Influence of Growing Region on Targeted Anthocyanin Profile, Color, Phenolics, and Antioxidant Activity of Grape Juice

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ABSTRACT:

Aims: To evaluate the influence of geographical origin on Anthocyanin Profile, Color, Phenolics, and Antioxidant Activity of Grape Juice cv. Manjari Medika (MM).

Study design: All the experiments were performed in triplicate. The secondary metabolite study was conducted as a randomized block design. The data were analyzed using SAS 9.3 (SAS Institute, Inc., Cary, NC) and results were expressed as means ± standard deviation (SD).

Place and Duration of Study: This research conducted at research station located at Vijayapura, from farmer plot of Nashik district, and the farm of ICAR-NRCG at Pune during March 2022.

Methodology: This study included Manjari Medika (MM) variety tasted against three different locations to evaluate their performance against targeted Anthocyanin Profile, Color, Phenolics, and Antioxidant Activity of Grape Juice cv. Manjari Medika (MM).

Results: The MM grape berries were harvested from three environmentally different locations in India i.e. Pune (MMJP), Vijayapura (MMJV), and Nashik (MMJN). MMJV, grown in region with high average temperatures and low rainfall showed higher levels of (393.65 ± 8.18 g/L) reducing sugar with lower levels of color intensity (49.69±0.55%), juice recovery (68.70±0.54%), and acidity (6.98±0.04 g/L) followed by flavonoids (0.023 g/L) than the regions with relatively low temperatures and high rainfall ((MMJP) and (MMJN)). In UHPLC-Oribitrap MS analysis, a total of 11 anthocyanins, which includes delphinidin-3-galactoside (Dp3Ga), cyanidin (Cy), peonidin-glucoside-4-Vinylphenol (Pn3Glu4VP), petunidin-3-glucoside (Pt3Glu), malvidin-3-glucoside (Mv3Glu), pelargonidin (Pg), petunidin-3-acetyl glucoside (Pt3ace), peonidin-3-acetyl glucoside (Pn3ace), malvidin-3-acetyl glucoside (Mv3ace), delphinidin (Dp) and petunidin (Pt) were detected in grape juice samples. MMJV. It is interesting to note that among the 11 anthocyanins detected, two compounds, petunidin-3-acetyl glucoside (Pt3ace) and peonidin-3-acetyl glucoside (Pn3ace), have been reported only in specific grape juice samples, i.e., Pt3ace in MMJV and Pn3ace in MMJN. Discriminatory compounds among the regions were petunidin-3-acetyl glucoside (Pt3ace), peonidin-3-acetyl glucoside (Pn3ace), and cyanidin (Cy) according to principal component analysis (PCA). UHPLC-MS results showed that the total anthocyanin content of the fresh juice in the cultivar tended to increase with altitude from sea level.

Conclusion: Grape juices from vineyard grown at different location have different levels of phenolic compounds, levels of anthocyanins etc. In comparison of MMJV (which were grown in regions with high average temperatures and low rainfall) with MMJP and MMJN (which were grown in regions with low average temperatures and high rainfall) had higher levels of reducing sugar (393.65 8.18 g/L) and lower levels of colour intensity (49.69 0.55%), juice recovery (68.70 0.54%), acidity (6.98 0.04 g/L), followed by flavonoids (0.023 g/L).

Keywords: Manjari Medika; Juice recovery; Environmental factor; Physiochemical composition: principal component analysis

1. INTRODUCTION

Among environmental factors such as temperature, water, radiation, and soil significantly impact agriculture, including viticulture and grape production (Suchkov et al., 2022). Climate change, influenced by these factors, affects grapevine growth, wine quality, and yield. (Gurpreet, Kaur and Gurpreet, Singh., 2019). The microclimate of grapevines plays a crucial role in determining wine characteristics, with air temperature and water availability influencing grape composition and volatile compounds in wines (Helena and Costa 2022). Additionally, soil conditions, light interception angles, and radiation distribution affect vine physiological activity and grape ripening (Kobus et al., 2020). Presently grapes are being cultivated in all continents except Antarctica. The grapevines have covered different regions of word from temperate to tropical conditions. The grape is an important cultural, economic, and ecological crop with the largest acreage of fruit crops globally (Ponti, et al., 2018). Grapes are a delicate, highly perishable fresh fruit with numerous reported health advantages (Kumar and Babu, 2021). Consequently, during harvest and distribution, grapes have a high incidence of depletion. The geographic origin, and thus the soil on which grapes are growing, is one of the most determinative factors to produce quality grapes. Although the suitability of grapevine cultivation in a particular region is largely determined by prevailing mean temperature ranged between 12 and 22 °C during the growing season. Other than mean temperature, growing season length, radiation levels, minimum temperature during winter, spring and fall frosts or soil water balance, etc. are also very important factors to grow quality grapes (Jones et al., 2012). The grapevine phenology determined by air temperature during the growing season and yield also affected by temperature (Bock et al. 2013).

