

# 1 **The impact of cold plasma on the phenolic composition and biogenic amine content of** 2 **red wine**

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18

## 19 **Abstract**

20 The effect of cold plasma (CP) on phenolic compound (PC) and biogenic amine (BA)  
21 contents of red wine was investigated for the first time. The influence of CP was compared  
22 with the effects of a wine preservation using potassium metabisulfite and a combined method.  
23 The PC profile was determined by UPLC-PDA-MS/MS while BAs using DLLME-GC-MS.  
24 Chemometric analysis also was used. The content of PCs was 3.1% higher in the sample  
25 preserved by CP treatment (5 min, helium/nitrogen) compared to a sample preserved by the  
26 addition of potassium metabisulfite (100 mg/L). On a positive note, CP treatment reduced the  
27 concentration of BAs in the wine samples. The lowest BA contents were recorded after 10  
28 min of cold plasma (helium/oxygen) treatment with the addition of potassium metabisulfite  
29 (1120.85 µg/L). The results may promote interest in CP as a potential alternative method for  
30 the preservation of wine and other alcoholic beverages.

31

32 **Keywords:** cold plasma; phenolic compounds; biogenic amines; chemometric analysis; wine  
33 preservation; red wine

34

## 35 **1. Introduction**

36 Wine is an alcoholic beverage, the tradition of production and consumption of which  
37 has been known around the world for centuries (Gajek et al. 2021). The recent climatic  
38 changes have brought about alterations in the geographical distribution of areas used for  
39 viticulture. As a consequence of global warming, a significant increase in the area of  
40 vineyards and wine production has been observed across Central and Eastern Europe,  
41 including Poland (Koźmiński et al. 2020). Wine is produced by fermentation of sugars  
42 contained in fruit using naturally occurring microorganisms or starter cultures. This product is  
43 a complex matrix consisting of water, alcohol, carbohydrates, organic acids, polyphenols,  
44 minerals and aromatic substances (Robles et al. 2019).

45 One of the most abundant and important groups of compounds found in wines are  
46 polyphenols. They are responsible for the color (anthocyanins), taste (tannins), and aroma of  
47 wines. Additionally, they show antioxidant activity, which makes them beneficial in the  
48 prevention of cardiovascular diseases and other chronic medical conditions (Snopek et al.  
49 2018). The polyphenol content of wine depends on the grapevine strain and the grape variety,  
50 the winemaking technology, the aging processes and the wine preservation methods used. Red  
51 wines are characterized by a higher content of polyphenolic compounds (PCs) compared to  
52 white wines, and thus show a higher antioxidant activity (Robles et al. 2019). Due to the  
53 growing consumer awareness of the health benefits associated with the consumption of  
54 polyphenol-rich products as well as the knowledge of the impact of these compounds on the  
55 final quality of a product, wine producers are looking for solutions that would minimize the  
56 loss of polyphenols during the entire winemaking process.

57 Besides health-promoting phenolic compounds, wines also contain biogenic amines  
58 (BAs), which may have a negative impact on human health. They are nitrogenous compounds  
59 that are mainly formed by the decarboxylation of amino acids, which in wine is the result of  
60 the activity of microbes such as yeast or lactic acid bacteria (LAB) (Smit and Maret du Toit,  
61 2012). The content of biogenic amines depends mainly on the concentration of amino acid  
62 precursors in a product's matrix, but also on pH as well as alcohol and sulfur dioxide  
63 contents, which directly affect the growth of microorganisms (Papageorgiou et al. 2018). In  
64 addition, the presence of amino acid precursors is influenced by the grape variety, the  
65 geographical region, vinification methods, and the aging process (Płotka-Wasyłka et al.

66 2018a). The biogenic amines most commonly found in wines include histamine (HIS),  
67 cadaverine (CAD), tyramine (TYR), 2-phenylethylamine (2-PE), putrescine (PUT), and  
68 tryptemine (TRP) (Esposito et al. 2019). High concentrations of biogenic amines in the final  
69 product may cause undesirable physiological effects in the consumer, such as headaches,  
70 nausea or tachycardia (Naila et al. 2010).

71 In order to prevent the negative effects of microorganisms on the quality of wine,  
72 methods of eliminating the undesirable microbes have been developed. Although classic  
73 thermal methods of food preservation still play a very important role in food technology, they  
74 are not suitable for vinification processes as they can negatively affect the unique taste, color  
75 and flavor of wine (Niu et al. 2019). Instead, sulfur dioxide, which has decontaminating and  
76 antioxidant properties, is commonly added to wine to remove unwanted microorganisms.  
77 However, despite its positive effects, it can cause allergic reactions in some consumers, which  
78 is why the World Health Organization (WHO) has introduced restrictions on its use. This has  
79 contributed to an increased search for new strategies to minimize or even replace SO<sub>2</sub>  
80 (Cordero-Bueso et al. 2019), but the problem of biogenic amines still remains unsolved.  
81 Therefore, scientists are looking for effective non-thermal preservation methods which allow  
82 to remove undesirable microorganisms without significantly affecting the final stability of the  
83 product (Puligundla et al. 2018).

84 Cold plasma is one of the most recent non-thermal methods used in sterilization  
85 processes. Numerous scientific publications confirm its effective antimicrobial activity, which  
86 is connected with the influence of reactive compounds, atoms in the excited and basic state,  
87 and UV photons on microbial cells (Bourke et al. 2017). Reactive compounds are produced  
88 by subjecting a working gas to various electrical discharges such as barrier discharge and  
89 corona discharge. Importantly, in the context of cold plasma applications in the food industry,  
90 the temperature of the free electrons in the working gas is lower than that of the other  
91 particles, which directly results in a slight increase in process temperature (Niedźwiedź et al.  
92 2019). However, there is limited information in the literature regarding the impact of cold  
93 plasma on the final quality of alcoholic beverages, which means this problem is worth delving  
94 into.

95 The objective of the present study was to investigate the effect of a new wine  
96 preservation method using cold plasma on the phenolic composition and biogenic amine  
97 content of red wine. An additional objective was to compare the effect of preserving wine  
98 samples using the traditional method (addition of potassium metabisulfite at 30 mg/L or

99 100mg/L) and a combined method (cold plasma and the addition of potassium metabisulfite at  
100 30 mg/L) with the effect of cold plasma alone. Wine sample storage was also considered in  
101 the study. In addition, chemometric analysis was conducted to discover specific relationships  
102 between the different wine preservation methods and the content of bioamines and selected  
103 phenolic compounds.

## 104 **2. Materials and methods**

### 105 **2.1 Chemicals and materials**

106 All reference materials used in the determination of the biogenic amines such as tryptamine  
107 hydrochloride, putrescine dihydrochloride, histamine dihydrochloride, tyramine  
108 hydrochloride, cadaverine hydrochloride and 2-phenylethylamine hydrochloride, as well as  
109 hexylamine (internal standard, IS), were purchased from Sigma-Aldrich (St. Louis, MO,  
110 USA). The derivatization reagent (isobutyl chloroformate) was purchased from Sigma-  
111 Aldrich. Ultrapure water was obtained from a Milli-Q water purification system (Millipore,  
112 Bedford, MA, USA). Stock solutions of BAs and IS (both at 1 mg/mL) were prepared daily in  
113 ultrapure water and stored at +4°C in silanized screw-capped vials with solid PTFE-lined caps  
114 (Supelco, Bellefonte, PA). Methanol, used as a dispersive solvent, was a high purity grade  
115 solvent purchased from Fluka. High purity grade chloroform, applied as an extractive solvent,  
116 was obtained from Sigma. 0.1 M HCl was supplied by Fluka. Other chemicals were of an  
117 analytical grade.

