



## Short communication

# Green, simple analytical method for total biogenic amines content determination in wine using spectrophotometry

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## ABSTRACT

A simple, green and equitable procedure for total biogenic amines (BAs) content determination was developed. The scientific novelty lies in the use of commercially available S 0378 dye, the reaction of which with BAs results in a colour change of the solution. Sample preparation and analysis were simplified to make the method suitable for routine analyses even in resource-scarce settings. The optimization of the method was carried out using a Box-Behnken response surface design. The developed method has satisfactory figures of merit for putrescine equivalent determination with  $R^2$  in the range of 0.9906–0.9933 and recovery between 99.7 and 101.2%. The method's greenness was assessed using AGREEprep. Finally, wine samples were analysed to demonstrate the applicability of the developed method.

## 1. Introduction

Biogenic amines (BAs) play a key role in several physiological processes in plants, such as fruit and flower development and cell division (Karovičová & Kohajdová, 2005). However, since the high content of certain amines in food products can be the result of poor quality of raw materials, inadequate food processing, and contamination (Ónal, 2007), the determination of BAs can give important information on spoilage and overall sanitary quality of food (Triki et al., 2018). Moreover, from the point of view of both consumer and producer, it is also important to monitor BAs concentration due to the fact that high levels of some, such as putrescine and cadaverine, can lower the sensorial quality of food. Because of numerous risks associated with the consumption of high amounts of BAs, the concentration of individual amines as well as total biogenic amines content in meat (Hernández-Jover et al., 1996; Wojnowski et al., 2019), fish (Tsai et al., 2006), beers (Kalac & Krizek, 2003), etc. has been extensively researched. However, these investigations were focused mostly on increasing the body of knowledge concerning the presence of biogenic amines in food and not on developing methods suitable for routine analyses. It is also worth noting that the current gold standards in the biogenic amines determination are various chromatography-based methods, which are relatively complicated, expensive and require skilful operators (Ahmad et al., 2020). Thus, the majority of the analytical methods for BAs determination is more suitable for laboratory use rather than for routine analysis in

industrial or retail settings.

While in the case of fish and fish products there are certain regulatory limits of e.g. histamine levels set by European legislation, there is no regulatory limit concerning the concentration of biogenic amines in wine (Visciano et al., 2014). However, monitoring the level of BAs is of particular importance in the case of wine and other alcoholic beverages, since the presence of ethanol and acetaldehyde might exacerbate the undesirable effect that amines can have on the quality and safety of food (Ancín-Azpilicueta et al., 2019). Because of that, the International Organization of Vine and Wine (OIV) recommends the reduction of BAs content in wine and other vine-based products (Ancín-Azpilicueta et al., 2019).

The aim of this study was to develop a simple, green and equitable procedure for total biogenic amines content determination that could be potentially used in routine wine analysis. The optimization of the method was carried out using the Design of Experiment (DoE). Then, the developed method was validated. The method's greenness was assessed using AGREEprep. Finally, red and white wine samples were analysed to demonstrate the applicability and validity of the developed methodology.

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## 2. Materials and methods

### 2.1. Reagents

Putrescine (PUT, 97.5 %) and triethylamine (TEA, 99.5 %) were purchased from Merck Life Science Sp. z o.o. (Merck, Poznań, Poland). The long-wavelength absorbing cyanine dye S 0378 ( $C_{37}H_{44}ClN_2NaO_6S_2$ ) was obtained from FEW Chemicals GmbH (Bitterfeld-Wolfen, Germany), while ethanol (EtOH, 99.8 %) was purchased from Avantor Performance Materials Poland (Gliwice, Poland). Ultrapure water for aqueous solutions and glassware washing was prepared using HLP5 Hydrolab demineralizer (Wiślina, Poland). A stock solution of putrescine (1.0 g/L) was prepared in ultrapure water, and the standard solution (0.5 g/l) was freshly prepared by diluting the stock solution.