At present the global revenue in the Grape Juice market amounts to ₹269.9bn in 2024. The market is expected to grow annually by 4.15% (CAGR 2024-2028). In the Grape Juice market, at-home volume is expected to amount to 1.3bn L by 2028. The Grape Juice market is expected to show a volume growth of - 0.6% in 2025. The average volume per person in the Grape Juice market is expected to amount to 0.17L in 2024 (Statista. (2024)). Phenolic compounds, proanthocyanidins, anthocyanins, and flavonoids are mainly found in grape juice. Anthocyanins are the phenolic compounds responsible for the red color of grape juice. The biosynthesis of these compounds is influenced by several climatic factors. Weather considerably governs the whole grapevine development process throughout its growth cycle, ultimately influencing the yield of biomass and berry characteristics (Makra et al., 2009; Fraga et al., 2015). Temperature is another important factor; its effect on grapevine growth, development, physiology, and berry composition has been studied for decades. The elevated temperature could lead to the over-ripening of grapes also (Sadras and Moran 2012). Biochemical processes for colour, flavour and aroma development in the grape berries are favoured by constant and moderate temperature during ripening stage. The environmental conditions, at berry maturity, as prevailing high temperatures results in decreased berry weight, titratable acidity, anthocyanins and TPI, and increased pH (Costa et al. 2020).

Precipitation is another key climatic factor in viticulture. The amount of annual precipitation and its seasonal distribution are also crucial to the evolution of the plant water status and have an impact on berry quality. Water stress leads to a wide range of effects that are also dependent on the grapevine development stage (Chacón-Vozmediano et al., 2020). All in all, a high level of solar radiation induces the synthesis and accumulation of sugar, phenol, and many aromatic compounds during maturation. The role of soils, the changes associated with soils, and the impacts resulting from these changes on the plant have been the least studied (Paustian et al., 2016). The above-mentioned insights commonly provide closer relationships between berry development and climate. Worldwide, the selection of grape varieties that can be grown in a particular region is based on climatic conditions.

In recent years in India, there has been a rapid expansion of grape growing regions for fresh consumption, juices and wine production. The climatic conditions vary greatly with increasing altitude and distance from the ocean from south to north, which might impact the accumulation of anthocyanins and other biochemical compounds in grapes. Considering the impact of location on grape quality, present investigation was carried out to understand effect of location specific grown Manjari Medika grapes on physical-biochemical properties as well as anthocyanin profiling of juice.

2. MATERIAL AND METHODS

2.1 Experimental plot and conditions:

The bunches of Manjari Medika vines were randomly harvested from the research station located at Vijayapura, from farmer plot of Nashik district, and the farm of ICAR-NRCG at Pune during March 2022. All three locations same number of bunches were maintained and recommended cultural practices were followed to maintain vines healthy and fruitfulness. Collected samples were transported in icebox to laboratory at ICAR-NRCG. To remove adhered soil and other particles, the bunches were washed with water properly. The experiment was conducted in a randomized block design with three replications.

2.2 Sample preparations:

Destemmed berries were collected in the plastic bag and crushed and squeezed manually. Liquids were separated through a muslin cloth. Samples were stored in the refrigerator and used for further analysis.

2.3 Physico-Biochemical Analysis

2.3.1 Color intensity (CI):

CI was determined according to the method proposed by Glories (1984) and was calculated after measuring the absorbance at 420, 520, and 620 nm as follows:

CI = A420 + A520 + A620 × 2.5.

2.3.2 Titratable Acidity (TA):

A standard solution of 0.1N NaOH was prepared. Simultaneously 5 mL of grape juice was transferred to an Erlenmeyer flask, diluted up to 50 ml, graduated with distilled water, and a few drops (nearly 1ml) of phenolphthalein indicator was added. The sample was titrated against the standard NaOH solution to obtain an endpoint at pH 8.2 that gives a consistent pink color for 30 seconds. The following equation was used to determine the TA as tartaric acid g/ L. All the experimental measurements were replicated 3 times.

$$\text{TA as tartaric acid (g/ L)} = \frac{V1\ X\ N\ X\ 75\ X100}{1000\ X\ V2}$$

Where,

VI = Volume in ml of standard NaOH required for titration

N = Normality of the standard NaOH

V2= Volume in one of the grape juice samples taken for the test

2.3.3 Total Soluble Solids (TSS):

TSS is the total sugar content. The value displayed is based on the ratio of the speed of light in a vacuum and the speed of light through the sample. Refractometer was used to measure the TSS of grape. The berries were squeezed, then the grape juice samples were measured using a portable handheld refractometer (Erma Refractometer, Japan) at room temperature. TSS value is described in % Brix. All the experimental measurements were replicated 3 times.

