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Effect of Growing Location on the Aroma Volatile Profile of 'Kinnow' Mandarin Juice

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Abstract: 'Kinnow' mandarin is a commercially important citrus fruit crop in North India. This study was undertaken to investigate the differences in the characteristic aroma volatile profile of 'Kinnow' mandarin juice as affected by growing locations from different agro-climatic zones. Forty-four volatile compounds were identified and quantified in 'Kinnow' juice using gas chromatography-mass spectrometry (GC-MS). Aroma volatile profiles were found to be significantly affected by the growing location of the fruit. High esters and aldehydes were present at locations Abohar, Lambi, and Amritsar (sub-tropical arid and sub-tropical semi-arid zones) whereas location Una (sub-tropical sub-mountainous zone) had higher levels of alcohols and alkanes. The principal component analysis (PCA) clustered together different growing locations from similar agro-climatic zones based on similar aroma volatiles. The results strongly suggest that the growing location has a significant and a prominent impact on the aroma volatiles of 'Kinnow' juice.

Keywords: Growing location, 'Kinnow', aroma volatiles, GC-MS, PCA, Heat map

I. INTRODUCTION

'Kinnow' mandarin is an interspecific hybrid of king tangor (C. nobilis) and willow leaf mandarin (*C. deliciosa*), which was introduced in India in 1945. 'Kinnow' is the major fruit crop of the Punjab state of India and the Punjab province of Pakistan with an annual production of 1.0 and 2.0 million tonnes, respectively [1,2]. The fruit is highly relished as fresh fruit and in the processed state due to its thin adherent peelable rinds, medium-sized and yellowish-orange colored attractive fruits, high juice content and distinct flavour. The quality of fruit grown across different growing locations in Punjab state is highly variable, thus affecting consumer perception. The major variation occurs in flavour volatiles, which is one of the most important functional phenotypes of the fruits. Flavour, a combination of taste and aroma is perceived as the blend of sweetness, sourness, bitterness, saltiness, acidity and aroma volatiles present in it [3]. The aroma of fruit is the combination of different volatiles and their concentration providing various kinds of floral, fruity, minty, woody, mushroom, etc. aromas [4]. Previous aroma studies in citrus have focused mainly on lemons, oranges, and grapefruits [5,6], though recently the focus has been shifted to mandarins. Mandarins have an aroma profile similar to that of orange as it seems to be a mixture of different volatiles rather than any single compound [7]. Previous studies have focussed on the effect of various factors, including variety and ripening [8], disease [9], and postharvest storage [10] on aroma volatiles in mandarins. However, none of the studies have shown the effect of the growing locations on aroma volatiles, though some studies on fruit quality and specific metabolites in mandarins have been reported [11,12].

Keeping this in mind, the present study was undertaken to understand the chemical basis behind the flavour variation observed in 'Kinnow' grown under different growing locations from different agro-climates zones. Abohar in the North-East from subtropical-arid (STA) and Bhunga in the South-West from subtropical-humid (STH) zones are the epicenters of 'Kinnow' industry in the Punjab state of India. Our goal was to report the effect of growing location on the identity and abundance of aroma volatiles contributing to the flavour quality by employing GC–MS technique.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Authentic standards of all volatile compounds (α-pinene, myrcene, α-phellandrene, 3-carene, p-cymene, limonene, ocimene, γ-terpinene, α-terpinolene, benzothiazole, aromadendrene, α-humulene, allo-aromadendrene, t-caryophyllene, valencene, ledene, linalool, 1,4-cineole, linalool oxide, 6-methyl-5-hepten-2-one, 2,3-pentanedione, cyclohexanone, benzaldehyde, octanal, hexanal, cyclohexane, tridecane, pentadecane, hexadecane, ethyl acetate, ethyl butyrate, methyl benzoate, methyl benzoate, methyl octanaoate, ethyl benzoate, ethyl caprylate, octyl acetate, methyl nonanoate, ethyl trans-2-octanoate, ethyl phenyl acetate, bornyl acetate, citronellyl acetate, ethyl decanoate, geranyl acetate, ethyl dodecanoate), GC-MS grade methanol and internal standard 1-pentanol were obtained from Sigma–Aldrich Co. (Bangalore, India).