### 2.2. Wine samples and preparation procedure

The non-filtered dry wine samples used in this work were commercially available and labelled as follows: BR (red, from Chile), GR (red, from Georgia), MR (red, from Uruguay), B (white, from Chile), M (white, from Uruguay), A (white, from New Zealand). Other wine characteristics, such as grape variety and alcohol content, are listed in Table S1. All wine samples were stored at room temperature, protected from light, and opened directly before the analysis.

All samples were analysed using the same protocol under optimized conditions. 1.6 mL of ethanol, 100  $\mu$ L of S 0378 solution (0.5 g/L) and 100  $\mu$ L of wine were mixed in a glass vial. 10  $\mu$ L of TEA was added, followed by filling the vial up to 2 mL with ultrapure water. The solution was then vortexed for 30 s at 2500 rpm. After that, the sample was placed in a water bath (70 °C) for 2 h. Finally, the sample was analysed using a spectrophotometer (Hach-Lange DR 3900, Colorado, United States) with absorbance measured at 650 nm. In cases where absorbance was higher than 1, samples were diluted 4-fold. Each sample was prepared in five replicates. Total biogenic amines content was then calculated as putrescine equivalent and expressed in mg PUT/L wine  $\pm$  standard deviation. A schematic representation of the method is shown in Fig. 1.

### 2.3. Optimization of the procedure

The novelty of the developed procedure lies in the application of the S 0378 dye. It reacts with primary amines according to the  $S_N1$  mechanism (Fig. 2) forming conjugate, which results in a visible change of solution's colour from green to blue (Gorris et al., 2011), with a maximum of the absorbance of resulting conjugate at 650 nm (Figure S2). The S 0378 dye was selected due to its proven ability to

react with biogenic amines as well as its excellent water solubility (Mobarez, Wongkaew, Simsek, Baeumner, & Duerkop, 2020).

The main parameters affecting the reaction of the S 0378 dye with biogenic amines are time and temperature of the reaction and type and volume of the solvent. Thus, it was decided to optimize them using a Design of Experiment. The experimental factors and factor levels were selected in preliminary studies based on the results of single-factor tests. Besides the above-mentioned factors, i.e. time, temperature, and type of solvent, it was decided to also assess the impact of the addition of TEA, since the use of triethylamine as a non-nucleophilic hydrogen chloride scavenger seems to make the formation of the amine-dye adduct more favourable.

The Response Surface Methodology (RSM) was used to evaluate the maximum efficiency of the S 0378 dye reaction with BAs in wine samples with the yield of the process expressed as the absorbance of the solution. The influence of four independent variables was assessed: the volume of ethanol (corresponding to 40–80 % of the total solution volume), trimethylamine volume (0–10  $\mu$ L), the temperature of the water bath (30–70 °C) and the reaction time (30–120 min). The design of experiment as well as the codes and levels of standardized variables are listed in Table S2.

### 2.4. Calibration curves and validation

Two different calibration curves of putrescine were prepared: 1–20 mg/L and 20–100 mg/L. The method described in 2.2 was then evaluated in accordance with quality assurance protocol, in which the following validation parameters were assessed: linearity, precision, sensitivity and accuracy. Description of the validation procedure can be found in Supplementary Materials.

### 2.5. Data analysis

The optimization of the sample preparation procedure was performed on wine samples enriched with putrescine (at 100  $\mu$ g/mL) using the response surface methodology (RSM) (Minitab 17, LLC, State College, Pennsylvania, USA). The design of experiment (DoE), namely the Box-Behnken design, was used to evaluate the optimal level and interaction effects of the four independent factors affecting the absorbance of the solutions. Factors in question were the volume of EtOH (99.8 %) solution [EtOH], the volume of trimethylamine (99.5 %) solution [TEA], time [Time] and temperature [Temperature] of the reaction. A total of 29 experiments (including 24 runs with factors examined at three levels (-1, 0, 1) and 5 centre points for the experimental error estimation) were carried out. The wine samples enriched with putrescine at 100  $\mu$ g/mL were analyzed in a randomized runs order. Three-dimensional response surface plots were generated in order to interpret the effects of four