2.3.4Total Reducing Sugars:

The reducing sugar content of the raw materials was assessed by the 3, 5-dinitro salicylic acid (DNS) method, based on Miller (1959), with modifications. 0.2ml of the sample was pipetted and the volume was made up to 1 mL with distilled water (DI H2O). 1 mL of 1% (v/v) DNS solution was added and heated in boiling water for 5 min. The samples were cooled to room temperature and 5 mL of DI H2O was added. The absorbance was measured at 540 nm using a Thermo Fisher Scientific Genesys 10S UV–Visible spectrophotometer. The results were expressed in grams per liter (L) of the sample (g/L) using an external calibration curve of glucose.

2.3.5 Estimation of Protein:

Total protein content was measured by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). 500 mg of the juice was made up to 10 ml with sodium phosphate buffer pH 7.5. The supernatant was separated by centrifugation at 5000 rpm for 5 minutes and used for protein estimation. The assay was carried out by making up the 0.2 ml extracts to 1 mL with DI H2O and 5 mL of solution A containing 50 ml of 2% sodium carbonate (Na2Co3) in 0.1N sodium hydroxide (NaOH) and 1ml of 0.5% copper sulfate (CuSo4) in 1% potassium sodium tartrate was added and mixed well. The reaction mixture was allowed to stand at room temperature for 10mins. Finally, 3 mL of solution B (Folin–Ciocalteu phenol reagent) was added and incubated for 30 min at room temperature in the dark. The colored developed was read at 650 nm. The total protein content of the juice was calculated from a calibration curve prepared with bovine serum albumin (BSA) and expressed as protein g/L of grape juice.

2.3.6 Estimation of phenols and tannins:

The total amount of phenols and tannins in methanol grape seed extracts was determined according to the Folin-Ciocalteu (FC) modified procedure (Al-Jadidi et al., 2015). Briefly, 50 μl of juices were taken and the volume was made up to 3 ml with DI H2O. Further, 0.5 ml of 1:1 FC and 2 ml of 20% Na2Co3 were added. The reaction mixtures were shaken well and incubated for 30 min in the dark. After the incubation, a UV-VIS spectrophotometer at a wavelength of 630 nm and 700 nm was used to measure the absorbance for phenols and tannins, respectively. The number of total phenols and tannins was calculated from the standard curve, and the results were expressed as gallic acid equivalents g/L of grape juice.

2.3.7 Estimation of flavonoids:

The total flavonoid content in the methanol grape seed extract was determined by using the aluminum chloride (AlCl3) colorimetric method with catechin as the reference standard. Briefly, 100 µl of crude grape seed extracts were diluted with DI H_2O up to 2.5 mL. Initially, 150 µl of 5% sodium nitrate solution was added, and then the same volume of 10% $AlCl_3$ was added. The reaction mixture was mixed thoroughly and incubated at room temperature for 6 mins. Finally, 1 mL of 1.0 M NaOH, followed by 1.2 mL of water was added and mixed well. The absorbance of the mixture was read at 510nm. The total amount of flavonoids was determined as catechin equivalents g/L of grape juice.

2.3.8 Anti-oxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH):

The protocol described by Arnous et al. 2001 was used for the determination of free radical scavenging activity. The methanol extract (100 µL) was mixed with 3900 µL of DPPH solution and incubated in dark for 90 min followed by measurement of absorbance at 515 nm. The suppression of absorbance of DPPH radical by sample antioxidants was compared with that of the Trolox standard. The antioxidant activity of DPPH radical was expressed as in percentage.

2.3.9 Chemicals and Reagent:

Sodium hydroxide (NaOH), phenolphthalein indicator, 3, 5-dinitro salicylic acid (DNS), glucose, di-Sodium hydrogen orthophosphate anhydrous (Na_2HPO_4), Sodium Dihydrogen ortho phosphate (NaH_2PO_4), sodium carbonate (Na_2Co_3) copper sulfate ($CuSo_4$) potassium sodium tartrate, Folin–Ciocalteu phenol reagent, bovine serum albumin (BSA), sulfosalicylic acid, ninhydrin, orthophosphoric acid; glacial acetic acid, aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox were purchased from Himedia.

2.4 Ultrahigh Performance Liquid Chromatograph (UHPLC)-Orbitrap Exactive mass spectrometer (MS)

2.4.1 Sample preparation:

Each juice sample was diluted (1:1) with water: methanol and injected into UHPLC - Orbitrap MS for qualitative identification of anthocyanins. Samples were prepared in triplicates and from each replicate, two technical injections were performed.

