

*Influence of pH and ionic strength on the color parameters and antioxidant properties of an ethanolic red grape marc extract*

Article

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1 **Influence of pH and ionic strength on the colour parameters and antioxidant properties**  
2 **of an ethanolic red grape marc extract**

3  
4 *(Abbreviated running title: Study of some medium factors influencing grape marc extract*  
5 *properties)*

6 Elena Cristea<sup>a</sup>, cristea.ele@gmail.com, elena.cristea@sa.utm.md, 9/9 Studentilor St, Chisinau,  
7 Republic of Moldova, MD-2045; <https://www.linkedin.com/in/elena-cristea-73991421/>,  
8 [orcid.org/0000-0001-8942-4288](https://orcid.org/0000-0001-8942-4288)

9 Rodica Sturza<sup>a</sup>, sturzar@yandex.ru, 9/9 Studentilor St, Chisinau, Republic of Moldova, MD-  
10 2045, [orcid.org/0000-0002-9552-1671](https://orcid.org/0000-0002-9552-1671)

11 Paula Jauregi<sup>b</sup>, p.jauregi@reading.ac.uk, Food Biosciences 2-54, Whiteknights, Reading,  
12 United Kingdom, RG6 6AP, [orcid.org/0000-0003-4438-191X](https://orcid.org/0000-0003-4438-191X)

13 Marius Niculaua<sup>c</sup>, niculaua@gmail.com, 9 Mihail Sadoveanu Alley, Iasi, Romania, 700490,  
14 [orcid.org/0000-0002-6331-8206](https://orcid.org/0000-0002-6331-8206)

15 Aliona Ghendov-Moșanu<sup>a</sup>, aliona.mosanu@tpa.utm.md, a\_mosanu@yahoo.com, 9/9  
16 Studentilor St, Chisinau, Republic of Moldova, MD-2045, [orcid.org/0000-0001-5214-3562](https://orcid.org/0000-0001-5214-3562)

17 Antoanela Patras<sup>d\*</sup>, apatras@uaiasi.ro, antoanelapatras@gmail.com, 3 Mihail Sadoveanu  
18 Alley, Iasi, Romania, 700490, [orcid.org/0000-0002-4054-4884](https://orcid.org/0000-0002-4054-4884), phone: 0040.720033945

19 <sup>a</sup>-Technical University of Moldova, <sup>b</sup>-University of Reading, <sup>c</sup>- Research Center for Oenology  
20 (Romanian Academy, Iași Branch), <sup>d</sup> - University of Agricultural Sciences and Veterinary  
21 Medicine „Ion Ionescu de la Brad”, Iasi, Romania

22 **Highlights**

- 23 • Gallic, protocatechuic, ferulic, chlorogenic and salicylic acids were identified  
24 • CaCl<sub>2</sub> decreased antioxidant activity, but enhanced colour intensity  
25 • Different pH values had slight influence on the antioxidant activity

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\* Corresponding author

26 **Keywords:** grape marc extract, antioxidant activity, colour parameters, polyphenols, pH,  
27 CIELab

28 **Abstract:** The aim of this paper was to investigate the influences of pH and several salts on the  
29 antioxidant activity and colour of an ethanolic grape marc extract. Furthermore, the phenolic  
30 content of the extract was analysed using HPLC and spectrophotometric methods, while the  
31 total antioxidant activity was assessed by reaction with ABTS radical. Gallic acid, procyanidin  
32 B1, polydatin, catechin, epicatechin, hyperoside, ferulic, chlorogenic, and salicylic acids were  
33 among the main identified polyphenols. Different pH values had slight influence on the  
34 antioxidant activity; the highest value being determined for the pH 3.7. The redness, blueness,  
35 chroma and hue were significantly enhanced at pH 3.7 and 2.6. The chromaticity decreased at  
36 pH=5.5 and pH=7.4, so the extract should be used with care in products with such media. The  
37 presence of salts did not significantly affect the antioxidant activity, except the higher  
38 concentration of CaCl<sub>2</sub>, which decreased antioxidant activity, but enhanced colour intensity.

39 **Practical application:** The data presented in this paper could be used for the development of a  
40 new food dye with antioxidant properties of natural origin. The optimal medium conditions, i.e.  
41 pH and ionic strength for the use of an ethanolic red grape marc extract, have been identified.  
42 The information could be used in product development and product formulation, especially  
43 when functional foodstuffs are envisaged. Consequently, this paper would be of significant  
44 interest for food chemists, food technologists, food manufacturers and especially manufacturers  
45 of food dyes and all those using natural substances in their production process.

## 46 **1. Introduction**

47 The “clean label” is a growing global trend and involves aspects, which range from  
48 sustainability of food production to use of non-synthetic ingredients (Global Food Forums,  
49 2017). The use of natural pigments is part of the latter and recent publications report that the  
50 global market will grow by 6.22% revenue until 2019 (Cortez et al., 2017). Furthermore, due  
51 to their structure and properties, these substances could play a double technological role in  
52 foods, i.e. act as both colourants and antioxidants. Many of these pigments/antioxidants can be  
53 sourced from the by-products of the food industry, which, at present, are not utilized at full  
54 potential. For example, in the process of winemaking, around 25% of the grape weight results  
55 in waste, most of which is afterwards composted and reintroduced in the vineyards (Dwyer et  
56 al., 2014). Studies suggest that, depending on the winemaking technique, around 70% of  
57 phenolics remain in that waste after processing (Ratnasooriya & Rupasinghe, 2012). These  
58 phenolics present also a source of valuable bioactive compounds, which may be used in

59 different pharmaceutical, nutraceutical, and food formulations. Traditionally, natural  
60 antioxidant extracts are intended for medical use, however, because of many uncertainties  
61 related to their bioavailability and metabolism, their application in food systems is more  
62 promising (Astley, 2003) where they can be used as antioxidants, colour compounds, and  
63 antimicrobial agents(Oliveira et al., 2013). If implemented globally, such trends could impact  
64 different aspects of the entire food chain namely contribute to a more sustainable agriculture,  
65 increase the availability of some important nutrients in diets and reduce the risk of certain  
66 degenerative diseases while improving the sensory properties of foodstuffs.