118 Analytical standards for phenolic profile determination such as cyanidin-3-O-glucoside,  
119 delphinidin-3-O-glucoside, isorhamnetin-3-O-glucoside, kaempferol-3-O-glucoside, malvidin-  
120 3-O-glucoside, myricetin-3-O-glucoside, peonidin-3-O-glucoside, petunidin-3-O-glucoside,  
121 quercetin-3-O-glucoside, quercetin-4'-O-glucoside, quercetin-3-O-rutinoside, (+)-catechin, (-  
122 )-epicatechin, (-)-epicatechin-3-gallate, procyanidin A1 and A2, trans-resveratrol, and trans-  
123 piceid were purchased from Extrasynthese (Lyon, France). Caftaric acid, caffeic acid, coumaric  
124 acid, gallic acid, caftaric acid, ferulic acid, protocatechuic acid, and p-coumaric acid were  
125 purchased from PhytoLab (Vestenbergsgreuth, Germany). Formic acid (LC-MS grade) was  
126 purchased from Fischer Scientific (Schwerte, Germany). Acetonitrile was purchased from  
127 POCH (Gliwice, Poland).

128

### 129 **2.2 Wine samples**

130 The red wine used in this study was produced at Dom Bliskowice Winery (Poland, Lublin  
 131 Province) from grapes of Regent and Rondo (1:1) varieties harvested in October 2019. The  
 132 wine was subjected to different preservation processes (control sample – not preserved; cold  
 133 plasma treatment; addition of 30 mg/L potassium metabisulfite; addition of 30 mg/L  
 134 potassium metabisulfite combined with cold plasma treatment; and addition of 100 mg/L  
 135 potassium metabisulfite). The samples were then analyzed immediately after preservation and  
 136 also after three months of storage (15°C, limited light) (Table 1).

137 Table 1. Characterization of samples and sample coding

<b>Sample</b>	<b>Preservation method</b>	<b>Cold plasma exposure time</b>	<b>Gas used for preservation</b>	<b>Storage</b>
<b>1</b>	no preservation	0	No	No
<b>2</b>	cold plasma	2	He / O <sub>2</sub>	No
<b>3</b>	cold plasma	5	He / O <sub>2</sub>	No
<b>4</b>	cold plasma	10	He / O <sub>2</sub>	No
<b>5</b>	cold plasma	2	He / N <sub>2</sub>	No
<b>6</b>	cold plasma	5	He / N <sub>2</sub>	No
<b>7</b>	cold plasma	10	He / N <sub>2</sub>	No
<b>8</b>	30mg/L potassium metabisulfite	0	No	No
<b>9</b>	cold plasma and 30mg/L potassium metabisulfite	2	He / O <sub>2</sub>	No
<b>10</b>	cold plasma and 30mg/L potassium metabisulfite	5	He / O <sub>2</sub>	No
<b>11</b>	cold plasma and 30mg/L potassium metabisulfite	10	He / O <sub>2</sub>	No
<b>12</b>	cold plasma and 30mg/L potassium metabisulfite	2	He / N <sub>2</sub>	No
<b>13</b>	cold plasma and 30mg/L potassium metabisulfite	5	He / N <sub>2</sub>	No
<b>14</b>	cold plasma and 30mg/L potassium metabisulfite	10	He / N <sub>2</sub>	No
<b>15</b>	100 mg/L potassium metabisulfate	0	No	No

16	no preservation	0	No	Yes
17	cold plasma	2	He / O <sub>2</sub>	Yes
18	cold plasma	5	He / O <sub>2</sub>	Yes
19	cold plasma	10	He / O <sub>2</sub>	Yes
20	cold plasma	2	He / N <sub>2</sub>	Yes
21	cold plasma	5	He / N <sub>2</sub>	Yes
22	cold plasma	10	He / N <sub>2</sub>	Yes
23	30 mg/L potassium metabisulfite	0	No	Yes
24	cold plasma and 30 mg/L potassium metabisulfite	2	He / O <sub>2</sub>	Yes
25	cold plasma and 30 mg/L potassium metabisulfite	5	He / O <sub>2</sub>	Yes
26	cold plasma and 30 mg/L potassium metabisulfite	10	He / O <sub>2</sub>	Yes
27	cold plasma and 30 mg/L potassium metabisulfite	2	He / N <sub>2</sub>	Yes
28	cold plasma and 30 mg/L potassium metabisulfite	5	He / N <sub>2</sub>	Yes
29	cold plasma and 30 mg/L potassium metabisulfite	10	He / N <sub>2</sub>	Yes
30	100 mg/L potassium metabisulfate	0	No	Yes

138

### 139 2.3 Cold plasma treatment of wine

140 Wine samples were exposed to cold plasma for 2, 5 and 10 min. Mixtures of helium and  
 141 nitrogen or helium and oxygen were used as the working gas. The samples were treated using  
 142 a DBD (Dielectric Barrier Discharge) plasma jet reactor. The volume of 50 ml of wine was  
 143 poured to a sterilized glass container and placed on a magnetic stirrer. To ensure homogenous  
 144 exposure to plasma treatment, samples were stirred with a PTFE stir bar placed inside the  
 145 sample. The DBD reactor consisted of a 1.4 mm internal diameter ceramic gas tube. Two  
 146 metal electrodes were located as follows: a ring-shaped high voltage electrode was positioned  
 147 10 mm from the end of the jet and A flat, copper PCB laminated electrode was used as the  
 148 ground. The latter electrode was placed on the magnetic stirrer, just beneath the sample  
 149 container. The distance between the end of the reactor's tube and the surface of the liquid was

150 2 mm. The flow rates of the substrate gas mixtures were 96 L/h of helium with 1.8 L/h of  
151 oxygen or nitrogen admixtures. The flow rates were adjusted by gas flow controllers  
152 (Automatic Works “ROTAMETR”, Gliwice, Poland). A schematic view of the experimental  
153 set-up is presented in Fig. 1. The mean power of the power supply was 6 W. For both gas  
154 mixtures, the sine-like voltage signals were quite similar, with a subtle difference in the  
155 maximum voltage, which was slightly higher in the case of the helium and oxygen mixture  
156 and ranged 8.3 kV.

157 A K-type thermocouple connected to a DT-847U meter was used to measure the temperature  
158 of the sample after plasma treatment. In the course of the experiment, the maximum registered  
159 temperature of the sample did not exceed 32°C, so the treatment can be considered a cold one.