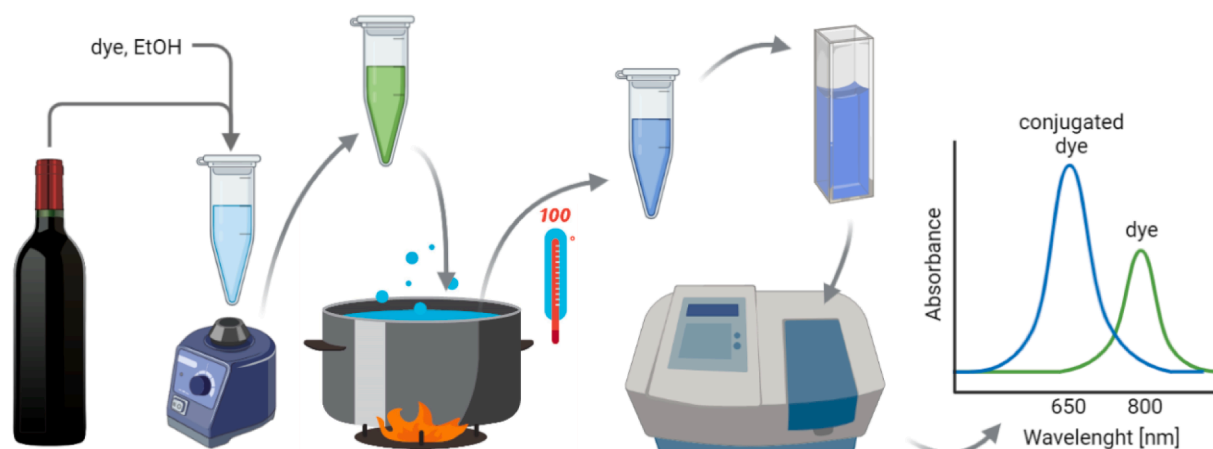


Fig. 1. Scheme of total biogenic amines content determination in wine samples.

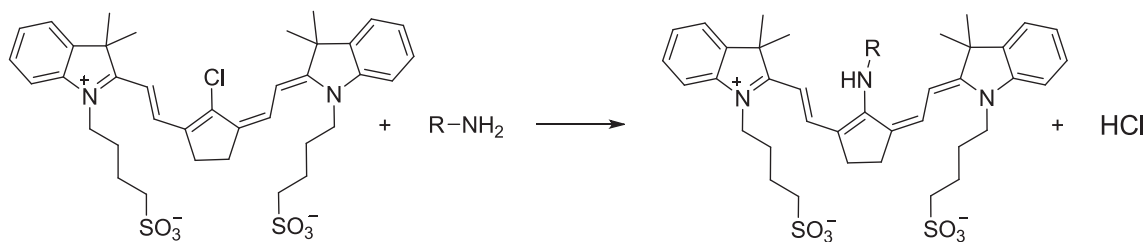


Fig. 2. Nucleophilic substitution of the S 0378 dye by a primary amine.

independent variables on the efficiency of S 0378 dye reaction with BAs. RSM was applied for optimization of the sample preparation process. The regression coefficients of intercept, linear, quadratic, and interaction terms involved in the model and their effects were tested statistically at probability levels of  $p \leq 0.05$  using one-way analysis of variance (ANOVA). In order to optimize the processing conditions based on the model desirability features, graphical and numerical analyses were used.

### 3. Results and discussion

#### 3.1. Method optimization

The yield of the reaction of the S 0378 dye with primary amines was expressed as the absorbance of the solution (consisting of ethanol, water, TEA, S 0378 solution and spiked sample) in order to assess the maximum efficiency of the process. The resulting polynomial for estimating the absorbance of the solution based on EtOH and TEA content, time and temperature of the reaction is given in Equation 1:

$$\text{Absorbance} = 0.880 - 0.01034 * [\text{EtOH}] - 0.0882 * [\text{TEA}] - 0.0253 * [\text{Temperature}] + 0.00129 * [\text{Time}] - 0.000065 * [\text{EtOH}]^2$$

$$- 0.00347 * [\text{TEA}]^2 + 0.000065 * [\text{Temperature}]^2 - 0.000049 * [\text{Time}]^2 + 0.000480 * [\text{EtOH}] * [\text{TEA}] + 0.000270 * [\text{EtOH}] * [\text{Temperature}] + 0.000030 * [\text{EtOH}] * [\text{Time}] + 0.002325 * [\text{TEA}] * [\text{Temperature}] + 0.000244 * [\text{TEA}] * [\text{Time}] + 0.000101 * [\text{Temperature}] * [\text{Time}]$$