67 Nevertheless, the pigments found in grape skins, such as the anthocyanins, degrade  
68 rapidly and form colourless or brown compounds(Ngo & Zhao, 2009), which is why it is  
69 important to consider the optimal technological conditions and other ingredients in foods which  
70 may influence their antioxidant activity and colour. The discovery of acylated anthocyanins and  
71 their stability has opened new pathways for food producers since these pigments present lower  
72 susceptibility to temperature, light and pH change. Caffeic, *p*-coumaric, ferulic, and sinapic  
73 acids, as well as a range of aliphatic acids like acetic, malic, malonic, oxalic, and succinic acid  
74 are some of the most important acylating agents(Bakowska-Barcak, 2005). Even though recent  
75 studies suggest that during alcoholic fermentation, the grape anthocyanins stabilise via  
76 interaction with other compounds, i.e. acetic acid originating from yeast metabolism(Campos,  
77 2009), a study on the stability of the antioxidant activity and colour of these newly formed  
78 compounds is necessary. Moreover, several physicochemical parameters could affect  
79 phenolics, in particular, the pH and the complexation with other compounds present in the food  
80 matrix (Cortez et al., 2017).

81 CIEL\*a\*b\* parameters are becoming increasingly popular among food scientists and  
82 processors for the description and standardisation of foodstuffs' colour. Although difficult at  
83 first glance, this colour space is in fact easy to interpret with little training and could save a  
84 great amount of expensive sensory work performed in industrial environments for simple colour  
85 match, if the data becomes available.

86 The objective of this study was to research the influence of pH and various ions on the  
87 antioxidant activity and colour of an ethanolic grape marc extract. This paper also offers some  
88 answers on the interactions and effectiveness of antioxidants/colourants in different food media.

## 89 **2. Materials and Methods**

### 90 **2.1 *Materials***

91 The red grape marc was sourced from a Moldovan winery. D (-)-quinic acid (98%), sinapic  
92 acid (98%), methyl 4-hydroxy-3-methoxycinnamate (99%), ABTS(2,2'-azino-bis(3-  
93 ethylbenzothiazoline-6-sulphonic acid) were obtained from Alfa Aesar (Germany), Folin-  
94 Ciocalteu reagent was provided by Merck (Germany), (+)-catechin(98%), morin hydrate,  
95 ellagic acid ( $\geq 95\%$ ), benzoic acid, quercetin, caffeic acid, (+)-rutintri-hydrate, syringic acid,  
96 ferulic acid, gallic acid (98%), protocatechuic acid, gentisic acid, parahydroxybenzoic acid,  
97 salicylic acid (99.9%), para-coumaric acid were purchased from Sigma (Germany, Japan,  
98 China). Procyanidin B1, procyanidin B2, polydatin, hyperoside were purchased from  
99 Extrasynthese (France). Trans-resveratrol was purchased from TCI Europe (Belgium).  
100 Quercetin ( $>95\%$ ) was obtained from Sigma-Aldrich. All the spectrophotometric measurements  
101 were made using Analytic Jena Specord 200 Plus spectrophotometer (Germany).

## 102 ***2.2 Extraction***

103 The marc was dried at the temperature up to  $65^{\circ}\text{C}$ , chopped up to a powdery state, and  
104 sieved. The initial samples were obtained by extraction in ethanol 50% (v/v) at the ratio 1g  
105 marc: 10 mL solvent, under stirring for 30 min at room temperature(Cristea et al., 2015). The  
106 extraction parameters have been optimised during the earlier stages of the research (unpublished  
107 data). These extracts were stored in the dark, at the temperature (t) of  $4^{\circ}\text{C}$ , and then used in the  
108 experiments involving pH modification and the addition of salts. Moreover, their composition  
109 was determined.

## 110 ***2.3 Studies on the ionic strength***

111 Three different salts widely used in food production, i.e. NaCl,  $\text{CaCl}_2$ ,  $\text{KNO}_3$  were added  
112 at different concentrations (0.001 M, 0.01 M, and 0.1 M). The extracts were then stored at  $t =$   
113  $4 \pm 1^{\circ}\text{C}$  for 12 hours, after which the antioxidant activity and the colour parameters ( $\text{CIEL}^*a^*b^*$ )  
114 were measured. The parameter  $(A-A_0)/A_0$  was calculated and expressed as a percentage, in  
115 order to assess the hyperchromic shift, where  $A$ =absorption after salt addition,  $A_0$ =absorbtion  
116 of the extract in the absence of the salts, both at  $\lambda=520$  nm (Gonzalez-Manzano et al., 2009;  
117 Malaj et al., 2013).

## 118 ***2.4 Studies on pH***

119 The extracts were brought to the following values of pH: 2.6; 3.7; 5.5; 7.4, and 8.0 using  
120 adequate buffers, i.e. buffer pH=3.7 (glycocoll and sodium chloride), buffer pH=5.5 (sodium  
121 citrate), pH=7.4 (PBS – phosphate-buffered saline and sodium dihydrogenphosphate), but also  
122 NaOH (0.1 M) and HCl (0.1 M), then stored at  $t = 4 \pm 1^{\circ}\text{C}$  for 12 hours. Control samples were

123 prepared by diluting the extracts with the same volumes of ethanol 50% (v/v) as the ones of the  
124 buffers used for pH adjustment. Afterwards, the antioxidant activity and the colour parameters  
125 (CIELab) were determined.

### 126 ***2.5 Antioxidant activity by reaction with ABTS radical***

127 The antioxidant activity of the extracts was measured using the assay with ABTS  
128 radical. ABTS was dissolved in distilled water to 7 mM concentration, after which the ABTS  
129 radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium  
130 persulfate and allowing the mixture to stand in the dark for 12-16 hours before use. Before  
131 analysis, the ABTS radical solution was diluted and equilibrated to an absorbance of 0.70  
132 ( $\pm 0.02$ ) at 734 nm. 2.0 mL of diluted ABTS radical solution were added to 20  $\mu$ L of sample  
133 then the absorbance was measured after 1 to 6 minutes after the initial mixing, using ethanol as  
134 a blank (Re et al., 1999). The results were expressed as mmol trolox equivalent (TE) /L, from  
135 a calibration curve (0-2000  $\mu$ mol/L;  $R^2=0.9974$ ).

### 136 ***2.6 Total polyphenols by Folin-Ciocalteu method***

137 The determination of total polyphenols was performed by introducing the following  
138 into a test tube: 0.2 mL of the sample, previously diluted; 6 mL of distilled water; 0.5 mL of  
139 Folin-Ciocalteu reagent. The mixture was vortexed, and after 1 min, 1.5 mL of aqueous  
140 sodium carbonate (20%) were added, the mixture was vortexed again and allowed to stay in  
141 the dark at room temperature for 120 min. The absorbance was measured at 750 nm through a  
142 path length of 1 cm against a blank prepared with distilled water in place of the  
143 sample (Singleton & Rossi, 1965). The results of total polyphenols were calculated from a  
144 calibration curve of gallic acid (0-500 mg/L,  $R^2=0.9988$ ), and expressed in mg equivalents of  
145 gallic acid (mg GAE).