#### 160 **2.4. Determination of polyphenolic compounds**

161 The protocole reported by Kapusta et al. (2018) was used to determine polyphenolic  
162 compounds in the wine samples. The qualitative and quantitative determination of the  
163 phenolic compound profile was performer using ultra-performance reverse-phase liquid  
164 chromatography (UPLC-PDA-MS/MS). The UPLC-PDA-MS/MS Waters ACQUITY system  
165 (Walters, Milford, MA, USA) used consisted of a sample manager, a binary pump manager, a  
166 column manager, a photodiode array (PDA) detector, and a tandem quadrupole mass  
167 spectrometer (TQD) with electrospray ionization (ESI). A BEH C18 column (100 mm × 2.1  
168 mm i.d., 1.7 µm, Waters) was used to separate the compounds. Wine samples were filtered  
169 before the analysis through a 0.45-µm Millipore filter and then injected onto the  
170 chromatographic column. The injected sample volume was 5 µL. The experiment was  
171 conducted in duplicate. Waters MassLynx software v.4.1 was used to collect and analyze the  
172 results. The results obtained are expressed in mg/L.

#### 173 **2.5 GC-MS dermination of biogenic amine content**

174 The protocole reported by Płotka-Wasyłka et al. (2018b) was used to determine biogenic  
175 amines (BAs) in the wine samples. Isolation of analytes was carried out simultaneously with  
176 their derivatization. The selected analytes were determined qualitatively and quantitatively  
177 using gas chromatography combined with mass spectrometry (GC-MS). A gas  
178 chromatography (GC) 7890A (Agilent Technologies, Santa Clara, CA, USA) system was  
179 interfaced with an inert mass selective detector (5975C, Agilent Technologies) with an  
180 electron impact ionization chamber (EI). A ZB-5MS capillary column (30 m × 0.25 mm I.D.,  
181 0.25 µm) supplied by Zebron Phenomenex was used for chromatographic separation. The

182 injection was performed in the splitless mode at 230°C. The interface was set at 250°C. The  
183 injected sample volume was 2 µl. Helium was the carrier gas with a constant pressure of 30  
184 psi. The oven temperature program was as follows: 50°C held for 1 min, ramped to 280°C at  
185 15°C /min and held for 9 min (total run time was 25.3 min). The analysis was carried out in  
186 the selected ion monitoring (SIM) mode. The MS parameters were set as follows: EI  
187 ionization with 70 eV energy; ion source temperature, 250 °C. All the ion fragments with  
188 their relative intensities at the specific retention times were considered as a valid confirmation  
189 criterion and were used to identify the selected BAs. An Agilent ChemStation was used for  
190 data collection and GC-MS control.

191 The optimized method was evaluated using the following validation parameters: linearity,  
192 precision, sensitivity and accuracy in accordance to quality assurance protocol. Linearity was  
193 examined by application of 10 different concentrations. Hexylamine was used as internal  
194 standard. Limits of detection (LODs) and limits of quantification (LOQs) were calculated to  
195 estimate the sensitivity of the methodology. Both LODs and LOQs were calculated  
196 from spiked samples ( $n=3$ ) and the minimum detectable analyte amount with a signal-to-noise  
197 ratio of 3 and 10, respectively, was established. The intra-day ( $RSD_I$ ) and inter-day ( $RSD_R$ )  
198 precision were determined by the application of five replicates of wine samples spiked at two  
199 levels (0.10 and 0.25 mg/L). In addition to validation parameters, recovery rates were  
200 estimated using the ratio of the peak areas of the spiked samples of known concentration of  
201 biogenic amines to those of spiked water solution ( $n=3$ ). The matrix effect (ME) of the  
202 optimized method was also evaluated by application the procedure described by Matuszewski  
203 et al. (Matuszewski, Constanzer, & Chavez-Eng, 2003). The ME was examined  
204 at a concentration level of 0.25 mg/L, and calculated by comparing the mean peak area  
205 of the analyte standards in the water solution (a,  $n=3$ ) with the mean peak area of an analyte  
206 spiked postextraction (b,  $n=3$ ). The following Equation was used:

$$207 \quad ME [\%] = \frac{b}{a} \times 100\% \quad (\text{Equation 1})$$

208 The MEs, were ranged from 79% and 99%. In general, ME has no impact on the qualitative  
209 and quantitative results of this method and can be omitted. Additionally, it was proven that it  
210 is justified to use an internal standard (IS) for calibration. Information on determined  
211 validation parameters and average recoveries (%) obtained with the optimized method in  
212 spiked wine samples are given in Table 1SI (Supporting Information).

213



## 214 **2.6. Chemometric analysis**

215 In the present study, multivariate statistical data mining was used to discover the  
216 specific correlations between the different wine preservation methods and determine the  
217 content of bioamines and selected phenolic compounds. The following chemometric methods  
218 were used for intelligent data analysis: cluster analysis (hierarchical and non-hierarchical or  
219 K-means clustering), two-way joining analysis, principal component analysis, and factor  
220 analysis. The analysis were performed using STATISTICA 8.0 software.

## 221 **3. Results and Discussion**

### 222 **3.1. Polyphenolic content**

223 Red wine is a rich source of phenolic compounds that exert beneficial effects on the  
224 human health due to their antioxidant properties. Many studies have been conducted which  
225 indicate that the profile of phenolic compounds in a wine depends on the geographical  
226 location of the vineyard, the type of grapes, the method of production and preservation, and  
227 storage time (Manns et al. 2013; Stój et al. 2020). To evaluate the effect of the preservation  
228 method and storage time on the phenolic compound content of red wine samples, UPLC-  
229 PDA-MS/MS was used. A total of 54 compounds were determined in the studied samples by  
230 UPLC: 24 anthocyanins, 7 flavonols, 12 flavon-3-ols, 7 phenolic acids, and 4 stilbenes  
231 (Supplementary Material – Table S.1). The Retention times, molecular ion masses and the  
232 basic MS2 fragments of the individual phenolic compounds are presented in Supplementary  
233 Material - Table S.2. The contents of selected phenolic compounds detected in our red wine  
234 samples are shown in Table 2.

235 The most abundant group were anthocyanins, especially malvidin 3-O-glucoside-5-O-  
236 glucoside, malvidin 3-O-glucoside and delphinidin 3-O-glucoside, a profile that is  
237 characteristic of Rondo variety wines (Stój et al. 2020; Kapusta et al. 2018). Anthocyanins are  
238 responsible for the hue and color stability of wine and are indicative of its final quality. The  
239 basic structure of anthocyanins is their aglycone part (Khoo et al. 2017). In the examined  
240 wine, derivatives of five aglycones were determined: delphicin, malvidin, petunidin, peonidin  
241 and cyanidin. Anthocyanins are unstable compounds that can undergo reversible  
242 transformations in aqueous environments due to pH changes, thus affecting the color of the  
243 product. In addition, these compounds may degrade during processing when exposed to  
244 various factors, such as temperature, oxygen, or light (He et al. 2012; Yue et al. 2021). This is  
245 consistent with our results, which indicated that the storage process as well as the preservation