Based on the analysis of the  $p$ -values of each component of the model, it was possible to conclude that all four linear coefficients, three square coefficients ( $[\text{EtOH}]^2$ ,  $[\text{TEA}]^2$ ,  $[\text{Time}]^2$ ), and three two-way interaction coefficients ( $[\text{EtOH}] \cdot [\text{TEA}]$ ,  $[\text{EtOH}] \cdot [\text{Temperature}]$ ,  $[\text{TEA}] \cdot [\text{Temperature}]$ ) were significant and indicative of a pattern of interactions between the studied variables.

The use of the Box-Behnken design resulted in six response surface plots for putrescine determination, which are graphical representations of the regression equation (Fig. 3). With the use of these plots, it is possible to visualize the relationship between the responses and the experimental parameter levels of variables, and the type of interaction between them.

All optimized parameters have a large impact on the efficiency of the reaction of the S 0378 dye with primary amines. The extraction

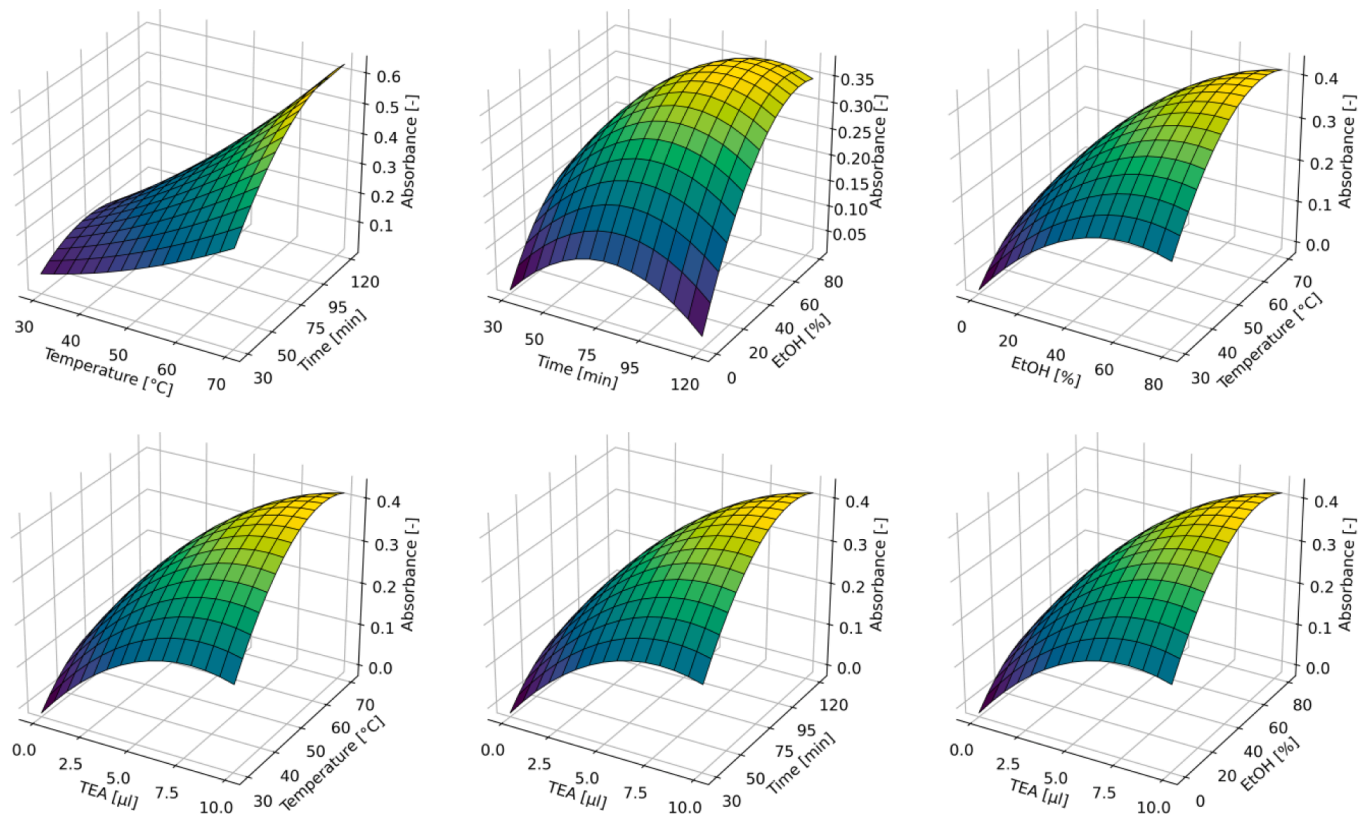


Fig. 3. Response surface plots of Box-Behnken DoE. The x and y-axis represent the variables, namely temperature, time, the volume of ethanol and trimethylamine, while the z is the standardized absorbance of the solution.

efficiency increased with the increase of all the parameters. The fact that absorbance is increasing in the whole range of selected parameters could suggest that the optimal values of the reaction's factors are not in the proposed ranges. The evaluated ranges of the continuous variables were selected based mostly on the preliminary studies, however, the value of the boundary conditions was also affected by practical limitations, e.g., the upper limit of the temperature range was set to not exceed the boiling point of the solvent. With the use of Box-Behnken design, it was possible to assess the impact of temperature, time as well as volume of TEA and EtOH and select their optimal values within these external constraints. Moreover, it was possible to significantly decrease the number of experiments and thus, reduce the impact of the optimization process on the environment.

