Biogenic amines in raisins of one vintage year: Influence of two chemical pretreatments (dipping in oak ash solution or potassium carbonate solution)

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Some biogenic amines (BAs) may be used as quality markers in grape derived products and they have key roles in human nutrition. In this study, therefore, two traditional dipping solutions, namely oak ash and potassium carbonate solutions applied before drying, were compared for their effects on the BA profile of Gök Üzüm raisins. The mean agmatine, putrescine, spermine, and serotonin content of Gök Üzüm raisins was lower in samples dried by dipping in potassium carbonate solution than samples dried by dipping in oak ash solution. There were no significant differences in contents of cadaverine, spermidine, histamine, tryptamine, dopamine, and norepinephrine between samples of both dipping solutions. Among the BAs under investigation, putrescine, agmatine, histamine, and tryptamine were the most abundant amines, whereas the lowest amine contents were those of cadaverine, spermine, spermidine, and norepinephrine. The mean putrescine, agmatine, spermine, and serotonin content of Gök Üzüm raisins was higher in samples dried by dipping in oak ash solution than in samples dried by dipping in potassium carbonate solution. Drying under shade by dipping in oak ash solution proved to be superior to dipping in potassium carbonate solution concerning Gök Üzüm raisin BAs and could be considered as an attractive pre-drying solution for the production of both raisins and perhaps other dried fruits.
Keywords: Gök Üzüm raisin, oak ash, potassium carbonate, biogenic amines, drying

Biogene Amine in Rosinen eines Jahrgangs: Einfluss zweier chemischer Vorbehandlungen (Eintauchen in Lösungen aus Eichenasche oder Kaliumcarbonat). Einige biogene Amine (BAs) können als Qualitätsmarker in aus Trauben gewonnenen Produkten verwendet werden und spielen eine Schlüsselrolle in der menschlichen Ernährung. In dieser Studie wurden daher zwei traditionelle Tauchlösungen, Eichenasche- und Kaliumcarbonatlösung, die vor dem Trocknen von Trauben angewandt wurden, auf ihre Auswirkungen auf das BA-Profil von Gök Üzüm-Rosinen verglichen. Der durchschnittliche Agmatin-, Putrescin-, Spermin- und Serotoningehalt von Gök Üzüm-Rosinen war in Proben, die durch Eintauchen in Kaliumcarbonatlösung getrocknet wurden, niedriger als in Proben, die durch Tauchen in Eichenaschelösung getrocknet wurden. Es gab keine signifikanten Unterschiede im Gehalt an Cadaverin, Spermidin, Histamin, Tryptamin, Dopamin und Noradrenalin zwischen den Proben beider Tauchlösungen. Unter den untersuchten BAs waren Putrescin, Agmatin, Histamin und Tryptamin die am häufigsten vorkommenden Amine, während Cadaverin, Spermin, Spermidin und Noradrenalin die niedrigsten Gehalte aufwiesen. Der durchschnittliche Putrescin-, Agmatin-, Spermin- und Serotoningehalt von Gök Üzüm-Rosinen war in durch Tauchen in Eichenaschelösung getrockneten Proben höher als in Proben, die durch Tauchen in Kaliumcarbonatlösung getrocknet wurden. Die Trocknung im Schatten nach Eintauchen in Eichenaschelösung erwies sich bei Gök Üzüm-Rosinen der Anwendung von Kaliumcarbonatlösung überlegen und könnte als attraktive Vortrocknungslösung für die Herstellung von Rosinen und möglicherweise anderer Trockenfrüchte angesehen werden.
Schlagwörter: Gök Üzüm-Rosine, Eichenasche, Kaliumcarbonat, biogene Amine, Trocknung

