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Detection of adulteration in market ghee using FT-IR Spectroscopy

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Abstract: ***Dairy ghee is a prominent synergistic fat product that is comprised of various health benefiting compounds such as milk fat globule membrane, conjugated linoleic acid, and short-chain free fatty acids. It emanates numerous beneficial actions on human heath are anticancer, antidiabetic, anticholesterolemic, antimicrobial, antioxidant, antiatherogenic and anitadipogenic properties. In order to increase the quantity for marketing purposes, ghee is adulterated with low quality fats. These adulterated low quality fats have long-chain fatty acids and trans-fats which causes cardiac diseases, obesity and renal dysfunction. Hence, the present study aimed to detect the adulterant in ghee by Fourier transform infrared spectroscopy method. It also shows the quantitative increase of adulterated animal body fat with ghee. The peak C=C increases with the raise of alkene concentration. The N-H bend peak, which represents amines, determines the trans-fat adulteration, causes health-related issues. Thus, FT-IR technique proved the ideal tools for detecting and estimating the degree of ghee adulteration.***
Keywords: FT-IR, Ghee, Adulteration, Commercial brands, Beef fat.

I. INTRODUCTION

Eat right is the major concern among the people to lead a healthy life style. FSSAI, FDA, regulations provoked that before consuming the food, whether the food having healthy and hygienic is the basic understanding about food[1].Since adulteration is the main drawback corresponding nutritious diet. Especially ghee adulteration hikes with all the cheapest fats and oils, because all fatty acids are similar in nature[2].

Ghee usage in various sector such as food shortenings, enhance the memory power, reduce pitta, associated with herbal and siddha products consumption for oral and topical absorption. It has immense benefits among the multicusine in the Indian foods[3]. Due to similarity, vanaspathi spiked with ghee apart from that animal body fat, vegetable oil, paraffin and wax added. However, it has several health benefits, genuine ghee is spiked to increase the quantity and shelf life[4]

Consumption of adulterated products possess various health complications. High-throughput, cost-effective robust method is needed for detecting each of the components in ghee at once. RP-HPLC is the method to detect the adulteration with standard sterols such as β- sitosterol has a marked raise using unsaponifiable matter present in the ghee. Authentication detection of animal body fat in milk fat is more difficult[5].

The thermal behavior of different melting point ranges for animal body fats (43 to 50°C) and ghee (30 to 44° C) determined by opacity test. It is claimed around 5% animal body fats in ghee. However, this test is specific for only animal fat and not vegetable oil, cotton tract ghee[6]. Methylene blue reduction testovercame this difficulty, but cotton tract ghee decolorize reagent. A cost effective, time reducing and non-laborious methods needed to check the adulteration.

FTIR sounds good for the detection of adulteration in ghee[7]. Mainly to discriminate cis fat and trans fat in ghee. This non-destructive method been used for most of the adulterated food products detection. Lard adulteration detection in chicken, beef and goat fat, variation in frequency considered: (A): 3009-3000, (B): 1418-1417, (C): 1116-1098 and (D): 968-966 cm-1. Advances in FTIR spectroscopy instrumentation and its combination with statistical techniques have made FTIR technique a powerful tool for determination of food quality and authenticity[8].

Main advantage of FTIR is its ability to analyze sample with little sample preparation, rapidity and capability to serve as ‘fingerprint’ technique. Apart from the application of FTIR in detection of adulteration of food, the technique has also received attention for use in the quantitative analysis of fats and oils. Because of the advantages of FT-IR, a study has been proposed to explore the possibilities of FT-IR for identification of adulteration in milk ghee.

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II. MATERIALS AND METHODS

A. Preparation of Sample

Raw milk was collected from the milk society, Thanjavur, Tamil Nadu, India. Cow ghee was prepared using the traditional method and preserved under 4°C for future purpose. Cheapest vegetable oil palm oil prepared from wood pressed extraction unit. Cheapest animal body fat ie beef fat cholesterol were procured from the local slaughter house. Commercial brands of seven ghee samples and were purchased from a local market and stored at 4°C.

B. Traditional Method of Ghee Preparation

In agricultural occupation, cow ghee preparation is one of the most widely income giving for farmers. Milk was boiled for long time without stirring leads that milk skin or lactoderm formation. This milk skin are preserved under 4°C and mixed with cold water using domestic mixer. After the milk skin been churned to a particular time. It has to wash using the cold water to remove the extraneous matter. Then the washed butter is strained and subjected to melt at 120°C for 10 minutes. Thus the clarified butter been separated with the continuous stirring and brown residue been filtered and removed using the metallic filter. Later it has to keep in refrigerator with airtight container for further studies. Some steps has to be concentrate are while clarifying butter for long time leads more scorching of brown residue which produces trans fats. A peculiar nutty flavor produced at Indian tradition ghee due to browning of milk solids.