### 3.2. Method validation

The method's linearity and sensitivity were assessed by calibration with standard solutions of putrescine. Different amounts of putrescine standard were added to wine in order to obtain 16 solutions with a concentration in two ranges (from 1 to 20 mg/L and from 20 to 100 mg/L), which were then subjected to the developed procedure. 1.6 mL of ethanol, 100  $\mu$ L of S 0378 solution (0.5 g/L), 10  $\mu$ L of TEA and 100  $\mu$ L of wine were mixed in a glass vial, which was then filled up to 2 mL with ultrapure water. The solution was then vortexed for 30 s at 2500 rpm, placed in a water bath (75 °C) for 2 h and then analysed using a spectrophotometer. Least squares linear regression was used to calculate the equations of the calibration curves and the determination coefficients ( $R^2$ ). Detailed information concerning analytical figures of merit is provided in Table 1. Good linearity at the wavelength of 650 nm was obtained for both ranges with a determination coefficient of 0.9906 for the first range and 0.9933 for the second range. Limits of detection and quantification were assessed based on the linear calibration equation and 10 blank samples. Calculations were made using the following equations:  $LOD = 3 \cdot (\sigma/S)$  and  $LOQ = 10 \cdot (\sigma/S)$ , where  $\sigma$  is the standard deviation and S is the slope of the calibration curve (Mörschbacher et al., 2018). Obtained limits of detection and quantification (0.29 and 0.98 mg/L for the first range) are higher than in the case of chromatography-based methods, where LOQ can range from 0.006 to 1.54 mg/L (Papageorgiou et al., 2018; Milheiro et al., 2019; Fabjanowicz et al. 2022). However, the aim of the study was to develop a procedure that will be simple and green, so that it can be applied for the routine analysis. Spectrophotometry is energy-efficient, the analysis takes only several seconds. What is more, the portability of the spectrophotometers as well as the ease of their use considerably facilitates on-site use. Developed method is also greener than chromatography-based methods, since the preparation of the samples is simplified and does not involve derivatization, which is potentially detrimental to the environment and the health of the analyst.