2.4.2 LC-MS [UHPLC-Orbitrap MS] conditions:

The experiment was performed using Ultimate 3000-series Ultrahigh Performance Liquid Chromatograph (UHPLC) hyphenated to Orbitrap Q-Exactive mass spectrometer (MS) (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separations were performed using an Ascentis Express C18 (100 × 2.1 mm, 2.7µm) column (Supelco). The mobile phase consisted of (A) - water (100%), and (B) - ACN (100%) with 0.1% formic acid in both phases. The gradient program was set as 0–2 min: 2%B, 2–11 min:98%B, 11–16 min:98% B, 16–17min:2% B, and 17–24 min: 2% B with a 0.4 mL/min flow rate. A heated-electro-spray ionization (H-ESI) source was used in positive polarity with the following parameters: sheath gas flow rate: 45; auxiliary gas flow rate: 8; sweep gas flow rate: 1; spray voltage: 3.50 kV; S-lens RF level: 50.0; capillary temperature: 320 ºC; S-lens RF level: 50.0; heater temperature: 300 ºC. The MS analysis was performed in full scan (70000 full widths at half maxima at m/z 200), followed by data-dependent MS/MS (ddMS2) at 17500 resolution (m/z 200) with stepped collision energy, operated at 18, 35, and 70 eV maintaining the automatic gain control (AGC) targeted 1e6.

2.4.3 Data processing:

Compounds were identified using a high-resolution accurate mass (HRAM) database generated in-house. The analytes were identified and confirmed by the HRAM measurement of the precursor and characteristic fragment ions, retention time, greater than 90% isotopic pattern match, and molecular formula. The tolerance limit for mass error was ±5 ppm and the retention time was ±0.1 min. It was above the threshold intensity of 5000. The reference standards (>99% purity, Sigma Aldrich, USA) solutions of Cyanidin-3-glucoside were injected to confirm the retention time, MS spectra, and MS/MS fragments. The LC-MS data files (n= 3) were processed by the Trace finder software (version 3.3, Thermo Fisher Scientific). The automated data processing involved compound identifications by comparison with a database of anthocyanins including their derivatives. The database provided the following specific information about compounds; molecular formula, adduct, monoisotopic molecular mass, and fragment mass. This database was initially provided by Thermo Fisher but was updated using open web-based sources- ChemSpider and Mass Bank- and literature published in authentic journals. The relative quantification of identified anthocyanins was determined using the response of Cyanidin-3-glucoside.

2.5 Statistical Analysis

To examine the differences in physico - biochemical properties and secondary metabolite profile across samples, we performed analysis of variance (ANOVA). The ANOVA analysis involved p-value correction. All the experiments were performed in triplicate. The secondary metabolite study was conducted as a randomized block design. The data were analyzed using SAS 9.3 (SAS Institute, Inc., Cary, NC) and results were expressed as means + standard deviation (SD). In case the results reflected a significant difference in anthocyanin profile across samples, multivariate statistical tools were applied to determine the exact cause of these differences. The pattern recognition tools of multivariate analysis including PCA (principal component analysis), log-transformed and auto-scaled (mean-centered and divided by the SD of each variable) datasets were used in Metaboanalyst 5.0 (a set of online tools for metabolomics data analysis). Correlation analysis was also performed and

3. RESULTS AND DISCUSSION

Climatic parameters

In our study, all the grapes chosen from the three vineyards was established on black cotton soil having average pH 7.5, medium black soil having pH 6.5 and medium black soils having average pH 7.8 at Pune, Nashik and Vijayapura district respectively, and followed similar cultivation practices. We referred to the variable factor of climate conditions to conduct reliable comparisons. The raw climate conditions were obtained from the data supply portal for climate research and services, Pune, and the Bengaluru meteorological center, under the maintenance of the India meteorological department, which belongs to the Ministry of Earth Sciences of the Government of India (GOI). Hence, Figure 1 represents the typical climate characteristics during the grape-growing season in these three regions in the year 2021-2022, including daily maximum and minimum temperatures, the daily average temperature, average humidity, and average rainfall. There was a gradually increasing daily maximum temperature in Vijayapura, which resulted in an increasing average temperature difference, which is negatively correlated with the average relative humidity of the city. The average temperature during the growing season for the Pune, Vijayapura, and Nashik regions was shown in Fig. 1. The vineyard of Nashik had a high average temperature and the same humidity as Pune, while the vineyard of Vijayapura had the same temperature with low humidity. The amount of rain tended to decrease with the increase in altitude and distance from the sea.

Physico-biochemical properties of MM grape berries from different locations:

As expected, due to the combinations of various climatic conditions, significant differences in the physical-biochemical properties were observed among the MM grape berries from different vineyard locations (Table 1). The average values showed that bunch weight ranged from 282 to 303 g, which was statistically insignificant between the different locations. The lowest bunch weight accounted for 282.45±5.48 g, in Pune, while the highest bunch weight was 303.63±9.90 g in Nashik, followed by Vijayapura (292.95±11.35g), the bunch weight, was directly proportionate in terms of yield of percent of juice recovery and color intensity. The average bunch weight reported by the grape research stations at Theni, under the governance of Tamil Nadu Agricultural University (TNAU), was similar to our data. Percent of juice recovery reported by Somkuwar et al., (2024) and Sharma et al., (2017) between 65-70%, supports our finding. The Indian Council of Agricultural Research-National Research Centre for Grapes (ICAR-NRCG) requires that grape juices have a minimum of 18°Brix; however, all the juices presented higher values. Due to the high average daily maximum temperature (31.9°C), the MMJP had higher mean values for acidity and TSS than the MMJV and MMJN, which indicates that the MMJP contains a greater number of solids. During growing season average maximum temperature and GDD were highest in Zhangye due to Merlot, Pinot and Vidal grapes berries from Zhangye location had higher TSS than Jiayuguan and Wuwei. (Yan et al., 2022). The °Brix/total acidity represents the balance between the acid and sweet taste of grape juice. In our studies, the TSS and total acidity values of the juices were similar, which indicates the quality of the grape juices was equal.