B. Fruit material and sampling

The fruit samples were collected from seven commercial orchards falling under agro-climatic zones: Abohar, Lambi, Tahliwala Jattan (North-East) falls under sub-tropical arid (STA), Chhauni Kalan, Bhunga (South-West) under sub-tropical humid (STH), Amritsar (Central) under sub-tropical semi-arid (STSA) and Una (border area) under sub-tropical sub-mountainous (STSM) agro-climatic zone (Table 1). Our focus was on two major 'Kinnow' growing regions of the Punjab state; North-East (Abohar, Lambi, and Tahliwala Jattan) from STA and South-West (Bhunga and Chhuani Kalan) from STH climatic zones. Rough lemon (C. jambhiri) was the common rootstock for all orchards. Commercially mature 'Kinnow' fruit were harvested in the mid-January from North-East region and in the last week of January from other regions when fruit attain soluble solids to acidity ratio higher than 13 [13]. At each location, 6 healthy trees were randomly selected and ten fruit were harvested from different parts of each tree. Sixty fruit from each location were transported in plastic crates to the laboratory at NABI, Mohali within 6–8 hours. Twenty fruit per replicate (three replicates) were used for analysis. After careful peeling, juice was extracted by hand-squeezing and passed through a strainer to remove seeds and fibrous material, stored in polypropylene tubes (50 mL) at –80°C until analysis.

Table 1 Agro-Climatic Conditions Of 'Kinnow' Growing Locations

Sampling location	Agro-climatic zone	Latitude and longitude	Altitude (m)	Temperature Range (°C)	Average Annual Rainfall (mm)	Soil type
Abohar	Sub-tropical arid	30.15°N; 74.19°E	187	0-42	210	Sandy loam
Lambi	Sub-tropical arid	30.06°N; 74.61°E	196	0-42	340	Sandy loam
Tahliwala Jattan	Sub-tropical arid	30.34°N; 74.21°E	190	0-42	210	Sandy loam
Chhauni Kalan	Sub-tropical humid	31.5°N; 75.94°E	301	0-41	600	Sandy loam and loamy sand
Bhunga	Sub-tropical humid	31.68°N; 75.81°E	301	0-41	600	Sandy loam and loamy sand
Amritsar	Sub-tropical semi-arid	31.63°N; 74.87°E	232	0-48	500	Sandy loam to loam
Una	Sub-tropical sub-mountainous	31.47°N; 76.27°E	369	0-40	1240	Sandy loam and loamy sand

Trewartha Climate classification has been used to define agro-climatic conditions of growing locations. Subtropical climates (located between 23.5 and 40° latitude) are characterized by warm to hot summers and cool winters with infrequent frost. The subtropical arid (STA) region has a dry climate where the rate of evaporation is more than the moisture received from precipitation (200-400 mm). Generally, the mean maximum and mean minimum temperatures are 46° C and 6° to 10° C, respectively.

In subtropical humid (STH) region, the climate is hot, usually humid summers and mild to cool winters. In most locations, the mean temperature of the coldest month is between 3 °C and 18 °C. The warmest month normally has a mean temperature of 22°C or higher. Rainfall (625 to 2500 mm) often shows a summer peak and monsoons are well developed. The subtropical semi-arid region has intermediate rainfall between arid and humid regions.

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C. Extraction of volatiles

Headspace solid-phase microextraction (HS-SPME) technique was used for extraction of aroma volatiles. The juice stored at -80 °C were thawed at ~22 °C. The sample vials containing 7 mL of juice and internal standard (7µL) were incubated in a water bath for 60 min at 65 °C. A 2–cm fused–silica SPME fibre coated with 100 µm polydimethylsiloxane was inserted into the headspace of the vial and exposed for 30 min using a manual SPME device (Supelco). The fibre was thermally desorbed in the injection port (splitless mode) for 10 min at 240 °C.

D. GC-MS/MS analysis

The separation of volatile compounds was achieved using an Agilent 7890A gas chromatograph (Agilent Technologies India Pvt. Ltd., New Delhi, India) instrument equipped with an SPB-5 MS capillary column (250 x 60 m x1.0 µm; Supelco), coupled with a triple quadrupole mass spectrometer (7000B QQQ, Agilent Technologies) operated in scan mode. The column oven was programmed to increase at 5 °C min^{-1} from the initial 40 to 100 °C, followed by 4 °C min^{-1} from 100 to 230 °C and then finally ramped at 5 to 250 °C, held for 15 min, with a total run time of 65 min. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. Transfer line, ion source, and quadrupole temperatures were set at 280, 230 and 150 °C, respectively. The mass spectrometer was used in scan mode between *m/z* 50 and 500 using electronic impact ionization at 70 eV.