Since raisins contain high levels of sugar, protein, vitamins, bioactive compounds, and various organic acids, they are often consumed for nutritional and medicinal purposes (Khiari et al., 2018). In the last decade, many studies have been published on the phytochemical properties and health benefits of raisin (Williamson and Carughi, 2010; Restani et al., 2016). The phytochemical properties of raisins have been found to be affected by many factors, including thermal and drying processes as well as environmental and agronomic conditions (Patras et al., 2010). In the drying process, the biochemical content of raisins has been reported to be affected by the several chemical pretreatments applied and drying techniques (Kamiloglu et al., 2016). Traditionally, raisins are dried by employing solar power, with the raw material being laid on structures such as on trays on the ground or racks. Other alternative drying methods used to obtain raisins from fresh grapes include shade drying and mechanical drying. Since no technical or mechanical means are used to start or accelerate the process, both direct sun and shade drying are natural drying methods (Vasilopoulou and Trichopoulou, 2014). Especially it should be emphasized, that raisin producers around the world are looking for alternative, however equally natural drying methods for higher quality end products. In this regard, an old but promising Gök Üzüm drying method has been revived in Turkey, i.e. drying under shade and cluster dipping mixtures (the samples are dipped in a solution of oak ash or potassium carbonate (70 to 90 °C; 5 to 10 sec) are used to accelerate the drying of raisins. Gök Üzüm is grown in the Yagcilar District of Hadim District of Konya, Turkey. This place is located in the Aladağ Region of the Taurus Mountains. In Gök Üzüm, raisins are produced through shade drying in attics, which have walls constructed which allow a warm breeze to flow freely through the attics. Compared to other methods or sundrying, those produced with this technology have a sweeter flavor and retain their original green color. Under these conditions, the drying process is completed in approximately 3 to 4 weeks. Drying process and light exposure are the parameters that differentiate the above-mentioned Gök Üzüm drying method; however, these parameters could be potential factors affecting the biogenic amines (BAs) content in the final product. Theoretically, shade drying could be expected to result in better quality products concerning their biogenic amines, however to the best of our knowledge, there has yet to be done a relevant study that scientifically proves such behavior in raisins.

Biogenic amines (BAs), which are low molecular weight nitrogenous compounds, are formed by amination or transamination of aldehydes and ketones or by the decarboxylation of free amino acids (Santos, 1996). The most known decarboxylase enzymes are tyrosine, histidine, lysindecarboxylases, glutamic acid, ornithine, and phenylalanine. BAs are endogenous and indispensable important components of living cells (ten Brink et al., 1990) and hereby, most foods, including vegetables and grains, display a variable concentration of these compounds depending on cultivation conditions, ripening degree, and cultivar (Mah et al., 2019). They are also found in various foods, especially in fermented food and other products, e.g. beer, sauerkraut, wine, cheese, meat, and fish (Henríquez-Aedo et al., 2012; Papageorgiou et al., 2018; Pradenas et al., 2016; Lee et al., 2016), and have heterocyclic, aliphatic and aromatic properties (Kim et al., 2019). BAs are sortable by their molecular structure: aliphatic polyamines (agmatine, spermine, and spermidine), heterocyclic monoamines (serotonin, tryptamine, and histamine), aromatic (phenylethylamine, adrenaline, tyramine, dopamine, and noradrenaline,) and aliphatic diamines (cadaverine and putrescine) (Eperjesi, 2010). They are naturally synthesized in several different ways, and there are some amines (tyramine, agmatine, and cadaverine) that can be synthesized only in one way, while others are produced in more than one way or after several sequential reactions (putrescine, histamine, spermine, and spermidine) (Nagy et al., 2018). On the other hand, the consumption of high concentrations of BAs, which are bioactive compounds with physiological activities, may produce various problems for human health such as kidney failure, hypo- or hypertension, anaphylactic shock, headache, and even death (Ancin-Azpilicueta et al., 2008; Anli and Bayram, 2009). Since the activities of diamine oxidase and monoamine oxidase, which are the enzymes responsible for BA metabolism, are reduced or inhibited by ethanol, the risk of such adverse effects increases during the consumption of alcoholic beverages (Anli and Bayram, 2009). In particular, high wine consumption worldwide has caused BAs to become a major concern for consumers. Various efforts are, in this context, being made to reduce BAs prevalence in fermented food products, and these, together with consumer demands for high-quality products, have forced producers to guarantee both, food safety and quality. Therefore, controlling the BA content in wine plays a key role in avoiding food safety and economic problems. However, the BA content is well documented for wine in general and interesting results have been reported in literature by many authors (Khiari et al., 2018; Emer et al., 2020; Yue et al., 2021), however we noticed the lack of a synthesis review regarding raisins and the effect of BA contents on quality characteristics.