Most fruits change their sugar content during maturity. The accumulation of sugar in Red Globe grapes is strongly affected by climatic conditions and alters their taste (Nan et al., 2022). Among the total reducing sugar contents of grape juices from different locations, 36% of the highest amount (393.65 g L-1) was found in MMJV, followed by 33% in MMJP (364.65 g L-1), and the lowest in MMJN (30%). Our results agree with reports that grapes grown in regions with high temperatures and low rainfall contain more sugar than grapes grown in other environments (Yan et al., 2022). Similar to TSS, the maximum amount of total protein was found in MMJP and the minimum in MMJN. MMJP had a high percentage, followed by MMJV and MMJN.

Determinations of total phenols, total tannins, and total flavonoids in seeds:

The end polyphenol compounds of the phenylpropanoids pathway are tannins, also called condensed tannins or proanthocyanidins (Rousserie et al., 2019). This reason might lead to the vast amounts of tannins and anthocyanins being accumulated in the plants, among other secondary metabolites. Our findings also suggested that the total tannins were higher than the phenols and flavonoids in all three samples. The total tannin content in the juices ranged from 0.330 g/L to 0.218 g/L (Figure 2). The highest amount of tannin content was detected in MMJP (41.8%), followed by MMJV (30.5%). The greatest reduction was observed in the MMJN (27.6%). Phenols, or phenolic compounds, include single or multiple aromatic rings containing one or more hydroxyl groups (Wenshi et al., 2023). Phenols, flavonoids, and tannins are considered secondary metabolites with unique sources of pharmaceutical actives, food additives, and flavors, along with other industrial applications that have many beneficial effects on human health (Somkuwar et al. 2024). In our findings, the highest amounts of phenols were observed in MMJP (0.238 g L-1) and MMJV (0.200 g L-1), but MMJN (0.170 g L-1) showed a lesser amount. In our study, flavonoids from grape seed extracts were 8.5–4.3 times lower than the phenols as well, which are the least present among other tested secondary metabolites. This, interestingly, MMJP presented higher amounts of flavonoids, followed by MMJN and MMJV.

Antioxidant activity of MM juices from different locations:

To determine the changes in antioxidant activity in MMJ from different locations, a DPPH assay was used in our research. The highest DPPH antioxidant activity was found to be in MMJP (128.14%). In MMJV and MMJN, the antioxidant activity was reported at 103.62% and 90.77%, respectively, which were 29% and 19% less than MMJP. Statistically significant decreases in the DPPH antioxidant activity value ($p<0.05$) were observed in all the samples. The antioxidant capacity was evaluated using the DPPH method on 8 grape juice samples, comprising table grapes and wine grape juices, ranging from 26.44 ± 1.56 to 32.62 ± 0.66 mg/mL (Rima et al., 2023).

Comparison of grapes anthocyanins from different locations:

In this study, a total of 11 anthocyanins were found in the MM grape juices from all three regions. Table 2 shows that there were obvious differences between the total anthocyanin content in MM from the three areas. The total anthocyanins in MMJV and MMJN were 465 and 381 ppm, which are much higher than the 242-ppm found in the same variety grown in MMJP. The anthocyanins of the MMJ chromatogram information include four primary anthocyanidins such as Cy, Pg, Dp, and Pt, three acetylated anthocyanidins, pyranoanthocyanins, and three anthocyanidins monoglycosides including two mono glucosides and one mono galactoside.