### 146 ***2.7 Total flavonoids by Folin-Ciocalteu***

147 The total flavonoid content was determined using formaldehyde precipitation in strong  
148 acidic medium, following the method described by Spranger et al. (2008). 2.5 mL of the extract  
149 were placed in a brown-coloured vial. Afterwards, 1.25 mL of HCl diluted with distilled water  
150 (50:50 by volume) and 1.25 mL of formaldehyde were added in the same vial. The mixture was  
151 left to stand for 24 hours at  $t=4^\circ\text{C}$ . After 24 hours, the mixture was filtered and the non flavonoid  
152 polyphenol content was determined by the method described in section 2.6 (Filimon et al.,  
153 2017). The total flavonoid content was calculated by the difference between the total content

154 of polyphenols previously determined and the polyphenol content remained after the flavonoids  
155 precipitation with formaldehyde.

### 156 **2.8 Total polyphenols by Abs 280**

157 The total polyphenolic content was also determined by measuring the absorbance at 280  
158 nm and expressed as mg equivalent of gallic acid (mg GAE) by construction of a calibration  
159 curve(0-50 mg/L,  $R^2=0.9958$ )(Patras et al., 2017), following the method described by Ribereau-  
160 Gayon et al., 2006.

### 161 **2.9 The content of anthocyanins by difference of pH**

162 The contents of total and monomeric anthocyanins were determined by reading the  
163 absorbance at 520 nm and 700 nm, 20 minutes after the addition of 4 mL of pH=1.0 (sodium  
164 acetate) and respectively, pH=4.5 (sodium citrate) buffer solutions to 1 mL of appropriate  
165 diluted sample(Giusti& Wrolstad, 2001 with modifications). The results were calculated with  
166 the following formulae and expressed as malvidin-3-glucoside equivalents (mg ME)/L extract:

167 Total anthocyanins, mg ME/L =  $(A_T \times MW \times d \times 1000)/(\epsilon \times l)$

168 Monomeric anthocyanins, mg ME/L =  $(A_M \times MW \times d \times 1000)/(\epsilon \times l)$

169  $A_T = (Abs_{520} - Abs_{700})_{pH\ 1.0}$

170  $A_M = (Abs_{520} - Abs_{700})_{pH\ 1.0} - (Abs_{520} - Abs_{700})_{pH\ 4.5}$

171 MW – molecular weight of malvidin-3-glucoside (493.4 g/mol)

172 d - dilution factor

173  $\epsilon$  - molar absorptivity of malvidin-3-glucoside ( $\epsilon = 37700$ )

174 l – pathlength (1 cm)

### 175 **2.10 Total cinnamic acids derivatives**

176 The content of total cinnamic acids derivatives was determined by acidifying 0.25 mL  
177 of extract with 0.25 mL acidified ethanol (0.1% in 95% ethanol) and 4.55 mL HCl (2%). After  
178 20 min, the absorbance was read at 320 nm and the results were expressed as caffeic acid  
179 equivalents (CAE) based on a calibration curve (0-50 mg/L,  $R^2=0.9994$ ) with standard of  
180 caffeic acid(Sant'Anna et al., 2012; Demir et al., 2014).



181 **2.11 Total flavonols**

182 The content of total flavonols was determined by acidifying 0.25 mL of extract with  
183 0.25 mL acidified ethanol (0.1% in 95% ethanol) and 4.55 mL HCl (2%). After 20 min, the  
184 absorbance was read at 360 nm and the results were expressed as quercetin equivalents (QE)  
185 based on a calibration curve (0-50 mg/L,  $R^2=0.9967$ ) with standard of quercetin(Sant'Anna et  
186 al., 2012; Demir et al., 2014).

187 **2.12 Colour parameters (CIEL\*a\*b\*)**

188 The CIEL\*a\*b\* parameters were determined using the Analytic Jena Specord 200 Plus  
189 (Germany) spectrophotometer, as mentioned previously. The calculations were made using the  
190 software WinASPECT PLUS provided by the same company. The transmittance of all the  
191 samples was measured between 380 nm and 780 nm, every nm, in optical glass cuvette with  
192 the path length of 1 mm using distilled water as reference. The illuminant was D65 and the  
193 observer at 10°. The results present three colorimetric coordinates, i.e. luminosity (L\*),  
194 red/green component (a\*), blue/yellow component (b\*) and two derived magnitudes, i.e.  
195 chromaticity (C\*), hue (H\*). The overall colour difference ( $\Delta E^*$ ) between the control and each  
196 extract with modified medium, by either addition of salt or pH change, was calculated, using  
197 the formula  $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ , where  $\Delta L^*$  - difference of luminosity  
198 between the control and the sample with modified medium,  $\Delta a^*$ - difference of red/green  
199 components between the control and the sample with modified medium,  $\Delta b^*$  - difference of  
200 blue/yellow component between the control and the sample with modified medium(OIV, 2013).

201 **2.13 HPLC analysis of polyphenols**

202 The polyphenol composition was analysed using the Agilent 1100 Series HPLC. The  
203 gradient was optimised using trifluoroacetic acid (TFA) as an eluent acidification of 1%  
204 CH<sub>3</sub>OH (A channel) and 50% CH<sub>3</sub>OH (B channel) acidified to 2.15 pH with TFA. The column  
205 system was composed of a pre-column SecurityGuard ULTRA Cartridges HPLC C18 for 4.6  
206 mm ID coupled to Kinetex 5  $\mu$ m C18 100 Å 250×4.6 mm columns manufactured by  
207 Phenomenex at 35°C. The injection volume was 20  $\mu$ L and the run time 90 min. The phases  
208 were A: H<sub>2</sub>O: CH<sub>3</sub>OH (99:1) and B: H<sub>2</sub>O: CH<sub>3</sub>OH (50:50), with a flow of 1.5 mL/min. The  
209 detection was carried out at 256 nm, 280 nm, 324 nm, and 365 nm. The gradient of elution was  
210 100% (A): for 10 min; 82% (A): 18% (B) for the next 10 min; 70% (A): 30% (B) for 10 min;  
211 65% (A): 35% (B) for 6 min; 40% (A): 60% (B) for 15 min; 20% (A): 80% (B) for 5 min; 100%  
212 (B) for 15 min. and 100% (A) for 10 min. The content of specific polyphenols was determined  
213 by comparison of retention times and peaks of the red grape marc chromatogram with the ones

214 from the chromatogram of a synthetic mixture containing the following standards: (+)-catechin,  
215 morin hydrate, ellagic acid, benzoic acid, quercetin, caffeic acid, (+)-rutintri-hydrate, syringic  
216 acid, ferulic acid, gallic acid, protocatechuic acid, gentisic acid, parahydroxybenzoic acid,  
217 salicylic acid, para-coumaric acid, D (-)-quinic acid, sinapic acid, methyl 4-hydroxy-3-  
218 methoxycinnamate, procyanidin B1, procyanidin B2, polydatin, hyperoside, and trans-  
219 resveratrol.