246 method used affected the final anthocyanin concentration. Analyzing the influence of the  
247 storage proces, we noted a 8.23 to 47.51 % reduction in the subtotal levels of these  
248 compounds in each of the tested samples compared to samples which had not been stored.  
249 Additionally, a lower decrease in the content of diglycoside anthocyanins was observed,  
250 which indicates that they exhibit a higher stability than monoglycoside anthocyanins (Table  
251 S.1). This observation is confirmed by numerous scientific reports (He et al. 2012; Kim et al.  
252 2010). The most stable molecule with the lowest level of reduction in all samples was  
253 cyanidin-3-O-glucoside-5-O-glucoside. By contrast, cyanidin-3-O-glucoside was the most  
254 susceptible to degradation, which was directly related to its structure. Malvidin-3-O-glucoside  
255 and peonidin-3-O-glucoside do not have hydroxyl groups in the ortho position, which makes  
256 them relatively more resistant to oxidation than cyanidin-3-O-glucoside (He et al.2012). Our  
257 results indicate that the content of each anthocyanin in both non-stored and stored samples  
258 was also dependent on the preservation method applied. In the present study, three  
259 preservation methods were used: cold plasma (variable process conditions), addition of  
260 potassium metabisulfite (30 mg/L or 100mg/L) and a method combining the use of cold  
261 plasma with the addition of potassium metabisulfite at 30 mg/L. In wine production, the  
262 standard method of wine preservation is sulfurization (Christofi et al. 2020). In the wine  
263 samples analyzed immediately after the addition of potassium metabisulfite (Table S.1 –  
264 samples no. 8 and 15), we observed a slight increase in the total content of anthocyanins  
265 compared to the control sample (no. 1). Moreover, the sample with the addition of 100 mg/L  
266 potassium metabisulfite had the highest content of anthocyanins (836.32 mg/L) compared to  
267 the other samples (580.36–811.73 mg/L). Sulfur compounds are used in vinification as  
268 antimicrobial and antioxidant agents. Furthermore, the addition of sulfur dioxide is thought to  
269 prevent enzymatic and non-enzymatic oxidation of wines (Esparza 2020). In our study,  
270 however, the total content of anthocyanins after the three-month storage period in samples  
271 subjected to sulfurization (Table S.1– samples 23 and 30) was similar to the control sample  
272 (sample 16); only the addition of 100 mg/L potassium metabisulfite caused a decrease in the  
273 content of these compounds by 5.77%. These results indicate that the application of potassium  
274 metabisulfite has a minimal effect on the reduction of the anthocyanin content. Despite their  
275 good preservative properties, sulfur compounds can have negative effects on consumer health  
276 causing allergic reactions in some consumers. Therefore, alternative wine preservation  
277 methods or combined methods are being sought to reduce the sulfate doses used (Christofi et  
278 al.2020). One of the new methods of food preservation, which we tested in this study, is cold  
279 plasma. To the best of our knowledge, there is no research so far regarding the effect of cold

280 plasma treatment on the content of phenolic compounds in red wine samples in comparison  
281 with the effect of this method combined with potassium metabisulfite. In our experiment, we  
282 evaluated the impact of cold plasma treatment time (2, 5, 10 min) and the type of working gas  
283 used (helium/oxygen and helium/nitrogen) on the profile of phenolic compounds in red wine  
284 samples. Additionally, we tested the effect of cold plasma treatment combined with potassium  
285 metabisulfite (30 mg/L) treatment. Our results showed that both the duration of the process  
286 and the type of gas used contributed to a change in the content of individual compounds. The  
287 analysis of the level of anthocyanins in unstored samples indicated that the application of cold  
288 plasma for 10 min with the mixture of helium/oxygen as the working gas resulted in the  
289 highest reduction in the total anthocyanin content compared to the control (Table S.1). To  
290 date, there are few reports in the literature explaining the mechanism of action of cold plasma  
291 on food products (Alves Filho et al. 2020; Gavahian et al. 2018). However, cold plasma  
292 generation is accompanied by light emission, cavitation processes, shock wave generation and  
293 free radical generation, which directly contributes to the degradation of many organic  
294 compounds including phenolic compounds (He et al. 2012). On the other hand, in wine  
295 samples exposed to cold plasma with helium/nitrogen as the working gas, an increase in  
296 anthocyanin concentration was observed, which was the larger the longer the samples were  
297 exposed to treatment. The total anthocyanin content after 2, 5, and 10 min was, respectively,  
298 707.23, 747.74, and 755.25 mg/L. Also, higher anthocyanin concentrations were recorded in  
299 the samples exposed to cold plasma with the addition of potassium metabisulfate compared to  
300 the same samples exposed to cold plasma alone (Table S.1). This was probably related to the  
301 protective effect of sulfate on anthocyanins discussed earlier in this section. We also observed  
302 a similar relationship related to the working gas used. Again, wine samples exposed to cold  
303 plasma generated using a mixture of helium and oxygen showed a higher reduction in the  
304 anthocyanin content compared to samples using a mixture of helium and nitrogen as the  
305 working gas. The disparities in the effect of the individual gases on anthocyanin stability were  
306 probably due to the fact that the different gases produced different reactive compounds during  
307 plasma generation. When oxygen is used in the working gas mixture, the plasma stream may  
308 contain hydrogen peroxide, hydroxyl radical, peroxy anion or singlet oxygen, all of which  
309 can cause significant degradation of anthocyanins (Arjunan et al. 2015). Since red wine is a  
310 complex matrix and undergoes various chemical processes, the effects of different  
311 preservation methods on phenolic compounds after three months of storage were also  
312 analyzed. Interesting results were observed in most of the samples exposed to 5 min of cold  
313 plasma. A higher content of some anthocyanins was noted compared to samples that were

314 plasma-treated for only 2 min (Table 2). In addition, the decrease in the anthocyanin content  
315 compared to the sample preserved by the same method but not stored was also lower than  
316 after a 2-min exposure to cold plasma. For example, for a 5-min cold plasma treatment with  
317 helium/nitrogen as the working gas, the total anthocyanin content before storage was 747.74  
318 mg/L and dropped after storage by 11.57 % to 661.23 mg/L. By contrast, a 2-min cold plasma  
319 treatment resulted in a 30.27 % reduction in anthocyanins compared to the non-stored sample  
320 (Table S.1). Moreover, higher anthocyanin contents were observed in the samples subjected to  
321 5 min of cold plasma treatment without potassium metabisulfite, which was an inverse  
322 relationship to that observed in the samples before storage. This may indicate that cold  
323 plasma, despite the initial degradation of anthocyanins, produces a better overall preservation  
324 effect than the mixed method. In addition, when samples with the same exposition time were  
325 compared, the anthocyanin content in samples exposed to cold plasma generated using the  
326 helium/nitrogen gas mixture was similar to that of the control sample and the sample with  
327 30mg/L potassium metabisulfite, and 4.34 % higher than that of the sample with 100 mg/L  
328 potassium metabisulfite.

329 The contents of phenolic acids such as gallic acid, protocatechuic acid, caftaric acid,  
330 cutaric acid, caffeic acid, coumaric acid and ferulic acid were also determined in the studied  
331 wine. Gallic acid was the most abundant of those compounds at concentrations from 9.52  
332 mg/L to 11.86 mg/L. In the samples before storage, the highest total content of phenolic acids  
333 was noted after a 2-min exposure to cold plasma – 24.68 mg/L (helium/nitrogen), a value that  
334 was 8.25% higher compared to the control sample (Table S.1). A study conducted on white  
335 wine by Lukić et al. (2019) also reported a slight increase in the content of some phenolic  
336 acids as a result of cold plasma exposure. Cold plasma also had a beneficial effect on the  
337 content of hydroxycinnamic acids in pomegranate juice (Herceg et al. 2016). Acids belonging  
338 to this group are characterized by a higher stability, which probably translates into their lower  
339 reactivity with the radicals formed during cold plasma generation.