E. Identification and semi-quantitation of volatile compounds

The Kovats method using n-alkanes (C7-C28) as external references were used for calculation of retention indices (Kovats indices) of sample compounds (Table 2) and were compared with those of authentic standards and literature [14]. To identify the peaks in the mass spectra, we compared our mass spectra with library entries (NIST/EPA/NIH Mass Spectral Library, version 2.0d; National Institute of Standards and Technology, Gaithersburg, MA, USA) as well as with mass spectra of authentic reference standards. Authentic standards were subjected to HS-SPME-GC-MS analysis under similar conditions as described for fruit samples; and spectra, retention time and retention index confirmed compound identities in samples. Semiquantification of compounds identified in this study was done by calculating relative percentage peak area of each compound. Samples were run in triplicate with a blank run between each location to ensure fibre cleanliness between samples.

Table 2 Kovats Retention Index (Ki) And Transition State For Each Volatile Compound Identified

S.No	Compounds	KI	Transition
1.	Alpha-Pinene	954	93.0
2.	Myrcene	985	95.1
3.	Alpha-Phellandrene	1017	93.0
4.	3-Carene	1024	93.0
5.	p-Cymene	1036	119.0
6.	Limonene	1045	68.1
7.	Ocimene	1049	93.0
8.	Gamma-Terpinene	1130	93.0
9.	Alpha-Terpinolene	1129	93.0
10.	Benzothiazole	1259	135.0
11.	Aromadendrene	1465	91.0
12.	Alpha-Humulene	1467	93.0
13.	Alloaromadendrene	1498	161.0
14.	trans-Caryophyllene	1465	93.0
15.	Valencene	1490	96.1
16.	Ledene	1508	107.0
17.	Linalool	1100	71.1
18.	1,4 Cineole	1039	93.0
19.	Linalool oxide	1099	59.1
20.	6-Methyl-5-Hepten-2-one	985	108.0
21.	2, 3-Pentanedione	700	43.1
22.	Cyclohexanone	901	98.1
23.	Octanal	1002	84.1
24.	Hexanal	784	56.1

25.	Cyclohexane	677	98.1
26.	Tridecane	1298	71.1
27.	Pentadecane	1590	57.1
28.	Hexadecane	1600	57.1
29.	Ethyl acetate	628	43.1
30.	Ethyl butyrate	796	71.1
31.	Methyl benzoate	1104	105.0
32.	Methyl octanoate	1326	74.1
33.	Ethyl benzoate	1183	105.0
34.	Ethyl caprylate	1191	88.1
35.	Octyl acetate	1149	43.1
36.	Methyl nonanoate	1221	74.1
37.	Ethyl trans-2-octanoate	1240	55.0
38.	Ethyl phenyl acetate	1252	91
39.	Bornyl acetate	1285	93
40.	Citronellyl acetate	1357	81
41.	Ethyl decanoate	1398	88.1
42.	Geranyl acetate	1382	93
43.	Ethyl dodecanoate	1579	74.1

F. Statistical analyses

Data were subjected to one-way analysis of variance (ANOVA) using SAS software (SAS Research & Development (India) Pvt. Ltd., Pune, India). The data acquired were normalized against an internal standard, $\log_e$ transformed and centered before being subjected to principal component analysis (PCA) and heat map representation. PCA and heat map were constructed using MarkerView software (version 1.2.1, SCIEX) and Multi Experiment Viewer (MeV) (version 4.8.1; Dana-Farber Cancer Institute, Boston, MA) softwares, respectively.

III. RESULTS AND DISCUSSION

A. Detection of volatiles

Forty-four volatile compounds were detected and identified in 'Kinnow' juice from seven different locations (Fig 1). Compounds were classified into four classes, viz., monoterpenes (10 compounds), sesquiterpenes (6 compounds), aldehydes (3 compounds), ketones (3 compounds), alcohols (3 compounds) and esters (4 compounds). Monoterpenes represented the major fraction of volatile compounds (83–94%), with limonene (orange like fruity odour), contributing to >80% of the total volatiles in 'Kinnow' from all the 7 locations. Other important monoterpenes included myrcene, terpinolene, and β-phellandrene. Valencene, aromadendrene, α-humulene, and ledene constituted the major sesquiterpenes; methyl nonanoate and methyl benzoate as major esters; octanal as major aldehydes and l, 4-cineole (Isocineole) as the major alcohol. Similar volatiles was identified in other citrus varieties like king mandarin, tangors, and tangelos [8, 10]. Miyazaki et al., 2011 [15] also reported higher levels of sesquiterpenes and esters than other mandarins in mandarin hybrids with a sweet-orange background (tangors).