The accuracy of the method was determined by a recovery test. Results of the analysis of wine samples spiked with putrescine standard at two concentration levels ( $n = 7$ ; 7 and 15 mg/L for the first range, 50 and 70 mg/L for the second range) were compared with the concentration of spikes themselves. Recovery rates were  $99.7 \pm 2.0$  % for the range of 1–20 mg/L and  $101.2 \pm 3.5$  % for 20–100 mg/L, which indicates that the developed procedure for total biogenic amines content determination in wine samples is characterized by high accuracy.

The intra-day repeatability ( $RSD_r$ ) was estimated based on the results

**Table 1**  
Validation parameters of developed methodology.

Concentration range [mg/L]	Linearity ( $R^2$ )	Intra-day repeatability (%RSD)		Recovery (%)	Inter-day repeatability (%RSD)		LOD [mg/L]	LOQ [mg/L]
1–20	0.9906	7 mg/L	15 mg/L	99.7 $\pm$ 2.0	7 mg/L	15 mg/L	0.29	0.98
		1.8	1.8		2.2	1.7		
20–100	0.9933	50 mg/L	70 mg/L	101.2 $\pm$ 3.5	50 mg/L	70 mg/L	1.4	4.5
		1.9	1.8		2.4	2.0		

of analysis of 7 replicates of wine samples fortified at two concentration levels (7 and 15 mg/L for the first range, 50 and 70 mg/L for the second range) on the same day. The inter-day repeatability ( $RSD_R$ ) was determined by analysis of samples from three different days over three weeks.  $RSD_r$  ranged from 1.8 % to 1.9 %, while  $RSD_R$  ranged from 1.7 % to 2.4 % (Table 1). Based on the results it can be concluded that biogenic amines are stable in the wine matrix and that the precision of the method is excellent.