340 In contrast to anthocyanins, the content of phenolic acids increased after storage in most  
341 samples (Table S.1). Interesting results were observed for the content of protocatechuic acid.  
342 In each sample after storage, the content of this acid increased compared to the non-stored  
343 samples. However, cold plasma treatment (10 min, helium/nitrogen) resulted in a substantial,  
344 up-to-4-fold increase in the content of this compound compared to non-stored samples (0.84  
345 mg/L). The lowest content of this compound was observed in samples with 100mg/L  
346 potassium metabisulfite (0.19 mg/L) (Table 2). The contents of other acids showed a similar

347 trend. Based on the literature data and our own results on the anthocyanin content, we can  
 348 assume that such a large increase in protocatechuic acid in samples exposed to cold plasma  
 349 was related to a decrease in the anthocyanin content. Under cold plasma treatment,  
 350 anthocyanins degrade to phenolic acids, and the main products of their decomposition are  
 351 protocatechuic, vanillic, syringic, and p-coumaric acids (Yang et al. 2018). Garofulić et al  
 352 (2015), who evaluated the effect of cold plasma treatment on the contents of anthocyanins and  
 353 phenolic acids in cherry juice, suggested that plasma acting on the food matrix for a short time  
 354 caused the dissociation of agglomerates or particles, leading to an increase in the content of  
 355 phenolic compounds.

356 In another experiment, we used ultraperformance chromatography to determine flavanols,  
 357 flavan-3-ols, and stilbenes in the examined red wine samples. The content of flavanols in the  
 358 wine was low and their total content ranged from 3.74 mg/L to 2.52 mg/L. The highest  
 359 concentration was recorded in the non-stored control sample, while the lowest concentration  
 360 was recorded after storage in the sample preserved by cold plasma (10 min, helium/nitrogen  
 361 working gas) with the addition of potassium metabisulfite. The most abundant flavan-3-  
 362 ols were (+)-catechin at 25.67 mg/L (sample no. 5) and procyanidin B1 at 10.46 mg/L (sample  
 363 no. 12). *Cis* - and *trans*-resveratrol were also determined in the studied wine samples. The  
 364 content of *cis*-resveratrol in the samples before and after storage was practically the same. A  
 365 slight increase in its content was observed after storage in the sample exposed to cold plasma  
 366 (10 min, helium/oxygen). An inverse correlation was noted for *trans*-resveratrol (Table S.1).

367 Table 2. Contents of selected phenolic compounds in red wine samples determined by UPLC-  
 368 PDA-MS/MS (n=2)

Sample no.*	3gM (mg/L)	3gD (mg/L)	3gC (mg/L)	3kGM (mg/L)	3kGPet (mg/L)	3kGPeo (mg/L)	PCA (mg/L)
<b>1</b>	191.76 ±	82.38 ±	2.96 ±	45.67 ±	18.99 ±	4.94 ± 0.26	0.16 ±
	3.02	5.62	0.01	1.13	1.84		0.00
<b>2</b>	142.6 ±	49.26 ±	1.91 ± 0.01	27.87 ±	13.35 ±	2.92 ± 0.26	0.2 ±
	3.02	5.60		1.07	1.75		0.00
<b>3</b>	153.32 ±	50.93 ±	2.11 ± 0.01	31.25 ±	15.38 ±	3.22 ± 0.28	0.22 ±
	3.24	5.79		1.19	2.02		0.00
<b>4</b>	126.35 ±	39.49 ±	1.67 ± 0.01	23.74 ±	11.36 ±	2.48 ± 0.22	0.2 ±
	2.67	4.49		0.91	1.49		0.00

<b>5</b>	157.68 ± 3.34	55.64 ± 6.33	2.03 ± 0.01	33.75 ± 1.29	16.33 ± 2.15	3.32 ± 0.29	0.2 ± 0.00
<b>6</b>	166.42 ± 3.52	57.24 ± 6.51	2.07 ± 0.01	34.97 ± 1.34	16.4 ± 2.15	3.61 ± 0.32	0.21 ± 0.00
<b>7</b>	170.08 ± 3.60	58.03 ± 6.60	2.07 ± 0.01	35.98 ± 1.38	17.17 ± 2.26	3.63 ± 0.32	0.21 ± 0.00
<b>8</b>	182.88 ± 3.87	64.70 ± 7.36	2.44 ± 0.01	39.71 ± 1.52	19.23 ± 2.53	4.03 ± 0.35	0.17 ± 0.00
<b>9</b>	177.2 ± 3.75	64.19 ± 7.30	2.25 ± 0.01	37.73 ± 1.44	18.94 ± 2.49	3.89 ± 0.34	0.2 ± 0.00
<b>10</b>	170.57 ± 3.61	58.29 ± 6.63	2.2 ± 0.01	37.34 ± 1.43	17.98 ± 2.36	3.70 ± 0.33	0.22 ± 0.00
<b>11</b>	150.23 ± 9.16	57.69 ± 9.27	1.91 ± 0.05	30.75 ± 3.14	11.63 ± 0.89	2.99 ± 0.24	0.17 ± 0.01
<b>12</b>	178.48 ± 10.88	75.63 ± 12.16	2.41 ± 0.06	39.46 ± 4.03	15.64 ± 1.19	3.80 ± 0.31	0.18 ± 0.01
<b>13</b>	166.59 ± 10.16	72.04 ± 11.58	2.23 ± 0.05	38.68 ± 3.95	15.28 ± 1.16	3.63 ± 0.30	0.18 ± 0.01
<b>14</b>	171.68 ± 10.47	69.42 ± 11.16	2.08 ± 0.05	38.43 ± 3.93	14.93 ± 1.14	3.66 ± 0.30	0.19 ± 0.01
<b>15</b>	190.82 ± 11.63	64.7 ± 13.17	2.43 ± 0.06	43.86 ± 4.48	17.36 ± 1.32	3.96 ± 0.32	0.13 ± 0.01
<b>16</b>	149.42 ± 9.11	61.47 ± 9.88	2.12 ± 0.05	30.64 ± 3.13	12.09 ± 0.92	2.94 ± 0.24	0.32 ± 0.02
<b>17</b>	106.81 ± 6.51	15.13 ± 2.43	0.41 ± 0.01	18.55 ± 1.90	7.43 ± 0.57	2.03 ± 0.17	0.79 ± 0.06
<b>18</b>	121.59 ± 7.41	40.16 ± 6.46	1.45 ± 0.04	22.42 ± 2.29	8.69 ± 0.66	2.27 ± 0.19	0.7 ± 0.05
<b>19</b>	54.59 ± 3.33	14.78 ± 2.38	0.52 ± 0.01	6.96 ± 0.71	3.05 ± 0.23	1.07 ± 0.09	0.8 ± 0.06
<b>20</b>	101.6 ± 6.19	11.50 ± 1.85	0.34 ± 0.01	17.1 ± 1.75	6.32 ± 0.48	1.88 ± 0.15	0.78 ± 0.06
<b>21</b>	138.68 ±	53.03 ±	1.67 ± 0.10	26.58 ±	13.18 ±	2.59 ± 0.01	0.73 ±