#### 220 ***2.14 Statistical analysis***

221 The mean values and the standard deviations were calculated from 3 parallel  
222 experiments using three extraction procedures for polyphenol composition and antioxidant  
223 activity analysis, and the same extract to study the influence of different treatments. One-way  
224 ANOVA and post-hoc Tukey test were used to distinguish between means and evaluate the  
225 results. The considered significance level was  $p \leq 0.05$ . All calculations were made using IBM  
226 SPSS Statistics 23.

### 227 **3. Results and Discussion**

#### 228 ***3.1 The phenolic content and the antioxidant activity of the original grape marc*** 229 ***extract***

230 The phenolic composition and the antioxidant activity of the initial extract were  
231 analysed and the results are presented in Table 1. These data demonstrate that the used red grape  
232 marc is an important source of antioxidants and could be of interest to food processors and  
233 consumers. In addition, the composition of extracts can be used to explain the changes in colour  
234 and antioxidant activity observed after pH change and the addition of salts.

#### 235 **Table 1. Composition of polyphenols and antioxidant activity of the grape marc extract** 236 **used for experiments (the results are presented as means $\pm$ standard deviations of three** 237 **experiments)**

238 The total concentrations of polyphenols measured by the two methods had comparable  
239 values, although the content of polyphenols obtained by Folin-Ciocalteu method was higher.  
240 It is documented that there are many interfering substances when the total polyphenol content  
241 is determined by the Folin-Ciocalteu method. Any substance with reducing properties such as  
242 reducing sugars, ascorbic acid, some proteins interact with the Folin-Ciocalteu reagent (Box,  
243 1983). In this way, this reagent determines not only the content of polyphenols, but the reducing

244 potential of a solution (Singleton & Rossi, 1965) and therefore it is considered suitable for the  
245 determination of the total antioxidant activity.

246 Other authors have obtained similar results, even though the content of different  
247 phenolics is greatly affected by several factors such as, grape variety, extraction method, type  
248 and volume of solvent and others. Negro et al. (2003) have obtained the values 4.19 g/100 g for  
249 polyphenols, 3.94 g/100 g for flavonoids and 0.98 g/100 g for anthocyanins, all the results being  
250 expressed as g/100 g dry marc. Their results for polyphenols and flavonoids are similar to those  
251 obtained in the present research, if the values are compared in the same units.

252 Sant'Anna et al. (2012) obtained the maximum total polyphenol extraction from wine  
253 marc at the solid-to-liquid ratio of 1 g dried marc to 50 mL of 50% ethanol. Moreover, the yields  
254 of extraction ranged from 11 to 22 mg GAE/g(Sant'Anna et al., 2012).

255 With regards to specific phenolics, gallic acid, procyanidin B1, catechin, epicatechin,  
256 ferulic acid methyl ester, hyperoside, polydatin, ferulic, chlorogenic and salicylic acids were  
257 the main compounds found in the grape marc extract. Tournmour et al., (2015) analysed grape  
258 pomace from Portuguese cultivars. The obtained values for antioxidant activity (ORAC) and  
259 total polyphenols were comprised between 906 and 2337  $\mu\text{molTE/g}$  and respectively,  $142.4 \pm$   
260  $1.1 \text{ mg GAE/g}$  of dry pomace, which are higher than the ones obtained in this study. The results  
261 of HPLC analysis revealed the presence of gallic acid, caffeic acid, (+) catechin, syringic acid,  
262 and (-)catechin, the latter two being the major identified compounds (Tournmour et al., 2015).  
263 Different results may be explained by the fact that the marc was obtained from different grape  
264 varieties and has resulted from different winemaking techniques(Apolinar-Valiente et al.,  
265 2015). Ramirez-Lopez and DeWitt (2014) have analysed commercial dried grape pomace by  
266 high-performance liquid chromatography electrospray ionization mass spectrometry and have  
267 determined a total of 16 phenolic compounds among which epicatechingallate, catechin  
268 hydrate, quercetin, caffeic, ferulic, gallic and protocatechuic acids, i.e. components or  
269 derivatives also identified in the present study.

### 270 *3.2 The effect of pH on the antioxidant activity and colour*

271 Figure 1 presents the change of the antioxidant activity after pH modification of the  
272 red grape marc extract.

273 **Figure 1. The dependence of the antioxidant activity on the grape marc extract's pH**  
274 **(errors bars present the standard deviation of three determinations, different letters**  
275 **designate statistically different results)**

276 Different values of pH had little influence on the antioxidant activity of the ethanolic  
277 grape marc extract. The highest value was determined for the pH=3.7 although not significantly  
278 different from the control which had the initial pH=4.4. The analysis between pairs of treatment  
279 revealed a statistically significant difference between the values found at pH=3.7 and pH=2.6;  
280 pH=3.7 and pH=5.5.

281 Altukaya et al. (2016) studied the influence of pH on the antioxidant activities of lettuce  
282 extract with quercetin, green tea extract, and grape seed extract. The authors found enhanced  
283 scavenging effect with increasing pH values and have explained this effect by the increase of  
284 the electron-donating ability upon deprotonation and stabilization in alkaline solutions  
285 (Altukaya et al., 2016). Saeedeh et al. (2007) have evaluated the antioxidant activity of  
286 drumstick leaves, mint leaves and carrot tuber extracts as well as its stability at different pH  
287 values, i.e. 4 and 9. The antioxidant activity of mint and carrot extracts was found to be higher  
288 at pH 9 than at pH 4, while the one of drumstick extract remained the same under both pH  
289 conditions (Saeedeh et al., 2007).

290 The antioxidant activity has been correlated with the number of hydroxyl groups and  
291 their hydrogen donating abilities (Lemanska et al., 2001; Jabbari & Gharib, 2012; Chen et al.,  
292 2014). Additional OH groups in ortho position increase the scavenging activity of polyphenols,  
293 especially at pH values superior to 4 (Altukaya et al., 2016). Thus, the structure of each phenolic  
294 compound must be taken into account when explaining the change of the antioxidant activity  
295 of the extract.