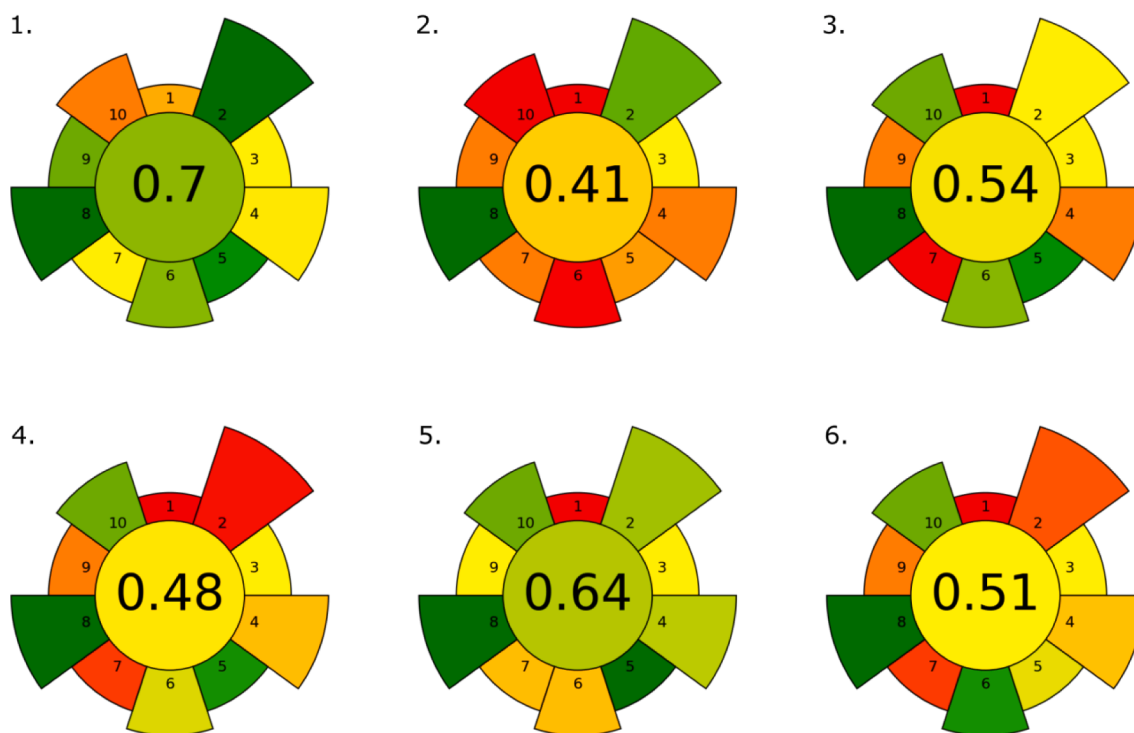
### 3.3. Greenness evaluation

The developed analytical procedure for the determination of BAs in wine samples was subsequently assessed in terms of greenness using Analytical Greenness Metric for Sample Preparation (AGREEprep) (Wojnowski, Tobiszewski, Pena-Pereira, & Psillakis, 2022). To evaluate its environmental impact, the developed approach was juxtaposed with five other methods for BAs determination chosen from the literature. Three liquid chromatography-based methodologies were included in the greenness evaluation since LC-based methods are seen as the gold standard for BAs determination. Since in order to reduce the time of the analysis as well as its impact on the environment, high-performance liquid chromatography and ultra-high-performance liquid chromatography are often implemented in amines determination, methods based on them were also included in the analysis. In addition, the greenness of gas chromatography- and capillary electrophoresis-based methods was assessed as well.

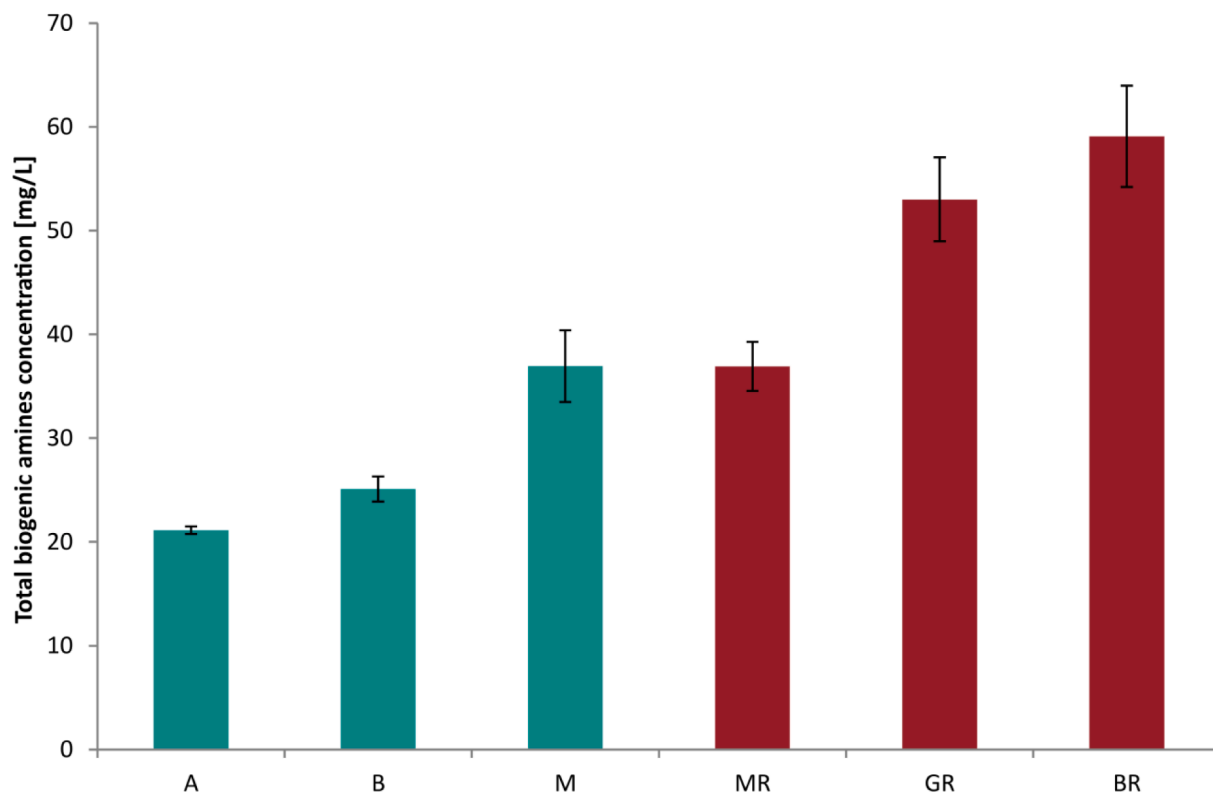
As shown in Fig. 4, methodologies often used for biogenic amines determination produced results far from satisfactory in terms of greenness. This is caused mainly by the use of chromatography since it is not only time- and energy-consuming, but also must be preceded by complicated sample preparation procedure involving, amongst others, derivatization. The proposed method obtained scored significantly higher in the greenness metric, since the preparation of the samples is simplified and does not involve derivatization, which is detrimental not only to the environment but potentially also to the health of the analyst. Another advantage of the developed procedure is the use of spectrophotometry, given that it is energy-efficient and the analysis takes only several seconds. Moreover, the portability of the spectrophotometers as well as ease of their use considerably increases their suitability of the method and facilitates on site use.