To our knowledge, there are no previous reports of the dynamics of the changes that occur in both biogenic amines profiles of Gök Üzüm raisin during the shade drying process to produce raisin. The main objective of this article was to compare the two traditional dipping solutions applied before the drying process, namely oak ash solution and potassium carbonate solution, for their effects on the biogenic amines profile of Gök Üzüm raisin. Additionally, the biogenic amines of Gök Üzüm have been reported here for the first time and we have tried to provide reliable nutritional information about this valuable raisin.

Materials and methods

Plant materials

In this study, Gök Üzüm grapes were harvested (20 to 21 °Brix) from their main production regions in Hadim, Turkey, in 2020. The study was conducted in a randomized complete block design with four replicates (six vines per rep.). After harvest, clusters were trimmed by removing damaged, small, infected, and immature berries with scissors.

Gök Üzüm grapes were obtained from Konya-Karaman (Karaman and Hadim Bozkir). Following the same procedure consisting of 24 randomly selected vines, approximately 20 kg of fresh grapes were collected at optimum maturity from the bottom, the middle and the top part of the cluster region.

A total of 5 kg (for six vines) of grape samples were taken from each replication (20 kg for a total of 24 vines). The vineyards were cultivated under similar irrigation, fertilization, disease, and pruning management. Vines were 15 to 20 years old with a planting density between 1100 and 1150 vines per hectare and with vine spacing of 3 m within a row and 3 m between rows. All the vines were grafted on Kober 5BB rootstocks and grown with the Goblet training system.

Pre-treatments before drying

To accelerate the drying of the fresh grapes, the clusters were dipped in solutions of oak ash or potassium carbonate at 70 to 90 °C for 5 to 10 sec. The oak ash solution contains 1v oak ash + 1v water and no other chemicals. The potassium carbonate solution contains 5 to 6 % K_2CO_3 + 0.5 to 1 % olive oil and water. After that, the clusters were allowed to dry in the shade in attics, which have walls constructed to allow the warm breeze to flow freely through the attics. In attics, the drying process was completed in approximately 3 to 4 weeks, and then they were dried till the moisture content was reduced to about 15 %. The study was set up in a randomized complete block design with four replicates (three bags of 300 g each were taken per rep.). A total of 900 g (for six vines) of raisin samples were taken from each replication (3600 g for a total of 24 vines). After drying had ended, raisin samples were placed in polyethylene bags with twelve separate portions of 300 g, sealed, and stored at 20 °C until analysis.

Analysis of biogenic amines

Minor modifications of a published derivatization-liquid chromatography method were performed to determine biogenic amines (BA) in raisin samples (Gómez-Alonso et al., 2007). The raisin samples were mixed with 10 µl of 1 g/l L-2-aminoadipic acid (internal standard), 315 µl of diethyl, 75 µl of methanol, 875 µl of 1 mol/l borate buffer (pH 9.0) and ethoxymethylenemalonate. This mixture of samples was sonicated for 30 min and then heated in a water bath at 70 °C for 2 h. Then, the mixture was cooled down to room temperature. The chilled mixture was filtered through 0.22 µm nylon filters and then 20 µl filtered samples were injected into HPLC. A Shimadzu LC-20AT LC system with a Venusil XSB C18 column (4.6 × 250 mm, 5 µm) (Shimadzu, Kyoto, Japan) was utilized to determine the BA derivatives. The mobile phase comprised (A) 25 mM acetate buffer (pH 5.8) containing 0.02 % sodium azide and (B) cetonitrile:methanol (4:1, v/v). The flow rate was set to 0.9 ml/min while maintaining the column temperature at 16 °C during the elution program. The elution was programmed as follows: 0 to 20 min, 90 % B isocratic; 20 to 30.5 min, 90 % B to 83 % B; 30.5 to 33.5 min, 83 % B isocratic; 33.5 to 65 min, 83 % B to 73 % B; 65 to 73 min, 73 % B to 28 % B; 73 to 78 min, 28 % B to 18 % B; 78 to 82 min, 28 % B to 0 % B; 82 to 85 min, 0 % B isocratic; 85 to 90 min, 0 % B to 90 % B; and 90 to 93 min, 90 % B isocratic. A model sample matrix, 5 g/l of tartaric acid in water (pH adjusted to 3.8 using 5 M NaOH) and consisting of 14% v/v ethanol, was mixed with the external BA standards. The standard matrix was analyzed under the same derivatization-liquid chromatography method. The quantitation of raisins was determined using the regression curves generated through the peak ratio of external over internal standards versus the concentrations of the external standards. The standard solution of the dansylated derivatives of putrescine, cadaverine, agmatine, spermine, spermidine, histamine, tryptamine, serotonin, dopamine, norepinephrine (Sigma-Aldrich Chemie, Steinhein, Germany) was used and all standards were diluted to 1 ml using 0.4 perchloric acid to give concentrations in the range 0.5 to 10 mg/ml. Biogenic amines concentration of samples was expressed in mg/kg dry weight.