All the detected anthocyanins from MMJ were not found in all regions. Dp3Ga, Cy, Pn3Glu4VP, Pt3Glu, Mv3Glu, Pg, Mv3ace, Dp, and Pt were common anthocyanins found in all of MMJ. Among them, Cy, Dp, and Pt were the three most abundant primary anthocyanidins in MMJ. A few anthocyanins, such as Pn3ace, were found only in MMJV and MMJN, and Pt3ace was found only in MMJV. The content of the monoglycosides of Dp, Pt, and Mv was expressed 13 times higher than the content of the acetylated monoglycosides of Pt, Pn, and Mv. Pt3ace and Pg were the least detected anthocyanins, followed by Cy, Pn3Glu4VP, Pn3ace, Pt, and Dp3Ga, which were detected in moderate amounts. However, Mv3Glu was the anthocyanin detected with the highest amount. Malvidin-3-glucoside (Oinin) is higher in all the other anthocyanins that have been found. Malvidin possesses ortho-positioned hydroxyl groups, which result in their comparatively higher resistance to oxidation. The principal component analysis (PCA) was performed based on different concentrations of anthocyanin in MMJ from different regions. The 3D score scatter plot shown in Figure 4A shows the clear separation of three individual clusters of samples. The first three principal components (PC) accounted for 99.8% of the total variance. PC1, PC2, and PC3 explained 65.5%, 33.6%, and 0.7% of the total variance, respectively. In general, MMJP was laid down on the PC1 axis. MMJV and MMJN were laid down on the left upper side, and MMJP was on the left lower side. For the PCA grouping (Figure 4B), the biplot showed a clear indication that MMJP was positively correlated with PC1, even though it was separated and differentiated from the other groups of samples based on the presence of significant anthocyanins. Among all the MMJs, the MMJV was more strongly correlated with PC2, which is grouped with Pt3ace. However, the MMJN is not correlated with PC2 based on the anthocyanins Pn3Glu4VP, Pn3ace, Mv3ace, and Mv3Glu pooled together, similar to the 3D score plot. Here, we performed a detailed cluster and heat map analysis of anthocyanins in MMJ from various locations so that all possible anthocyanins were profiled (Figure 5). The colors in the figure indicate relative metabolite concentrations, with maroon indicating high concentrations and blue indicating low concentrations. Each MMJ shows either a significantly high or low concentration of anthocyanins. The relative concentration of differential anthocyanins in MMJs shown in the heat map distinguishes the different locations. The anthocyanins such as Mv3ace, Mv3Glu, and Pn3ace are highly detected in MMJV and MMJN, however, those were detected less in MMJP. Interestingly, the highest amounts of Cy and Pg were found only in MMJP, while only traces were found in other samples. As we can see from the dendrogram, the presence of a group of anthocyanins is also based on their geographical locations.

Table 1. . Physico-Biochemical properties of MM grape berries from different locations

Physico - Biochemical properties	Locations		
	Pune	Vijayapura	Nasik
Bunch weight (g)	282.45±5.48[b]	292.95±11.35[ab]	303.63±9.90[a]
Juice recovery (%)	66.13±0.82[c]	68.70±0.54[b]	71.2±0.59[a]
Color Intensity (%)	76.62±2.55[b]	79.69±0.55[c]	85.9±1.93[a]
Acidity (g/L)	8.4±0.21[a]	6.98±0.04[b]	7.07±0.13[b]
Total soluble solids (°Brix)	23.67±0.34[a]	20.20±0.37[b]	19.95±0.64[b]
Total reducing sugar (g/L)	364.65±8.31[b]	393.65 ± 8.18[a]	326.25±1.70[c]
Total protein (g/L)	1.78±0.02[a]	1.43 ± 0.02[b]	1.39±0.04[c]

**Values are represented as mean ± S.D. Alphabetical characters show significant differences in each row (P≤ 0.05).*

Table 2. Anthocyanin compounds identified by UHPLC-Orbitrap MS in the grape juice extracts of Manjari medika.

S.No.	Compounds	Abbreviation	Formula	m/z (Apex)	RT	Concentration (ppm)		
						Pune	Vijayapura	Nashik
1.	Delphinidin-3-galactoside	Dp3Ga	C21H21O12	465.10	1.33	10.13	20.68	5.92
2.	Cyanidin	Cy	C15H11O6	287.05	1.55	7.92	3.49	3.71
3.	Peonidin-glucoside-4-Vinylphenol	Pn3Glu4VP	C30H27O12	579.15	1.93	5.47	4.78	6.27
4.	Petunidin-3-glucoside	Pt3Glu	C22H23O12	479.12	2.98	20.20	38.59	20.15
5.	Malvidin-3-glucoside	Mv3Glu	C23H25O12	493.13	3.91	164.35	338.19	301.53
6.	Pelargonidin	Pg	C15H11O5	271.06	5.39	1.10	0.90	0.97

7.	*Petunidin-3-acetylglucoside*	*Pt3ace*	$C_{24}H_{25}O_{13}$	*521.13*	*6.49*		*0.84*	
8.	*Peonidin-3-acetylglucoside*	*Pn3ace*	$C_{24}H_{25}O_{12}$	*505.13*	*6.84*		*10.11*	*8.77*
9.	*Malvidin-3-acetylglucoside*	*Mv3ace*	$C_{25}H_{27}O_{13}$	*55.14*	*6.85*	*9.31*	*19.50*	*20.64*
10	*Delphinidin*	*Dp*	$C_{15}H_{11}O_{7}$	*303.05*	*7.12*	*15.40*	*20.91*	*9.12*
11	*Petunidin*	*Pt*	$C_{16}H_{13}O_{7}$	*317.07*	*7.61*	*8.60*	*7.78*	*4.37*