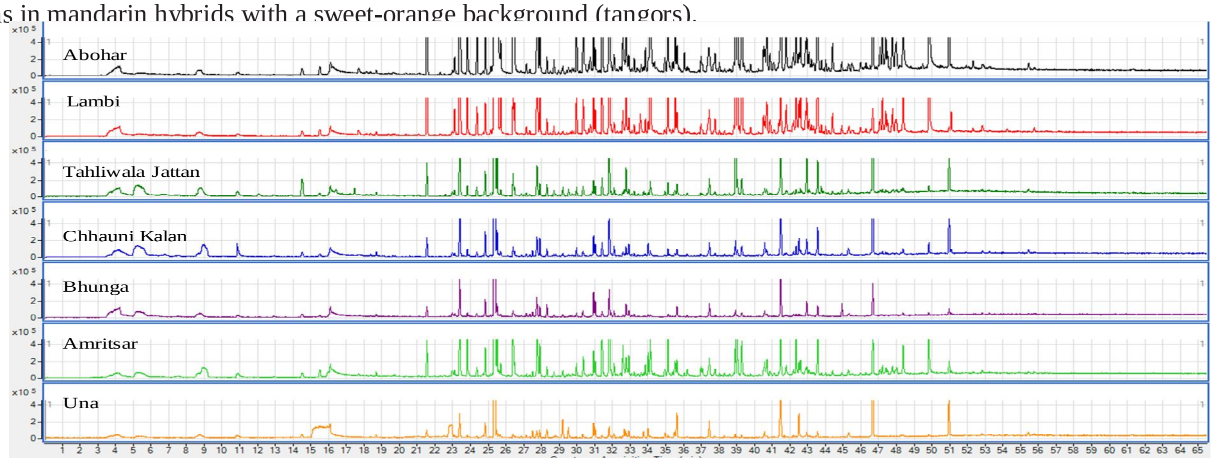

Fig 1. Chromatogram showing variation in aroma profile from seven different locations

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B. Variation in volatiles due to climate of different locations

Total ion chromatograms (TIC) of volatile compounds in 'Kinnow' from seven growing locations are shown in Fig.1 indicating the difference in quality and intensity of the components. Though, qualitatively the volatile composition of the fruits from all the seven sampling localities selected for the present study was similar; there were semi-quantitative differences in the volatiles. The volatile compounds in 'Kinnow' juice from locations Bhunga, Chhauni Kalan, and Una (STH and STSM zones) showed lower peak intensity and percentage peak area as compared to other locations. The representation of the percentage peak area values in the heat map clearly shows the differences in the volatiles compounds in 'Kinnow' juice from all the seven locations (Fig 2). Monoterpenes are major in all the locations due to the presence of limonene which covers >80% of the total peak area. There is no variation in monoterpenes across locations as indicated by the heat map except slightly lower levels in location Una (STSM zone). The locations Bhunga and Chhauni Kalan (STH zone) have higher sesquiterpenes, alcohols, and alkanes as compared to other locations. Whereas, higher esters and aldehydes are present in locations Abohar, Lambi, and Amritsar (STA and STSA zones). Location Una (STSM) climate has lowest levels of monoterpenes and sesquiterpenes but higher levels of alcohols, alkanes, and esters. This variation in aroma volatiles may be attributed to the variation in latitude and altitude (Table 1), which in turn influence factors such as solar radiation, temperature, and humidity, and water availability associated with each location. These factors can affect crop growth and internal quality characteristics of the harvested product, including flavour [16].

Fig 2. Heat map based on % peak area showing semi-quantitative variation in aroma from seven growing locations. Blue represents minimum %peak area and red represents maximum %peak area

C. Multivariate analyses

The PCA score plot based on volatile compounds showed broader segregation of fruit from seven growing locations (Fig.2). The principal components 1 (PC1) and 2 (PC2) accounted for 57.9 and 12.3% of the data variance for aroma volatiles, respectively (Fig. 2) and collectively explained 70.2% of the total variance. In general, PCA of volatile metabolites could discriminate the impact of growing location by clustering together fruit samples from regions with similar volatile profile together and separate from those with the different volatile profile. The locations Abohar, Lambi, Tahliwala Jattan (STA zone) clustered together with location Amritsar (STSA zone) indicating that locations from similar agro-climatic zone have similar aroma profile. Locations Bhunga and Chhauni kalan (STH zone) form a separate cluster which clearly shows that aroma profile of these locations is different from the other locations due to being present in the different agro-climatic zone. Location Una (STSM zone) does not fall in any cluster and lies separately indicating its different aroma profile from all other locations. These variations could be attributed to the agro-climatic different between all the seven locations which may influence different factors causing variation in the aroma profile of 'Kinnow' juice.