Statistical Analysis

Descriptive statistics for the studied variables (characteristics) were presented as mean and standard deviation. Kolmogorov-Smirnov normality test was performed to test for normality of the variables. After normality test, Mann-Whitney U test was used to compare two groups due to violation of the normality assumption. Nonlinear principal component analysis was performed to determine the association between the groups and characteristics. Statistical significance level was considered as 5 % and SPSS (ver: 21) statistical program was used for all statistical computations.

Results and discussion

Biogenic Amines

Although significant progress has been made in understanding the BA contents of wine and table grapes over the past few decades (Marques et al., 2008; Emer et al., 2020; Gomes et al., 2021), one question still remains a mystery among researchers: How do BAs change in raisins? Therefore, we determined the putrescine, agmatine, spermine, serotonin, histamine, cadaverine, spermidine, dopamine, tryptamine, and norepinephrine contents in Gök Üzüm grapes. In our study, BA contents of this raisin as affected by pre-treatments applied before drying are presented in Table 1. The mean putrescine, agmatine, spermine, and serotonin content of Gök Üzüm was higher in samples dried by dipping in oak ash solution than in samples dried by dipping in potassium carbonate solution. There was no significant difference between the two dipping solutions in terms of cadaverine, spermidine, histamine, tryptamine, dopamine and norepinephrine.Studies have shown that biogenic amines (spermidine, serotonin, spermine, and dopamine) found in foods are powerful antioxidant compounds with higher antioxidant properties than natural antioxidants (Borges et al., 2019). His also known by researchers that the serotonin found in fruit is defined as an antioxidant and has beneficial effects on human health (as a neurotransmitter) (Islam et al., 2016). In our study, the serotonin profile of berries dried after dipping in oak ash was approximately twice that of grapes treated with potassium carbonate solution.

Among the BAs under investigation, putrescine, agmatine, histamine, and tryptamine were the most abundant amines, whereas the lowest amines among them were cadaverine, spermine, spermidine, and norepinephrine. In all raisin samples analyzed for both dipping solutions, the dominant amine was putrescine, whereas the lowest amines were spermine and norepinephrine. Although the formation of BA in raisins has not been well documented in literature, our results are in line with the findings reported with variable concentrations of BA in different types of wines (Marques et al., 2008; Emer et al., 2020; Yue et al., 2021).

Tab. 1: BA contents of Gök Üzüm grapes dried after dipping in different solutions (mean ± SD)

	Dipping solutions		
Biogenic Amines (mg/kg DW)	**Oak Ash**	**Potassium Carbonate**	**P-value**
Putrescine	27.51 ± 2.09	22.043 ± 2.13	0.034
Cadaverine	2.81 ± 0.30	3.377 ± 0.94	0.384
Agmatine	15.37 ± 1.09	12.712 ± 0.76	0.026
Spermine	3.04 ± 0.09	1.84 ± 0.32	0.003
Spermidine	3.66 ± 0.13	3.266 ± 0.30	0.109
Histamine	12.74 ± 1.22	13.089 ± 1.44	0.763
Tryptamine	9.59 ± 1.89	9.076 ± 1.50	0.730
Serotonin	9.35 ± 0.77	5.88 ± 0.38	0.002
Dopamine	8.35 ± 0.93	7.432 ± 1.54	0.430
Norepinephrine	2.91 ± 0.19	2.538 ± 0.42	0.232