296 **Table 2. CIEL\*a\*b\* parameters' dependence on the pH of the grape marc extracts**  
297 **(results are expressed as means±standard deviation, different letters designate**  
298 **significantly different results between pairs of test and control for each value of pH)**

299 The highest decrease in luminosity was observed for the pH values of 2.6, 3.7, and 8.8.  
300 Furthermore, the redness, the blueness, the chroma and the hue angle were significantly  
301 enhanced at acidic pHs namely 2.6 and 3.7, values found in products such as lemon juice,  
302 vinegar, various syrups, and fruit juice, ketchup, fermented dairy products, pickled vegetables,  
303 various preserves, respectively. These changes are explained by the fact that the flavylum  
304 cation is stabilised by the excess of H<sup>+</sup>. On the other hand, the red/green (a\*) and blue/yellow  
305 (b\*) parameters were shifted towards green and yellow values, respectively, at pH≥7.4, because  
306 of the degradation of the red and blue pigments. A significant decrease of chroma which  
307 translates as colour quality was observed for pH=5.5 and pH=7.4. The results obtained for

308 pH $\geq$ 5.5 suggest that grape marc extract should be used with great care in foods with such media.  
309 Ready-to-eat meals, bread, nectars, soups and sauces, cheese, and tea are among the products  
310 with a pH $>$ 5.5. All these modifications caused significant changes in the overall colour,  
311 although of different nature, in both acidic and alkaline media. In strong acidic medium, the  
312 colour has been enhanced, while in alkaline medium it changed from red-blue to green-yellow.  
313 According to Gonnet (2001) and Martinez et al. (2011), these colour changes will be perceived  
314 by the human eye.

### 315 ***3.3 The influence of the ionic strength on the antioxidant activity and CIEL\*a\*b\**** 316 ***parameters***

317 The antioxidant activity was not affected significantly by the presence of added salts.  
318 Only the higher concentration of CaCl<sub>2</sub> decreased its value from 29.59 mmolTE/L to 17.30  
319 mmol TE/L, modification which was found to be statistically significant (fig. 2). The decrease  
320 of the antioxidant activity (fig. 2) caused by the addition of 0.01 M of calcium chloride was  
321 also close to significance threshold. Subsequent verification using Scheffé and Holm-Bonferoni  
322 multiple comparisons showed that the value is significantly different when the treatments are  
323 only compared to the control. Therefore, CaCl<sub>2</sub> could have a negative impact on the antioxidant  
324 activity of the grape marc ethanolic extract when concentrations equal or higher than 0.01 M  
325 are added.

326 **Figure 2. Change of the antioxidant activity for different salts and different**  
327 **concentrations added to the grape marc extract (error bars present the standard**  
328 **deviations of three replicates, different letters designate statistically different results)**

329 Several studies show that flavonoids also act as metal chelators, thus the interaction  
330 between metal ions and flavonoids may change their antioxidant activity (Jabbari & Gharib,  
331 2012). Jabbari & Gharib (2012) have determined that metal chelation with cerium (IV)  
332 enhances the radical scavenging activity of flavonoids. These results were not confirmed for  
333 the ions studied in the present experiment.

334 The most significant effect on all colour parameters was exerted by calcium chloride  
335 (table 3). Moreover, it was observed that the higher the concentration of the added salt, the  
336 higher the effect on the colour of the extract. The addition of this salt caused mainly a decrease  
337 of luminosity and an increase of redness, which resulted in enhanced chromaticity and  
338 important overall colour differences. Considering the fact that certain salts could modify the  
339 pH of a solution, the latter was measured after the addition of calcium chloride (table 3). The

340 results show a gradual decrease of pH, with a difference of 1.2 between the control and the  
341 extract containing 0.1 M CaCl<sub>2</sub>. Nonetheless, the same concentration of salt reduced the pH of  
342 distilled water from 7.8±0.2 to 7.5±0.2 when 0.1 M of calcium chloride was added, while the  
343 other two tested concentrations, i.e. 0.001 M and 0.01 had an insignificant effect on water pH.  
344 This difference in pH could be attributed to the formation of partially dissociated complexes  
345 between calcium and anions of carboxylic and weak acids (Joseph, 1946). Consequently, the  
346 enhancement in colour would be attributed to the stabilization of the flavylum ion in acidic  
347 medium, however the overall colour difference in this case is greater than in the case of pH  
348 modification using buffer solutions (table 2). The enhancement of colour through the addition  
349 of metals and divalent ions was documented by other authors(Cortez et al., 2017). This  
350 phenomenon could also be explained by the processes of polymerization and complexation  
351 between anthocyanins and metal ions(Negro et al., 2003). The significant increase of colour  
352 quality and intensity is interesting and could be used in the creation of new food dyes of natural  
353 origin. Furthermore, it would be interesting to investigate if such colourant could act as a  
354 calcium delivery system, if used in calcium-enriched dairy products such as yoghurts. Thus,  
355 detailed kinetic and nutritional studies are recommended.

356 **Table 3. Change of CIEL\*a\*b\* parameters for different salts and different concentrations**  
357 **added to the grape marc extract (standard deviations are based on three replicates,**  
358 **different letters designate significantly different results)**

359 Ngo & Zhao (2009) have studied the stabilization of anthocyanins on thermally  
360 processed red d'Anjou pears through complexation with Sn in the presence of hydrochloric  
361 acid, formaldehyde, and tannic acid. The treatment resulted in red pigments and although their  
362 nature is unknown, all four reagents were required to provide the stabilization. Polymerization  
363 was considered by the authors as the main responsible reaction. Furthermore, the authors  
364 observed both bathochromic and hyperchromic shifts when Sn was added alone (Ngo & Zhao,  
365 2009). Other metals that have been studied are tin, copper, aluminium, magnesium, and  
366 potassium in the quest to obtain a natural blue food dye from anthocyanins(Yoshida et al., 2009;  
367 Cortez et al., 2017). Only, a slight influence on the blueness of the extract was observed in this  
368 study.

369 The parameter  $(A-A_0)/A_0$  was also calculated for the extracts treated with different salts  
370 (fig. 3). A drastic hyperchromic shift was observed when CaCl<sub>2</sub> was added to the extract, the  
371 intensity of which increased with the greater concentration of the salt. Given  $\lambda=520$  nm, the

372 anthocyanins are the main molecules involved. The analysis of the results has shown that the  
373 addition of calcium salts in a concentration of 0.01 M can significantly improve the colour of  
374 the extract without affecting its radical-scavenging ability.