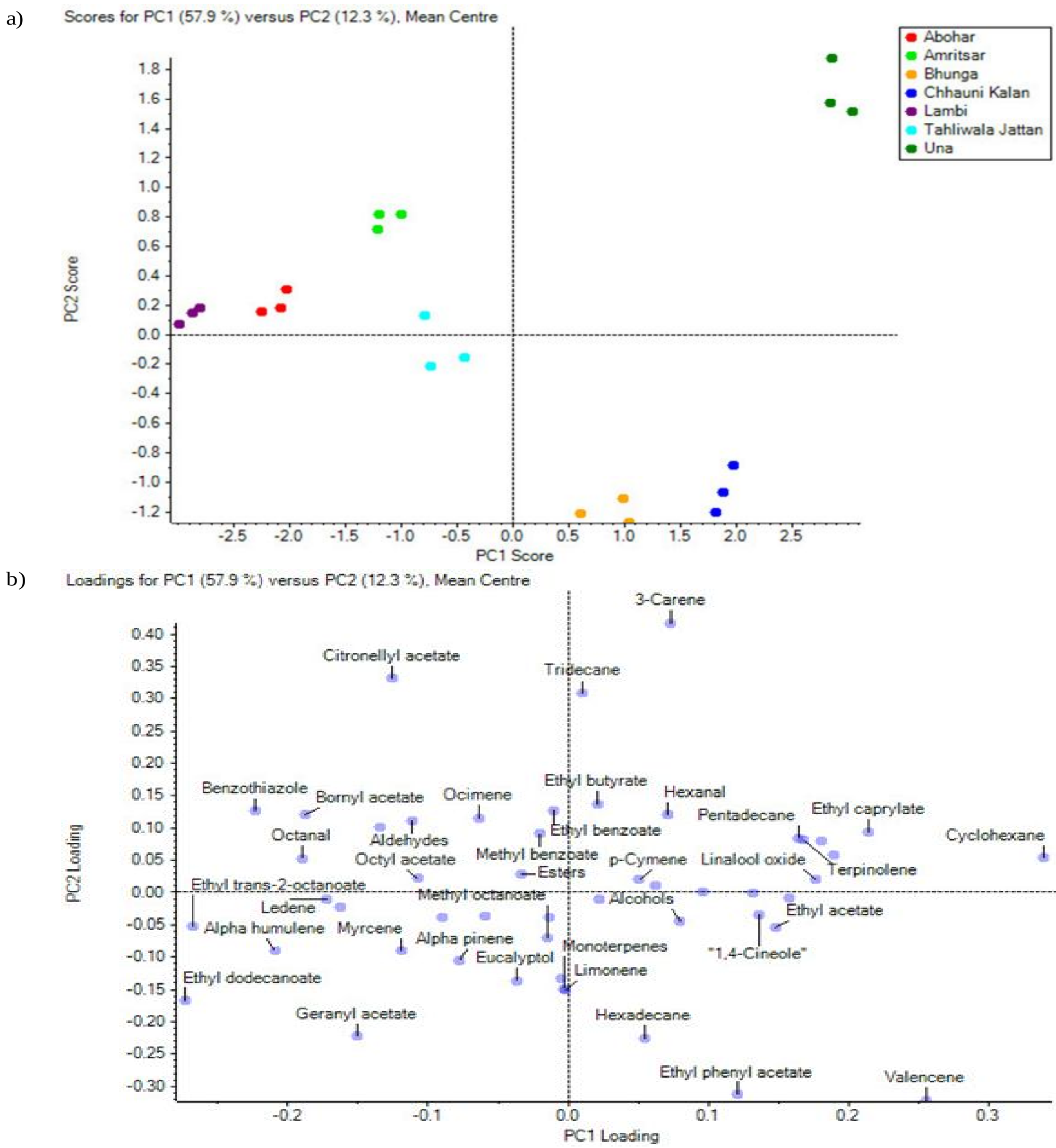

Fig 3.Principal component analysis(PCA) score plot (a) and loading plot (b) of 'Kinnow' discriminated by growing locations based on aroma volatiles. PCA score plots (filled circle red, Abohar; filled circle green, Amritsar; filled circle orange, Bhunga; filled circle blue, Chhauni Kalan; filled circle purple, Lambi; filled circle light blue, Tahliwala Jattan; filled circle dark green, Una)

IV. CONCLUSION

This study showed that growing location (different agro-climatic zones) had a significant influence on the aroma volatiles in 'Kinnow' juice. Between seven locations examined, Abohar, Lambi, and Amritsar (STA and STSA zone) showed the highest content of total aroma volatiles as compared to location Una (STSM zone), which showed the lowest. The information generated on the impact of agro-climatic locations on aroma volatiles of 'Kinnow' juice may provide a useful guide for selecting growing location towards improving fruit flavour and quality.