However, the definition of raisin quality should pay attention to the BA contents mainly due to both human health and commercial interests. Regulatory limits for BAs have not yet been established by the OIV (Organization International de la Vigne et du Vin) for both raisins and wines, however Lehtonen (2002) reported that for Austria 10 mg/l, Belgium 5 to 6 mg/l, France 8 mg/l, Germany 2 mg/l, Holland 3.5 mg/l and Switzerland 10 mg/l are recommended as the maximum limit for histamine in wine. A legal limit for histamine in wine neither exists in Austria, nor in the European Union (Lehtonen, 2002). In other foods, on the other hand, there is no legal limit for histamine, however it can reach concentrations of 758 mg/kg in sauces, 400 mg/kg in fermented sausages, and 1850 mg/kg in cheese (European Food Safety Authority, 2011). These levels are much higher than the cytotoxicity threshold detected in the present work for histamine. This finding supports the previous hypothesis that histamine concentrations above 400 mg/kg are possibly dangerous (Taylor, 1985). Other authors have also suggested more recently that histamine concentrations of 500 mg/kg in food may be harmful to human health (Kylián et al., 2009; Shukla et al., 2011). Although our results for histamine are lower than the limit values for other foods, the maximum limit of BAs generally considered safe for consumers is not yet known for raisins. The amines generally assumed to be the most toxic among BAs are histamine and tyramine (European Food Safety Authority, 2011); however, as far as we know, there is no scientific data to confirm this assumption. Despite the increase in putrescine, agmatine, histamine, and tryptamine with dipping solutions in our study, these values do not cause any problems related to fruit quality however are well below the values considered harmful to human health. For these, it has been reported that the level becomes toxic when the dose consumed is higher than 2000, 500 and 301.8 mg/kg of body weight for putrescine, histamine and tryptamine, respectively (Linares et al., 2016; Gomes et al., 2021), and our results are considerably lower than these values.

We opted to compare the obtained results by PCA to create a descriptive model for the grouping of amines as a function of dipping solution applications and to allow better visualization of the profiles of these compounds during drying. The dispersion of BAs according to PC1 and PC2 is shown in Figure 1. PC1 and PC2 accounted for 86.06 % of the total variance. Putrescine, agmatine, histamine, and tryptamine showed the highest levels in response to the application of drying for both dipping solutions, grouping in PC1 and PC2. Additionally, PC1 was the first main component responsible for the largest variance (52.60 %) in the data, and it is apparent that most of the amines showed high levels with the application of the dipping solutions, grouping in PC1. The variables spermin, tryptamine, putrescine, agmatine, and dopamine were the ones that presented the highest positive factor loadings in PC1, whereas the amine spermidine presented a negative loading factor in PC1, presenting a profile different from most of the analyzed amines. Analyzing possible relationships between different biogenic amines revealed a marked positive correlation between histamine, tryptamine, and serotonin, as well as spermine, putrescine, and agmatine (Fig. 2). The relationship between putrescine-tyramine (Herbert, 2005), histamine-tyramine (Soufleros, 1998), or all three amines (Romero, 2002) have been described in different studies for wines. We also could not verify a close linkage between cadaverine, tryptamine, and phenylethylamine (Garcia-Villar, 2007). To date, no research has been found on the relationship between BAs in grapes, therefore it is worth noting that the interpretation of interrelated amines is quite difficult.

