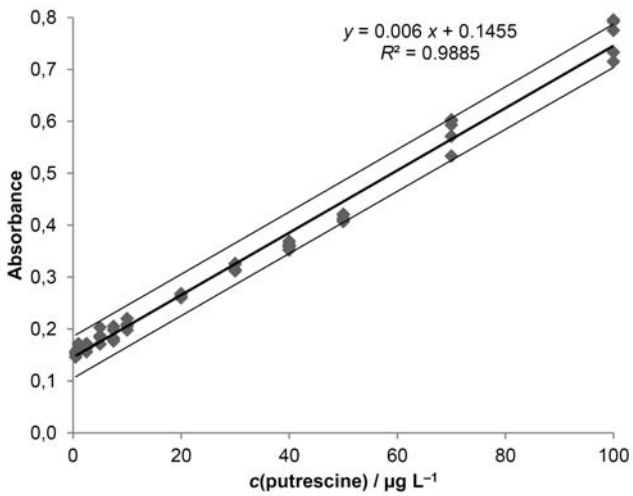

Fig. 3 Absorbance of S 0378 dye after reaction with different putrescine amounts.

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4. Conclusions

Based on the results of the experiment it was possible to conclude that chromogenic dye can be applied in the determination of total biogenic amines content. The proposed methodology is relatively simple and not requiring expert knowledge. This suggests that it could be applied not only in the analytical laboratory but also could be used in production and distribution centres.

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