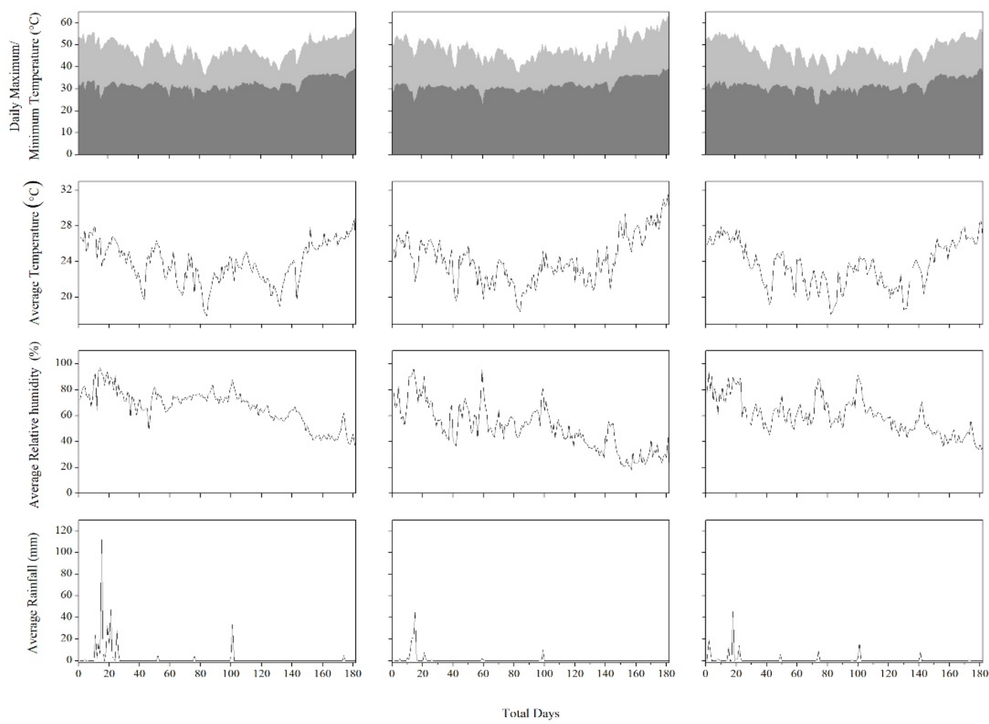

Fig. 1.Climate parameters in the three locations were sampled during grape growing seasons from October, 2021 to March, 2022.

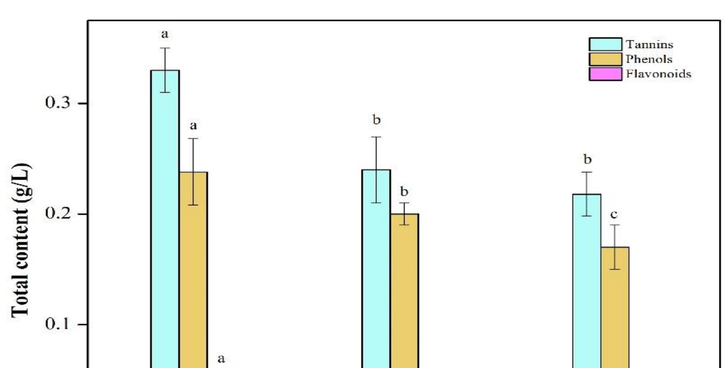

Figure 2. Total tannin (blue), total phenol (yellow), and total flavonoid (magenta) the content in fresh juice of MM grapes. Each value is a mean (± SD) of three replicates. The different letters on the same bar show a significant difference according to Anova's test at $p \leq 0.05$.

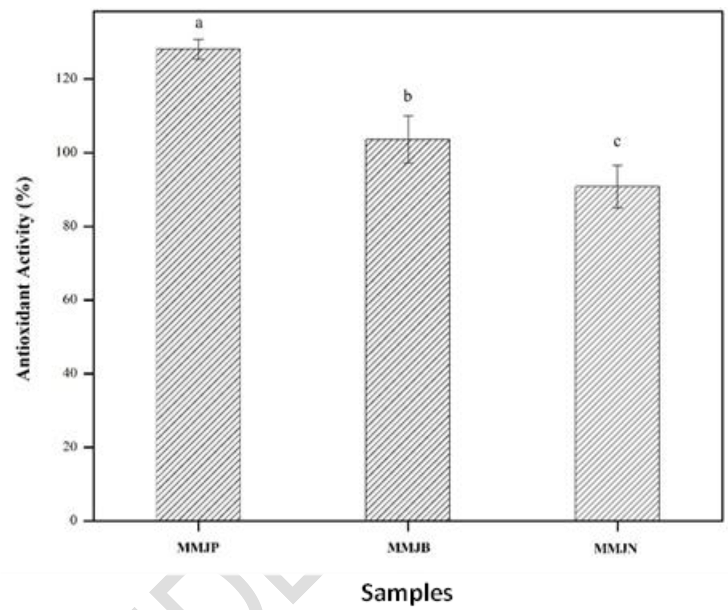

Figure 3 DPPH antioxidant activity of fresh grape juice and antioxidant activity is expressed as in the percentage. Each value is a mean (± SD) of three replicates. The different letters on the same bar show a significant difference according to Anova's test at $p \leq 0.05$.