	2.00	6.97		0.43	2.12		0.02
<b>22</b>	79.88 ± 1.15	12.89 ± 1.69	0.39 ± 0.04	11.36 ± 0.18	5.78 ± 0.93	1.44 ± 0.01	0.84 ± 0.02
<b>23</b>	143.55 ± 2.07	61.70 ± 8.11	2.28 ± 0.14	28.27 ± 0.46	14.36 ± 2.31	2.83 ± 0.01	0.25 ± 0.01
<b>24</b>	92.75 ± 1.34	7.81 ± 1.03	0.25 ± 0.02	14.19 ± 0.23	7.29 ± 1.17	1.63 ± 0.01	0.81 ± 0.02
<b>25</b>	97.01 ± 1.40	11.90 ± 1.56	0.33 ± 0.02	15.12 ± 0.25	7.74 ± 1.24	1.71 ± 0.01	0.79 ± 0.02
<b>26</b>	76.38 ± 1.10	19.42 ± 2.55	0.67 ± 0.04	11.26 ± 0.18	5.9 ± 0.95	1.4 ± 0.01	0.80 ± 0.02
<b>27</b>	84.70 ± 1.22	9.37 ± 1.23	0.28 ± 0.02	12.73 ± 0.21	6.49 ± 1.04	1.52 ± 0.01	0.84 ± 0.02
<b>28</b>	100.49 ± 1.45	23.42 ± 3.08	0.67 ± 0.04	16.68 ± 0.27	8.91 ± 1.43	1.83 ± 0.01	0.83 ± 0.02
<b>29</b>	97.29 ± 1.40	34.25 ± 4.50	1.18 ± 0.07	17.31 ± 0.28	8.86 ± 1.42	1.92 ± 0.01	0.74 ± 0.02
<b>30</b>	132.67 ± 1.91	57.73 ± 7.58	2.07 ± 0.13	26.45 ± 0.43	13.35 ± 2.15	2.7 ± 0.01	0.19 ± 0.00

369 3gM – malvidin 3-O-glucoside; 3gD – delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-  
370 glucoside; 3kGM – malvidin 3-O-(600-O-coumaryl)-glucoside; 3kGPet – petunidin 3-O-(600-  
371 O-coumaryl)-glucoside; 3kGPeo – peonidin 3-O-(60 0-O-coumaryl)-glucoside; PCA –  
372 protocatechuic acid

373 \* the coding of the samples is shown in Table 1

### 374 3.2 Biogenic amine content

375 DLLME-GC-MS was applied to determine the concentrations of biogenic amines in  
376 the red wine samples analyzed. The results are presented in Table 3. Six biogenic amines were  
377 identified: TRP, PUT, HIS, TYR, CAD and 2-PE, with histamine having the highest  
378 concentrations in all samples. This finding corresponds with the results reported by other  
379 researchers who indicate that histamine is the most abundant biogenic amine in wines (Plotka  
380 et al. 2018). High concentrations of histamine in a product can cause negative health effects in  
381 the consumer, so it is important to use methods that will reduce the content of this compound  
382 in the food matrix (Esposito et al. 2019). In our experiment, the highest HIS content was  
383 found in the unpreserved control sample (before storage: 818 ± 34 µg/L; after storage: 821 ±

384 30 µg/L). A significantly lower content of this compound was observed in the sample that had  
 385 been exposed to cold plasma for 10 min using a helium/oxygen mixture as the working gas  
 386 ( $584 \pm 34$  µg/L) in combination with the addition of 30 mg/L potassium metabisulfite. Also  
 387 after three months of storage, the HIS content of this sample did not change significantly ( $586$   
 388  $\pm 33$  µg/L). When the effect of the wine preservation method on the content of other biogenic  
 389 amines was analyzed, in all cases the 10-min application of cold plasma (helium/oxygen as  
 390 working gas) with 30 mg/L potassium metabisulfite resulted in the highest reduction in the  
 391 level of these compounds. Moreover, this effect persisted after storage. To date, the literature  
 392 provides no information on or explanation of the effect of cold plasma on the content of  
 393 biogenic amines in wine. However, because the formation of these compounds depends  
 394 mainly on the microorganisms present in the food matrix (Restuccia et al. 2018), it can be  
 395 assumed that cold plasma, which has a well-proven biocidal activity against unwanted  
 396 microorganisms, indirectly contributes to the reduction of biogenic amines in food products  
 397 (Bourke et al. 2017; Lu et al. 2014). Our results also showed that the efficiency of cold  
 398 plasma in reducing biogenic amines in wine samples was affected by the duration of treatment  
 399 and the type of working gas used. Increasing the duration of the process to 10 min and the use  
 400 of a mixture of helium and oxygen as the working gas favourably affected the elimination of  
 401 these compounds from the product matrix. The influence of the duration of the process as well  
 402 as the type of gases used on the sterilizing efficiency of cold plasma has also been  
 403 demonstrated by other authors. Hou et al. (2019), who sterilized blueberry juice using cold  
 404 plasma for 2, 4 and 6 min, recorded the highest reduction in *Bacillus* spp. populations after the  
 405 time of 6 min. Also our previous study on the effects of cold plasma on *Lentilactobacillus*  
 406 *hilgardii* cells showed that increasing the duration of the process as well as using a mixture of  
 407 helium and oxygen as the working gas resulted in higher cell reduction than using a mixture  
 408 of helium and nitrogen (Niedzwiedz et al. 2020).

409 Table 3. Concentrations of selected biogenic amines determined in wine samples by DLLME-  
 410 GC-MS;  $n=3$

411

Sample no.*	TRP (µg/L)	PUT (µg/L)	HIS (µg/L)	TYR (µg/L)	CAD (µg/L)	2-PE (µg/L)
1	$4.089 \pm 0.012$	$489 \pm 25$	$818 \pm 34$	$27.74 \pm 0.16$	$58.73 \pm 0.15$	$18.70 \pm 0.054$
2	$3.670 \pm 0.011$	$475 \pm 24$	$799 \pm 31$	$27.58 \pm 0.17$	$54.15 \pm 0.12$	$18.68 \pm 0.049$
3	$3.578 \pm 0.008$	$455 \pm 25$	$734 \pm 37$	$27.34 \pm 0.17$	$52.21 \pm 0.12$	$18.73 \pm 0.047$