### 3.4. Real samples analysis

The developed analytical method was used for putrescine equivalent determination in selected wines (Fig. 5). Six different wines (three white and three red) were analysed in five replicates using the proposed approach. The level of the total biogenic amines content was in a range of approx. 20–60 mg/L, which seems to be in accordance with information found in the literature (Papageorgiou et al., 2018). While there is no regulatory limit concerning the concentration of BAs in wine, there are regulations regarding histamine content in fish and fish-based products. Levels up to 200 mg/kg in fresh fish and 400 mg/kg in fishery products are deemed safe. It is worth noting, that in the case of wines, the total biogenic content is significantly lower than 200 mg/L (ranging from a few ng/L to 67 mg/L), which is why it was decided to evaluate the total biogenic amines content in lieu of histamine (Visciano et al., 2014; Martuscelli et al., 2013; Papageorgiou et al., 2018). What is



**Fig. 4.** Results of greenness evaluation of 6 analytical methodologies for BA's determination in wines: 1 – proposed procedure, 2 – DI-SPME-GC-MS (Papageorgiou et al., 2018), 3 – UPLC-MS/MS (Angulo et al., 2020), 4 – HPLC-PDA (Mitar et al., 2018), 5 – MEKC-LIF (Uzaşçı et al., 2012), 6 – SALLE-HPLC-FLD (Ramos et al., 2014). The higher the score, the greener the sample preparation method.



**Fig. 5.** Total biogenic amines concentration in red and white wines (depicted in burgundy and teal, respectively).

more, the concentration of BA's in red wines was higher than in white wines, which was to be expected since the process of their production differs (Guo et al., 2015). The highest concentration of biogenic amines

( $59.1 \pm 4.9$  mg/L) was found in the red wine from Chile (BR), while the lowest ( $21.14 \pm 0.36$  mg/L) in the white wine from New Zealand (A).

The use of spectrophotometry in the developed procedure facilitates



the process of total biogenic amines determination, which allows for fast, uncomplicated and cost-effective analysis. Furthermore, since the procedure involves only a few straightforward operations in the sample preparation step, it can be performed by people without prior analytical chemistry experience, which further increases the potential availability of the method. Thus, the application of the proposed procedure can significantly increase the sustainability and equitability of total biogenic amines determination.

#### 4. Conclusions

The majority of methods for biogenic amines determination in wine are chromatography-based and thus, are time- and resource-intensive, and have to be performed by a skilful operator. There is a lack of easy, straightforward methods for routine analysis, also in at-line and out of lab scenarios. The proposed method is aimed at filling this gap.

The developed procedure for total biogenic amines content in wine determination is simple to use and green. The use of spectrophotometry drastically increases the accessibility of the proposed procedure which would otherwise require educated operation, costly equipment and infrastructure, thus promoting equitable analytical chemistry. Simplification of the sample preparation stage translates, through the reduction of the number of analytical steps in a procedure, to reduced consumption of samples and reagents, resulting in a greener and more sustainable alternative to other analytical techniques for the determination of the total BAs content.

#### CRedit authorship contribution statement

**Kaja Kalinowska:** Conceptualization, Methodology, Investigation, Writing – original draft. **Marek Tobiszewski:** Writing – review & editing, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2022.134457>.

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