375 **Figure 3. Change of  $(A-A_0)/A_0$  entity in the grape marc extracts with different salts**  
376 **added in different concentrations (error bars present the standard deviations of three**  
377 **replicates, different letters designate statistically different results)**

378 The capacity of anthocyanins to form metal complexes is related to the ortho-dihydroxyl  
379 arrangement on the B ring. While the glucosides of cyanidin, delphinidin and petunidin can  
380 form such complexes, the ones of malvidin, pelargonidin and peonidin cannot, therefore it is  
381 unlikely that pigment-metal complexes play a significant role in the colour of grape marc  
382 extracts, some authors suggest (Boulton, 2001). Molecular stacking is necessary and special  
383 conditions are required to achieve stable colour formation. Previous studies indicate that the  
384 chirality of stacking is influenced by the glucosyl residues. Moreover, glycosyl residues are  
385 indispensable in metal-anthocyanin complex formation (Cheynier et al., 2012).

386 A research on the composition and structure of the anthocyanins present in the extract,  
387 as well as a study on the interactions between the respective anthocyanins, the weak acids  
388 present in the extract and calcium ions are recommended.

#### 389 **4. Conclusions**

390 This study has brought further proof that grape marc extracts contain high amounts of  
391 polyphenols from different classes, which results in a fairly high antioxidant activity. The main  
392 identified phenolics were gallic acid, polydatin, procyanidin B1, ferulic acid methyl ester,  
393 catechin, epicatechin, hyperoside, ferulic, chlorogenic, and salicylic acids.

394 The results of this research have shown that the presence of several ions does not  
395 significantly affect the antioxidant activity. The most noticeable effect was exhibited by high  
396 concentration of  $\text{CaCl}_2$ , which decreased antioxidant activity from 29.59 mmol TE/L to 17.30  
397 mmol TE/L. Furthermore, calcium salts have also shown the most significant effect on all  
398 colour parameters, by visibly enhancing the colour of grape marc extract. This phenomenon  
399 was explained by the complexation between anthocyanins and metal ions or the decrease of pH.  
400 The increase of colour intensity is potentially interesting for the food industry since it could be  
401 exploited for the formulation of new food dyes.

402 Different pH values had little influence on the antioxidant activity of the ethanolic grape  
403 marc extract. The highest value was determined for the pH=3.7, although, not significantly  
404 different from the control sample. The redness, blueness, the chroma and the hue angle were  
405 also significantly enhanced at this value of pH, as well as at pH=2.6. The colour of the extract  
406 was however affected in a negative way by pH values higher than 5.5, which is why the extract  
407 should be used with care in products with such media.

#### 408 **Conflict of interest**

409 The authors have declared no conflict of interest.

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#### 414 **ORCID**

415 *Antoanela Patras* <http://orcid.org/0000-0002-4054-4884>

#### 416 **References**

417 Altukaya, A., Gokmen, V., & Skibsted, L. H. (2016). pH dependent antioxidant activity of  
418 lettuce (*L. sativa*) and synergism with added phenolic antioxidants . *Food Chemistry*, *190* ,  
419 25-32.

420 Apolinar-Valiente, R., Romero-Cascales, I., Gomez-Plaza, E., Lopez-Roca, J. M., & Ros-  
421 Garcia, J. M. (2015). Cell wall compounds of red grapes skins and their grape marcs from  
422 three different winemaking techniques. *Food Chemistry*, *187* , 89-97.

423 Astley, S. B. (2003). Dietary antioxidants - past, present and future? *Trends in Food Science*  
424 *& Technology*, *14* , 93-98.

425 Bakowska-Barcak, A. (2005). Acylated anthocyanins as stable, natural food colorants - A  
426 review . *Polish Journal of Food and Nutrition Sciences* , *14/55* (2), 107-116.

427 Boulton, R. (2001). The Copigmentation of Anthocyanins and Its Role in the Color of Red  
428 Wine: A Critical Review. *Am. J. Enol. Vitic.*, *52:2* , 67-87.

429 Campos, F. (2009, July). Studies on the interaction between phenolic compounds and lactic  
430 acid bacteria from wine. *PhD thesis* . Porto, Portugal: Escola Superior de Biotechnologia -  
431 Universidade Catolica Portuguesa.



432 Chen, C., Xue, H., & Mu, S. (2014). pH dependence of reactive sites of curcumin possessing  
433 antioxidant activity and free radical scavenging ability studied using the electrochemical and  
434 ESR techniques: Polyaniline used as a source of the free radical . *Journal of Electroanalytical*  
435 *Chemistry*, 713 , 11-27.

436 Cortez, R., Luna-Vital, D. A., Margulis, D., & Gonzalez de Mejia, E. (2017). Natural  
437 Pigments; Stabilization Methods of Anthocyanins for Food Applications . *Coprehensive*  
438 *Reviews in Food Science and Food Safety*, 16 (1), 180-198.

439 Cristea, E., Sturza, R., & Patraş, A. (2015). The influence of temperature and time on the  
440 stability of the antioxidant activity and colour parameters of grape marc ethanolic extract. *The*  
441 *Annals of the University Dunarea de Jos of Galati, Fascicle VI – Food Technology* , 39 (2),  
442 96-104.

443 Demir, N., Yioldiz, O., Alpaslan, M., & Hayaloglu, A. A. (2014). Evaluation of volatiles,  
444 phenolic compounds and antioxidant activities of rose hip (*Rosa L.*) fruits in Turkey. *LWT -*  
445 *Food Science and Technology*, 57 , 126-133.

446 Dwyer, K., Hosseinian, F., & Rod, M. (2014). The Market Potential of Grape Waste  
447 Alternatives. *Journal of Food Research*, 3:2 , 91-106.

448 Filimon, R.V., Filimon, R.M., Nechita, A., Băetu, M.M., Rotaru, L., Arion, C., Patraş, A.  
449 (2017). Assessment of quality characteristics of new *Vitisvinifera L.* cultivars for temperate  
450 climate vineyards. *ActaAgricScand B*, 67(5), 405-415.

451 Giusti, M. M., & Wrolstad, R. E. (2001). Characterisation and measurement of anthocyanins  
452 by UV-visible spectroscopy. *Current protocols in food analytical chemistry* , F1.2.1-F1.2.13.