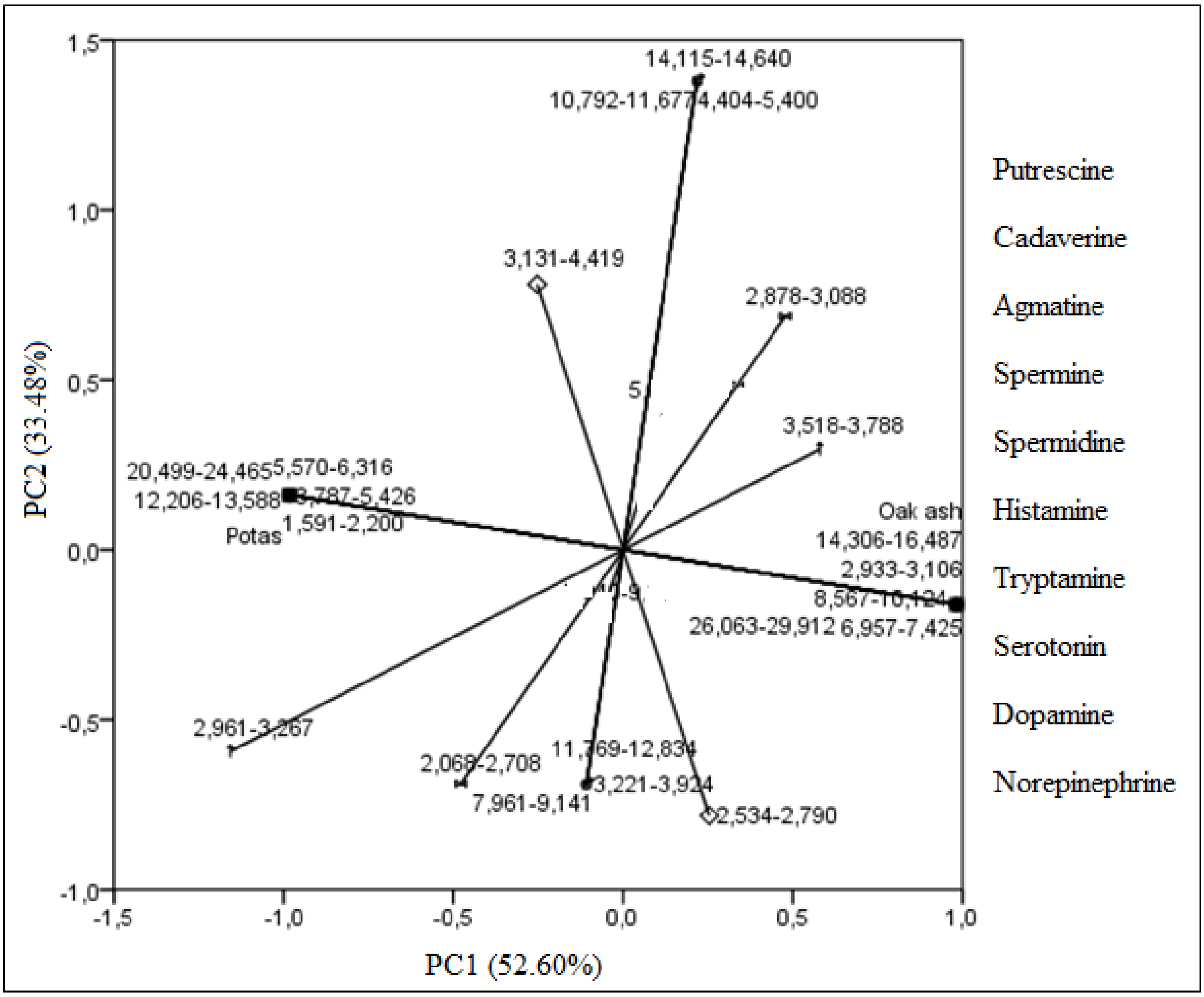

Fig. 1: Two-dimensional projection and values from amines in the first two principal components of Gök Üzüm raisins dried after dipping in different solutions