<b>4</b>	3.551 ± 0.009	449 ± 23	732 ± 36	26.43 ± 0.13	52.01 ± 0.14	18.63 ± 0.050
<b>5</b>	3.662 ± 0.010	471 ± 22	784 ± 29	27.51 ± 0.18	53.94 ± 0.13	18.71 ± 0.048
<b>6</b>	3.589 ± 0.008	466 ± 27	741 ± 33	27.44 ± 0.16	52.27 ± 0.11	18.75 ± 0.044
<b>7</b>	3.540 ± 0.010	457 ± 22	742 ± 34	26.78 ± 0.17	52.22 ± 0.13	18.66 ± 0.051
<b>8</b>	2.918 ± 0.008	344 ± 25	654 ± 34	<LOD	48.29 ± 0.16	23.74± 0.044
<b>9</b>	2.705 ± 0.011	324 ± 23	627 ± 38	<LOD	44.54 ± 0.14	23.77± 0.047
<b>10</b>	2.678 ± 0.013	299 ± 20	622 ± 33	<LOD	39.79 ± 0.12	23.68± 0.050
<b>11</b>	1.972 ± 0.006	278 ± 19	584 ± 34	<LOD	38.09 ± 0.14	23.76± 0.048
<b>12</b>	2.802 ± 0.014	348 ± 24	654 ± 38	<LOD	43.87 ± 0.16	23.72± 0.051
<b>13</b>	2.732 ± 0.016	320 ± 21	641 ± 32	<LOD	40.17 ± 0.13	23.63± 0.047
<b>14</b>	2.052 ± 0.008	291 ± 19	601 ± 36	<LOD	37.89 ± 0.11	23.69± 0.052
<b>15</b>	3.878 ± 0.013	466 ± 23	773 ± 30	<LOD	52.42 ± 0.17	25.88± 0.054
<b>16</b>	4.086 ± 0.011	490 ± 24	821 ± 30	27.71 ± 0.15	58.66 ± 0.18	18.78 ± 0.044
<b>17</b>	3.674 ± 0.010	479 ± 22	794 ± 29	27.66 ± 0.16	54.05 ± 0.14	18.75 ± 0.043
<b>18</b>	3.581 ± 0.012	457 ± 24	739 ± 35	27.91 ± 0.14	52.18 ± 0.13	18.79 ± 0.051
<b>19</b>	3.560 ± 0.010	449 ± 21	732 ± 33	26.38 ± 0.12	52.09 ± 0.18	18.60 ± 0.044
<b>20</b>	3.669 ± 0.010	476 ± 26	789 ± 31	27.79 ± 0.21	53.99 ± 0.15	18.77 ± 0.044
<b>21</b>	3.593 ± 0.013	471 ± 23	748 ± 27	27.49 ± 0.18	52.30 ± 0.17	18.70 ± 0.038
<b>22</b>	3.547 ± 0.011	457 ± 20	739 ± 31	26.85 ± 0.15	52.28 ± 0.10	18.71 ± 0.047
<b>23</b>	2.915 ± 0.009	349 ± 24	658 ± 33	<LOD	48.33 ± 0.16	23.81± 0.056
<b>24</b>	2.711 ± 0.013	332 ± 21	629 ± 38	<LOD	44.50 ± 0.19	23.84± 0.031
<b>25</b>	2.684 ± 0.012	309 ± 24	617 ± 31	<LOD	39.83 ± 0.10	23.77± 0.062
<b>26</b>	1.979 ± 0.011	279 ± 19	586 ± 33	<LOD	38.04 ± 0.14	23.85± 0.045
<b>27</b>	2.811 ± 0.017	353 ± 22	659 ± 36	<LOD	43.95 ± 0.11	23.77± 0.044
<b>28</b>	2.729 ± 0.014	320 ± 19	646 ± 31	<LOD	40.20 ± 0.17	23.56± 0.039

<b>29</b>	2.058 ± 0.009	289 ± 21	613 ± 35	<LOD	37.84 ± 0.15	23.71± 0.057
<b>30</b>	3.874 ± 0.012	469 ± 25	773 ± 34	<LOD	52.47 ± 0.20	25.93± 0.061

412 TRP – tryptamine, PUT – putrescine, HIS – histamine, TYR – tyramine, CAD – cadaverine,  
413 2-PE – 2-phenylethylamine

414 \* the coding of the samples is shown in Table 1.

### 415 **3.3 Chemometric analysis**

416 The major goal of multivariate statistical data mining was to reveal hidden specific relations  
417 between differently treated (different preservation conditions) wine samples (a total of 30  
418 cases) characterized by 13 chemical variables (bioamines and phenolic compounds). Another  
419 important task was to find similarity patterns depending on the storage conditions and, beyond  
420 that, to identify specific chemical descriptors responsible for the classification of the different  
421 wine samples.

422 The following chemometric methods were used in the intelligent data analysis:

- 423 • Cluster analysis (hierarchical and non-hierarchical or K-means clustering);
- 424 • Two-way joining;
- 425 • Principal components analysis and factor analysis.

426 Hierarchical clustering was performed on standardized input data (z-normalization), with  
427 squared Euclidean distance as a similarity measure, using Ward's method of linkage and  
428 Sneath's significance test. Fig. 2 A1 shows a hierarchical clustering dendrogram of the 13  
429 chemical variables. Three major clusters were identified at Sneath's significance level of  
430 1/3Dmax:

431 C1: 3gM, 3kGM, 3kGPeo, 3kGPet, 3gD, 3gC – phenolic cluster;

432 C2: HIS, PUT, TRP, CAD, TYR – amine cluster;

433 C3: 2PE, PCA – mixed cluster.

434 The hierarchical clustering of the chemical variables identified three patterns of similarity  
435 which could be conditionally determined as phenolic, amine and mixed clusters. There was a  
436 good separation between the phenolic and the amine variables, which indicated that both  
437 groups of variables had a separate impact on the quality of the different wine samples which  
438 was unrelated to the preservation or storage conditions. Fig. 2 A2 shows a hierarchical

439 dendrogram linking 30 wine samples (with different preservation and storage conditions).  
440 Three major clusters of cases were formed (under the same clustering conditions):

441 C1: 17, 19, 20, 22, 24, 25, 26, 27, 28, 29 – samples after storage and preservation by plasma  
442 and by plasma in combination with potassium metabisulfite;

443 C2: 8, 9, 10, 11, 12, 13, 14, 23 – samples before storage with preservation by plasma and  
444 potassium metabisulfite;

445 C3: 1, 2, 3, 4, 5, 6, 7, 15, 16, 18, 21, 30 – samples before storage with plasma preservation.

446 Cluster 1 mainly included samples after storage preserved by plasma and plasma plus  
447 potassium metabisulfite. Cluster 2 chiefly consisted of samples before storage but preserved  
448 by plasma or by potassium metabisulfite. Cluster 3 aggregated 12 plasma-preserved samples  
449 before storage. The clustering of the wine samples showed separation into patterns which  
450 differed in the treatment and storage conditions.

451 K-means clustering is a non-supervised clustering method in which clusters are not  
452 formed spontaneously but according to a preliminary hypothesis regarding the possible  
453 number of clusters. This a priori segmentation is based on an algorithm which selects  
454 centroids in the dataset under a predefined distance measure. The results of K-means  
455 clustering for the formation of 3 clusters of variables and 3 clusters of cases were identical to  
456 those obtained by hierarchical clustering. The members of the non-hierarchical clusters were  
457 the same. This is illustrated in Table 3 in Supplementary Materials (S.3) which shows cluster  
458 membership data for cases and variables along with the respective distances between the  
459 members in each identified cluster. It was important to reveal the role of the chemical  
460 variables as specific descriptors for each of the identified clusters. Fig. 3A presents the  
461 average values of each chemical variable for each cluster. The cluster which included plasma-  
462 preserved samples before storage (C1 in the plot below) was characterized by the highest  
463 levels of amines, moderate (rather high) levels of phenolic compounds and low levels of 2-  
464 PE and PCA. The cluster with samples before storage, preserved by plasma and potassium  
465 metabisulfite (C2 in the plot below) was characterized by the lowest levels of amines, the  
466 highest levels of phenolic compounds, the highest level of 2-PE, and the lowest level of PCA.  
467 The cluster with samples stored after preservation by plasma and by plasma in combination  
468 with potassium metabisulfite (C3 in the plot below) was characterized by moderate levels of  
469 amines, the lowest levels of phenolic compounds, moderate levels of 2-PE and the highest  
470 levels of PCA. It is readily seen that the storage conditions led to changes in the levels of all

471 the chemical variables, which additionally depended on the preservation treatment used. In  
472 general, the levels of phenolic compounds fell after storage, whereas levels of amines were  
473 high before storage and plasma preservation but decreased substantially following  
474 preservation with potassium metabisulfite or after storage.