453 Global Food Forums . (2017). *Clean Label Trends*. Retrieved April 22, 2017, from  
454 <https://www.globalfoodforums.com/>: [https://www.globalfoodforums.com/food-news-](https://www.globalfoodforums.com/food-news-bites/clean-label-trends/)  
455 [bites/clean-label-trends/](https://www.globalfoodforums.com/food-news-bites/clean-label-trends/)

456 Gonnet, J. F. (2001). Colour effect of co-pigmentation of anthocyanin revisited-3. A further  
457 description using CIELab differences and assessment of matched colours using the CMC  
458 model. *Food Chemistry*, 75 , 473-485.

459 Gonzalez-Manzano, S., Duenas, M., Rivas-Gonzalo, J. C., Escribano-Bailon, T., & Santos-  
460 Buelga, C. (2009). Studies on the copigmentation between anthocyanins and flavan-3-ols and  
461 their influence in the colour expression of red wine. *Food Chemistry*, 114 , 138-146.

462 Jabbari, M., & Gharib, F. (2012). Solvent dependence on antioxidant activity of some water-  
463 insoluble flavonoids and their cerium (IV) complexes. *Journal of Molecular Liquids*, 168 ,  
464 36-41.

465 Joseph, N.R. (1946). The dissociation constants of organic calcium complexes. *J Biol Chem.*  
466 *164*, 529-541.

467 Lemanska, K., Szymusiak, H., Tyrakowska, B., Zielinski, R., Soffers, A. E., & Rietjens, I. M.  
468 (2001). The influence of pH on antioxydant properties and the mechanism of antioxidant  
469 action of hydroxyflavones. *Free Radical Biology & Medicine*, 31 , 869-881.

470 Malaj, N., De Simone, B. C., Quartarolo, A. D., & Russo, N. (2013). Spectrophotometric  
471 study of malvidin-3-O-glucoside with p-coumaric, vanillic and syringic acids . *Food*  
472 *Chemistry*, 141 , 3614-3620.

473 Martinez, J. A., Melgosa, M., Perez, M. M., Hita, E., & Neguerela, A. I. (2011). Note. Visual  
474 and Instrumental Color Evaluation in Red Wines. *Food Science and Technology*  
475 *International*, 439-444.

476 Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity  
477 from red grape marc extracts. *Bioresource Technology*, 87 , 41-44.

478 Ngo, T., & Zhao, Y. (2009). Stabilization of anthocyanins on thermally processed red d'Anjou  
479 pears through complexation and polymerization . *LWT - Food Science and Technology*, 42 ,  
480 1144-1152.

481 OIV. (2013). Determination of chromatic characteristics according to CIE. CIELab. In OIV,  
482 *International Methods of Wine and Must Analysis, Volume 1*.

483 Oliveira, D. A., Salvador, A. A., Smania Jr, A., Smania, E. F., Maraschin, M., & Ferreira, S.  
484 R. (2013). Antimicrobial activity and composition profile of grape (*Vitis Vinifera*) pomace  
485 extracts obtained by supercritical fluids. *Journal of Biotechnology*, 164 , 423-432.

486 Patras, A., Stoleru, V., Filimon, R.V., Padureanu, S., Chelariu, E.L., Biliaderis, C. (2017).Influence of  
487 sodium and maturity stage on the antioxidant properties of cauliflower and broccoli sprouts. *Not Bot*  
488 *HortiAgrobo.*, 45(2), 458-465.

489 Ramirez-Lopez, L. M., & DeWitt, C. A. (2014). Analysis of phenolic compounds in  
490 commercial dried grape pomace by high-performance liquid chromatography electrospray  
491 ionization mass spectrometry. *Food Science and Nutrition*, 2(5) , 470-477.

492 Ratnasooriya, C., & Rupasinghe, V. (2012). Extraction of phenolic compounds from grapes  
493 and their pomace using beta-cyclodextrin. *Food Chemistry*, 134(2) , 625-631.

494 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999).  
495 Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free*  
496 *Radical Biology & Medicine*, 26 , 1231-1237.

497 Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of*  
498 *Enology - Volume 2, The Chemistry of Wine Stabilization and Treatments*. Chichester: John  
499 Wiley and Sons, Ltd.

500 Saeedeh, A. D., Vishlakshi Devi, D., & Urooj, A. (2007). Evaluation of antioxidant activity of  
501 some plant extracts and their heat, pH and storage stability . *Food Chemistry*, 100 , 1100-  
502 1105.

- 503 Sant'Anna, V., Brandelli, A., Marczak Damasceno Ferreira, L., & Tessaro, I. C. (2012).  
504 Kinetic modeling of total polyphenol extraction from grape marc and characterization of  
505 extracts. *Separation and Purification Technology*, 100 , 82-87.
- 506 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with  
507 phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16 , 144-158.
- 508 Yoshida, K., Mori, M., & Kondo, T. (2009). Blue flower color development by anthocyanins:  
509 from chemical structure to cell physiology. *Natural Product Report*, 26 (7), 884-915.
- 510

511 **Table 1. Composition of polyphenols and antioxidant activity of the grape marc extract used**  
 512 **for experiments (the results are presented as means±standard deviations of three**  
 513 **experiments)**

<b>Polyphenols and antioxidant activity</b>	<b>Value (per 1 L of extract and 100 g grape marc)</b>
Total polyphenols (Folin-Ciocalteu)	3749±128 mg GAE
Total polyphenols (Abs280)	2791±70 mg GAE
Total flavonoids	3699±70 mg GAE
Total anthocyanins	138±2 mg ME
Monomeric anthocyanins	116±2 mg ME
Cinnamic acids derivatives	446±21 mg CAE
Flavonols	358±15 mg QE
<b>Individual polyphenols</b>	
Gallic acid	7.40±0.60 mg
Protocatechuic acid	0.48±1.60 mg
p-hydroxybenzoic acid	Traces
Procyanidin B1	3.90±0.30 mg
m-hydroxybenzoic acid	0.14±0.01 mg
Catechin	29.10±13.20 mg
Vanillic acid	1.20±0.80 mg
Procyanidin B2	0.30±0.10 mg
Epicatechin	5.10±0.00 mg
Ferulic acid	4.00±2.00 mg
Sinapic acid	0.48±0.16 mg
Trans-resveratrol	Traces
Hyperoside	4.50±0.00 mg
Cis-resveratrol	0.12±0.00 mg
Ferulic acid methyl ester	24.40±8.50 mg
Quercetin	0.30±0.20 mg
Caffeic acid	Traces
Chlorogenic acid	2.80±0.00 mg
Polydatine	7.20±0.00 mg
Salicylic acid	70.00±0.00 mg
<b>Antioxidant activity</b>	<b>29.59±0.00 mmolTE</b>