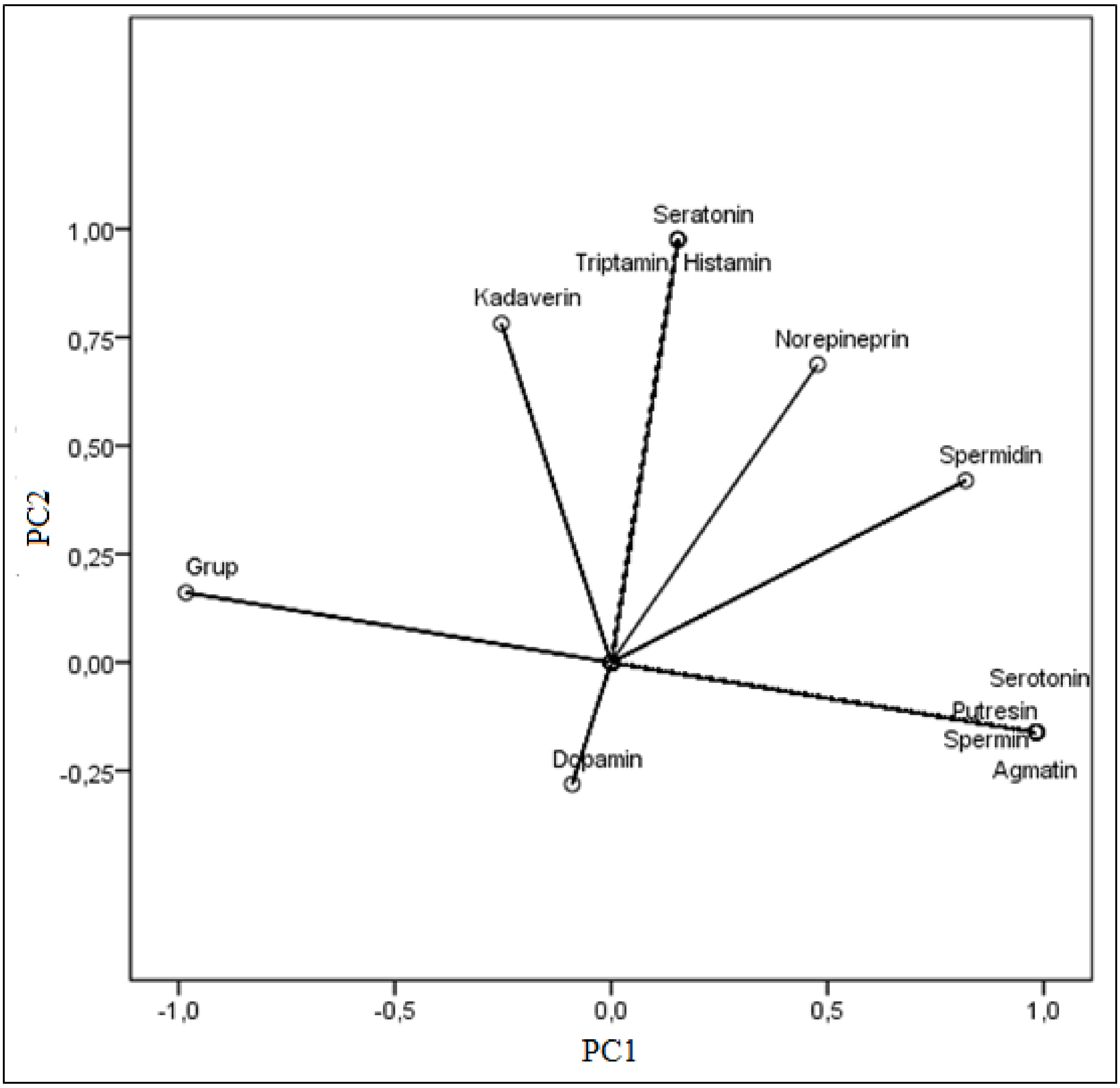

Fig. 2: Relationship between two-dimensional projection and amines in the first two main components of Gök Üzüm raisins dried after dipping in different solutions

Conclusions

The raisins produced from the Gök Üzüm variety showed an interesting potential for human consumption, considering that samples analyzed after drying in shade by dipping in oak ash solution had a high concentration of beneficial BAs. Additionally, these raisins exhibited low levels of undesirable BAs, and are safe for human consumption, even for people with allergic sensitivities. The raisins from samples dried by dipping in oak ash solution showed higher mean agmatine, putrescine, spermine, and serotonin content compared to samples dried by dipping in potassium carbonate solution. To sum up, drying under shade after dipping the grapes in the oak ash solution may be considered an appealing and effective method for the production of 'Gök Üzüm' grapes - and possibly other dried fruits - with enhanced amine contents; natural structure, low cost, and ease of application are other advantages of this dipping solution.

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