V. ACKNOWLEDGEMENTS

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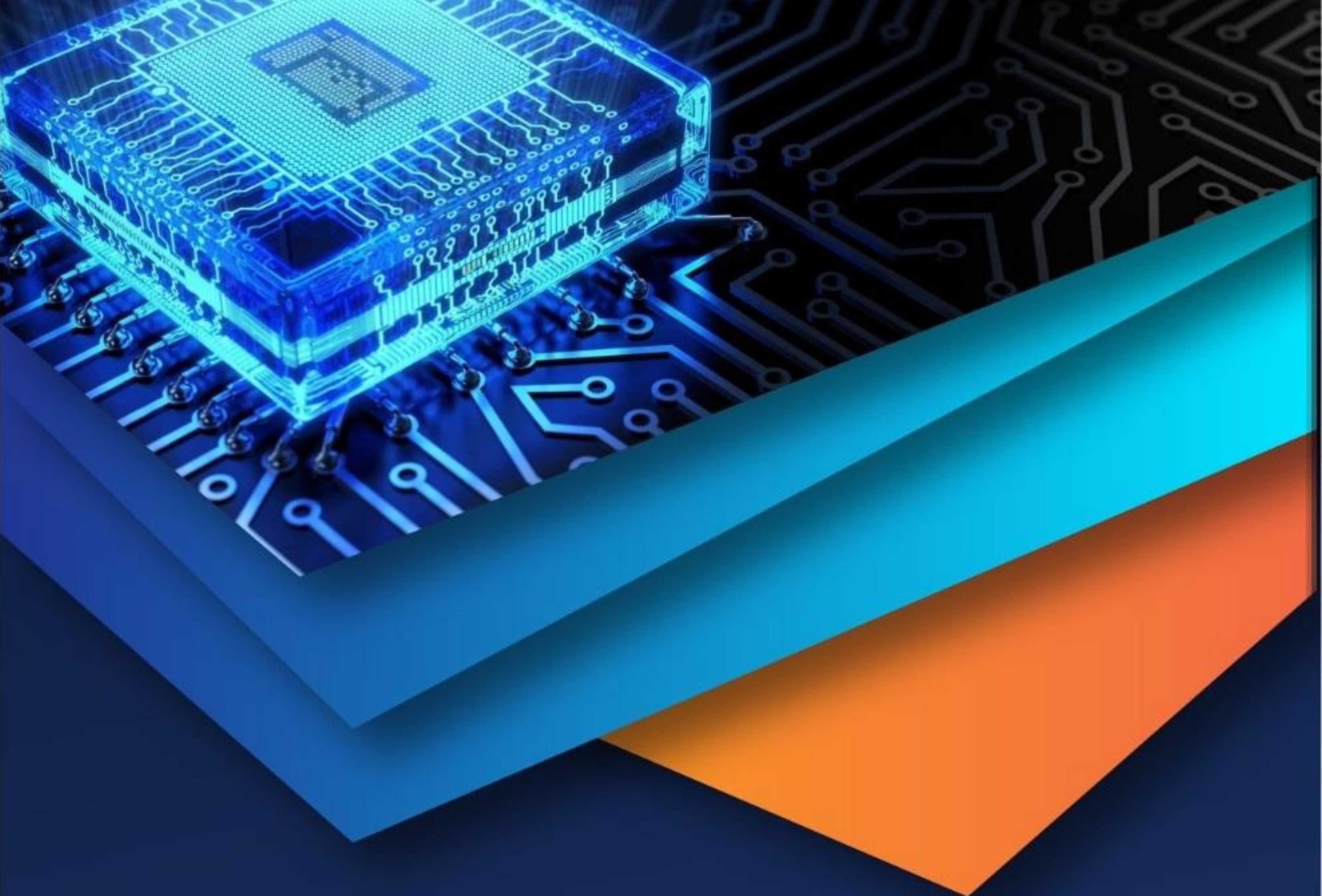

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