514

515

516 **Table 2. CIEL\*a\*b\* parameters' dependence of pH (results are expressed as means±standard**  
 517 **deviation, different letters designate significantly different results between pairs of test and**  
 518 **control for each value of pH)**

<b>CIEL*a*b*</b>	<b>L*</b>	<b>a*</b>	<b>b*</b>	<b>C*</b>	<b>H*</b>	<b>ΔE*</b>
<b>parameters</b>						
<b>Control 2.6</b>	86.9±0.8 <sup>a</sup>	11.5±0.4 <sup>a</sup>	-2.7±0.2 <sup>a</sup>	11.8±0.3 <sup>a</sup>	-4.12±0.51 <sup>a</sup>	
<b>pH=2.6</b>	72.1±2.2 <sup>b</sup>	48.1±3.1 <sup>b</sup>	-5.3±0.1 <sup>b</sup>	48.4±3.1 <sup>b</sup>	-9.07±0.50 <sup>b</sup>	39.56±3.04
<b>Control 3.7</b>	83.5±0.1 <sup>a</sup>	14.9±0.1 <sup>a</sup>	-4.7±0.1 <sup>a</sup>	15.6±0.1 <sup>a</sup>	-3.05±0.01 <sup>a</sup>	
<b>pH=3.7</b>	81.4±4.5 <sup>a</sup>	22.2±3.2 <sup>b</sup>	-3.6±0.7 <sup>b</sup>	22.4±3.2 <sup>b</sup>	-6.19±0.42 <sup>b</sup>	8.25±5.42
<b>Control 5.5</b>	74.4±0.8 <sup>a</sup>	22.4±0.6 <sup>a</sup>	-5.5±0.3 <sup>a</sup>	23.0±0.6 <sup>a</sup>	-4.00±0.09 <sup>a</sup>	
<b>pH=5.5</b>	79.6±0.1 <sup>b</sup>	11.7±0.1 <sup>b</sup>	-1.0±0.1 <sup>b</sup>	11.7±0.1 <sup>b</sup>	-11.80±1.60 <sup>b</sup>	12.72±0.88
<b>Control 7.4</b>	74.4±0.8 <sup>a</sup>	22.4±0.6 <sup>a</sup>	-5.5±0.3 <sup>a</sup>	23.0±0.6 <sup>a</sup>	-4.00±0.09 <sup>a</sup>	
<b>pH=7.4</b>	77.2±0.1 <sup>a</sup>	-0.7±0.2 <sup>b</sup>	9.3±0.3 <sup>b</sup>	9.3±0.3 <sup>b</sup>	-0.46±1.02 <sup>b</sup>	27.58±0.81
<b>Control 8.8</b>	83.5±0.1 <sup>a</sup>	14.9±0.1 <sup>a</sup>	-4.7±0.1 <sup>a</sup>	15.6±0.1 <sup>a</sup>	-3.05±0.01 <sup>a</sup>	
<b>pH=8.8</b>	81.3±0.2 <sup>a</sup>	-3.2±0.1 <sup>b</sup>	14.7±0.5 <sup>b</sup>	15.1±0.4 <sup>a</sup>	-0.16±0.26 <sup>b</sup>	26.62±0.41

519

520

521 **Table 3. Change of CIEL\*a\*b\* parameters for different salts and different concentrations**  
 522 **(standard deviations are based on three replicates, different letters designate significantly**  
 523 **different results)**

Salt and concentration	L*	a*	b*	H*	C*	ΔE*
Control	65.60±0.12 <sup>cd</sup>	30.00±0.19 <sup>ab</sup>	-7.14±0.09 <sup>cd</sup>	-4.12±0.08 <sup>b</sup>	30.84±0.16 <sup>ab</sup>	-
NaCl 0.001 M	65.76±0.14 <sup>cd</sup>	31.61±0.33 <sup>ab</sup>	-7.66±0.18 <sup>bcd</sup>	-4.05±0.85 <sup>b</sup>	32.52±0.35 <sup>ab</sup>	1.70±0.17
NaCl 0.01 M	65.79±0.52 <sup>cd</sup>	31.95±0.37 <sup>ab</sup>	-7.81±0.21 <sup>b</sup>	-4.01±0.07 <sup>b</sup>	32.89±0.40 <sup>ab</sup>	2.07±0.45
NaCl 0.1 M	65.88±0.10 <sup>c</sup>	35.99±0.28 <sup>b</sup>	-9.18±0.10 <sup>ab</sup>	-3.83±0.01 <sup>b</sup>	37.14±0.30 <sup>b</sup>	6.33±0.09
KNO <sub>3</sub> 0.001 M	68.96±0.68 <sup>e</sup>	27.89±0.81 <sup>a</sup>	-5.75±0.50 <sup>a</sup>	-4.80±0.31 <sup>ab</sup>	28.48±0.88 <sup>a</sup>	4.20±0.93
KNO <sub>3</sub> 0.01 M	68.52±0.15 <sup>de</sup>	29.54±0.52 <sup>ab</sup>	-6.29±0.22 <sup>cd</sup>	-4.62±0.09 <sup>ab</sup>	30.21±0.55 <sup>a</sup>	3.08±0.36
KNO <sub>3</sub> 0.1 M	67.27±0.20 <sup>de</sup>	32.44±0.47 <sup>b</sup>	-7.23±0.10 <sup>cd</sup>	-4.41±0.01 <sup>ab</sup>	33.24±0.48 <sup>ab</sup>	2.96±0.29
CaCl <sub>2</sub> 0.001 M*	59.82±2.68 <sup>b</sup>	45.55±6.70 <sup>c</sup>	-9.80±0.88 <sup>a</sup>	-4.56±0.29 <sup>ab</sup>	46.60±6.74 <sup>c</sup>	16.80±7.03
CaCl <sub>2</sub> 0.01 M**	51.59±0.72 <sup>a</sup>	64.65±1.15 <sup>c</sup>	-9.40±0.42 <sup>a</sup>	-6.84±0.42 <sup>a</sup>	65.33±1.08 <sup>d</sup>	37.44±1.79
CaCl <sub>2</sub> 0.1 M***	47.46±0.94 <sup>a</sup>	69.00±0.19 <sup>c</sup>	-6.25±1.07 <sup>d</sup>	-11.24±2.10 <sup>a</sup>	69.29±0.16 <sup>d</sup>	43.02±1.28

524 \*pH=4.1±0.1 after the addition of the salt; \*\*pH=3.7±0.1 after the addition of salt;

525 \*\*\*pH=3.2±0.1 after the addition of salt

526

527

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