475 The relationship between the chemical variables and the wine samples is shown  
476 additionally in the plot of the results of two-way joining cluster analysis, in which variables  
477 and cases are in respective correspondence (Fig. 3B).

478 The plot confirms the conclusions above about the determination of specific chemical  
479 descriptors for the wine sample clusters.

480 Both chemometric methods are very similar and their basic task is to find hidden  
481 factors (principal components or factors) responsible for the structure of the data matrix.  
482 Additionally, they are typical projection methods and, as such, lead to a dimensionality  
483 reduction of the system under consideration. In the working algorithm, the data matrix is  
484 decomposed into a factor loading matrix and a factor score matrix, the former presenting the  
485 newly defined special directions in the variables space, and the latter – the new coordinates of  
486 the objects. Both of these matrices need to be correctly interpreted in order to find specific  
487 relationships between objects and variables. In our dataset, two latent factors were responsible  
488 for the data structure. The first of them, which explained 51.3 % of the total variance of the  
489 system, could be tentatively named the “phenolic factor”, and the second factor, with 40.5 %  
490 of explained variance could be called the “amine factor”. This is largely consistent with the  
491 results of cluster analysis. Table 4 (Supplementary Materials S.4), in which statistically  
492 significant loadings are given in bold, shows that the variables 2-PE and PCA are reversely  
493 correlated to the rest of the significant factor loadings with regard to factor 1 and factor 2, and  
494 this specificity corresponds to the formation of the mixed cluster in cluster analysis. An  
495 interpretation of the data in the loadings table leads to the conclusion that the data structure is  
496 dependent on two latent relationships between the variables – a relationship between phenolic  
497 compounds as a similarity group and a relationship between biogenic amines as another  
498 similarity pattern. The graphical plot of the factor loadings in Fig. 2B clearly illustrates these  
499 relationships. Both clusters of variables are well-defined, and the more specific role of 2-PE  
500 as opposite to the amine group and PCA as opposite to the phenolic group is indicated. The  
501 factor scores plot illustrates the formation of three patterns of similarity between the wine  
502 samples. It matches the hierarchical and K-means clusters of wine samples almost perfectly.



503

#### 504 4. Conclusion

505 In this study, for the first time, the effect of cold plasma on the content of phenolic  
506 compounds and biogenic amines in red wine was evaluated with respect to storage time. In  
507 addition, the effect of cold plasma was compared with the traditional method of preservation  
508 (addition of 30 mg/L and 100 mg/L of potassium metabisulfite) and a combined method (cold  
509 plasma with 30 mg/L of potassium metabisulfite). In general, cold plasma treatment caused a  
510 decrease in the total content of phenolic compounds in the wine samples subjected to three  
511 months of storage. However, the application of cold plasma for 5 min with helium/nitrogen as  
512 the working gas reduced the content of these compounds by only 2.85 % compared to the  
513 control. Moreover, the content of phenolic compounds was 3.1% higher in the sample  
514 preserved by this method compared to the sample preserved by the addition of potassium  
515 metabisulfite at a dose of 100 mg/L. Additionally, cold plasma increased the content of  
516 phenolic acids in the studied samples. Importantly, the use of cold plasma resulted in a  
517 reduction of biogenic amines, which can cause adverse health reactions in the consumer. The  
518 highest degree of reduction was observed in the samples exposed to 10 min of cold plasma  
519 (helium/oxygen). Our results indicate that the influence of the storage process as well as the  
520 preservation method on the phenolic profile and the content of biogenic amines is not  
521 unambiguous and depends mainly on the chemical properties of the individual compounds.  
522 However, the reported effects of cold plasma and cold plasma combined with the addition of  
523 potassium metabisulfite on the analyzed compounds allow us to assume that in the future  
524 these methods can be successfully used to reduce the use of SO<sub>2</sub> in winemaking.

525 To conclude, cold plasma may become an alternative method for the preservation of wine or  
526 other alcoholic beverages in the future, ensuring adequate product safety and preserving the  
527 pro-health values of these products. However, further research is needed to optimize the  
528 process conditions of cold plasma treatment.

529 CRediT authorship contribution statement

530

531 **Iwona Niedźwiedź:** Conceptualization, Project administration, Investigation, Methodology,  
532 Validation, Writing – original draft, Visualization. **Justyna Plotka-Wasyłka:** Methodology,  
533 Software, Validation, Resources, Writing – original draft, Writing – review & editing.  
534 **Ireneusz Kapusta:** Methodology, Software. **Vasil Simeonov:** Methodology, Software,



535 Writing – original draft, Writing – review & editing. **Anna Stój:** Resources, Validation.  
536 **Adam Waśko:** Conceptualization, Writing – review & editing. **Joanna Pawlat:** Resources,  
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538 Conceptualization, Project administration, Writing – review & editing  
539

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## 666 Figure Captions

667 Figure 1. A. Experimental set-up for plasma treatment of wine: 1– plasma jet reactor; 2 –  
668 sample in a glass container; 3 – magnetic stirrer; 4 – high voltage power supply; 5 – gas  
669 flow controller. B. Voltage signal between electrodes for the selected gas mixtures.

670 Figure 2. A. Hierarchical dendrogram. 1) clustering of 13 chemical variables 2) –  
671 clustering of wine samples. B. 1) Plot of factor loadings. 2) Plot of factor scores. 3gM –  
672 malvidin 3-O-glucoside; 3gD – delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-glucoside;  
673 3kGM – malvidin 3-O-(600-O-coumaryl)-glucoside; 3kGPet – petunidin 3-O-(600-O-  
674 coumaryl)-glucoside; 3kGPeo – peonidin 3-O-(60 0-O-coumaryl)-glucoside; PCA –  
675 protocatechuic acid; TRP – tryptamine, PUT – putrescine, HIS – histamine, TYR –  
676 tyramine, CAD – cadaverine, 2-PE – 2-phenylethylamine.

677 Figure 3. A. Plot of means for each variable for each identified cluster B. Correspondence  
678 between wine samples and chemical variables. . 3gM – malvidin 3-O-glucoside; 3gD –  
679 delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-glucoside; 3kGM – malvidin 3-O-(600-O-  
680 coumaryl)-glucoside; 3kGPet – petunidin 3-O-(600-O-coumaryl)-glucoside; 3kGPeo –  
681 peonidin 3-O-(60 0-O-coumaryl)-glucoside; PCA – protocatechuic acid; TRP –  
682 tryptamine, PUT – putrescine, HIS – histamine, TYR – tyramine, CAD – cadaverine, 2-  
683 PE – 2-phenylethylamine.

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