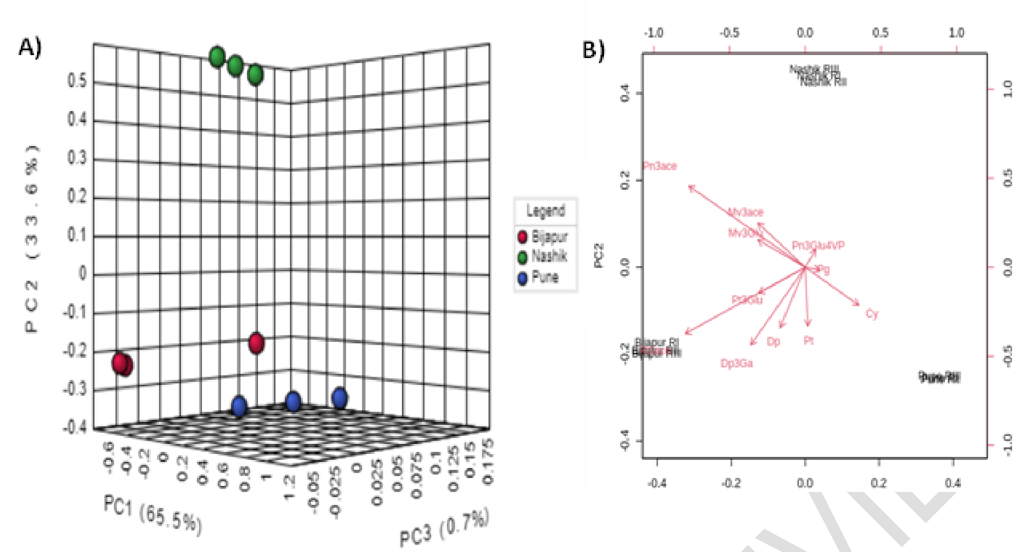

Figure 4. Principal components analysis (PCA) of MMJ from various locations are shown in A) 3D score plot between the first three principal components (PC1, PC2, and PC3). B) Biplot between the selected PCs from the MMJ from various locations between the two principal components (PC1, and PC2). The explained variances are shown in brackets.

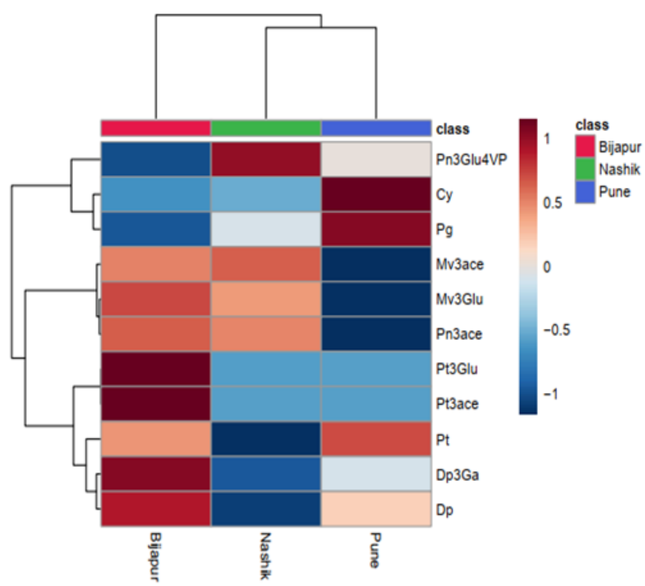

Figure 5. Hierarchical clustering heat map of the anthocyanins of MM juices from various locations.

4. CONCLUSION

'Manjari Medika' grape juice from three different location has adequate physical and chemical quality. It is one of the cultivars that is most frequently used to produce juice since it has the desired sensory characteristics. Grape juices from vineyard grown at different location have different levels of phenolic compounds, levels of anthocyanins etc. In comparison of MMJV (which were grown in regions with high average temperatures and low rainfall) with MMJP and MMJN (which were grown in regions with low average temperatures and high rainfall) had higher levels of reducing sugar (393.65 8.18 g/L) and lower levels of colour intensity (49.69 0.55%), juice recovery (68.70 0.54%), acidity (6.98 0.04 g/L), followed by flavonoids (0.023 g/L). Thus, it is relevant to study the impact of climate factors on the quality parameters of grape juice. It has been found that a number of climatic conditions have an impact on the development of bioactive compounds. Different climatic factor effect on grapevine growth, development, physiology, and berry composition also.

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