C. FTIR Spectrum Analysis

Before the analysis, the samples are subjected to keep in room temperature to remove the ice crytals and maintain 60°C in hot air oven to remove the excess moisture. Priory the instrument ATR-FTIR spectrometer Schimadzu IR tracer-100 was calibrated. The samples which are subjected to analysis were kept in a sample holder. Using the non destructive equipment, scanned with $4cm^{-1}$ resolution in the range of 4500-500 cm^{-1} region. Sample plates kept clean by chloroform using tissue paper thoroughly. On a sequential basis, the samples were taken by dropper and were analyzed at the rate of 45 scans/minute. Before and after analyzing the sample, the sample holder of the instrument was cleaned using tissue paper with chloroform to avoid contamination from one sample to another sample. The spectrogram data projected been saved after the analysis and functional group studied for discrimination of samples[10].

III. RESULTS AND DISCUSSION

The animal fatwhich were collected from the local slaughterhouse and rendered in the form of oil. At room temperature the fat becomes solid and liquid at molten stage at 60° C. The smoke point of clarified butter fat 240°C for ordinary compressed butter 175°C. It has extended shelf life, when kept in airtight container can be preserved at room temperature for months. Spectroscopic techniques were enunciate qualitative and quantitative analysis of chemicals and bonds corresponding the characteristic peaks absorbtion.to reduce the amount of total trans fat has been followed by health concerned organization. Fatty acids single or double bonds configuration with respect to partial hydrogenation from industries, naturally produced from ruminants or by food processing are strictly prohibited. Among thesamples ghee with various brands showing that 721.38 cm^{-1}, 1377.17cm^{-1}, 1463.97cm^{-1}, 2852.72cm^{-1}, 922.16cm^{-1}, 2953.02cm^{-1}. The spectrum of ghee 582.5cm^{-1}, 721.38cm^{-1}, 966.34cm^{-1}, 1099.43cm^{-1}, 1112.93cm^{-1}, 1159.22cm^{-1}, 1236.37cm^{-1}, 1298.09cm^{-1}, 1377.17cm^{-1}, 1417.68cm^{-1}, 1463.97cm^{-1}, 1743.65 cm^{-1}, 2852.72 cm^{-1}, 2922.16 cm^{-1} and 2953.02cm^{-1} was recorded in FTIR region[11]. For groundnut oil, unique ester at 1712.79cm-^{1}, whereas for the entire sample have shown the presence of ester at 1159.22cm-^{1}. At 1097.5 cm-^{1} strectching observed for ether in sample at C-O stretch. Only vanaspathi have shown wave number at 966cm^{-1} for anhydride related to transfatty acid, which is present in ghee and vanaspathi.

The common functional group is depicted in Figure 6. The peak 721.38cm^{-1}, the functional group obtained may be aliphatic chloro compound, CH_2, CH_3 symmetric bending vibration 9 aliphatic groups, corresponding to 1377.17cm^{-1} peak. Bending vibration of CH_2 and CH_3 aliphatic groups, nitrosamines at 1463.97cm-^{1}at C-H stretch. Aromatics and ketones were found at all tested samples with C- H stretch at 1236.37cm^{-1} and 1145cm-^{1}. The band at 966 cm^{-1}, responding C=C vibrations, specific Trans fats acts as marker present in vanaspathi has not been found in the ghee samples illustrated in the table 3. Hydrogenated fat has the highest trans content associated with a rich hardness index[12]. These HC=CH functional groups are responsible for the complex form in vanaspathi so it leads to unhealthy issues like indigestion, cancer, obesity-related diseases[13]. Many researchers stated that the Trans fatty acids intake leads to cardiovascular diseases, breast cancer, nervous disorder, pregnancy duration reduced, preeclampsia risks, and vision problems for infants, colon cancer and causes allergy[14]. Raising the intensity and area vary depending on the sample melting time and temperature factor. Feed of the cow, lactation flow, breed of the cattle and preparation of ghee are the fluctuating concern for ghee adulteration detection. Among these parameter marker identification is the crucial role.

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Peak intensity and peak area analysis are given in Table.1 and 2 Similarly, various researches found some transfats in the hydrogenated fats[16]. Although vegetable oil has saturated fatty acids, total fat in cookies did not increase significantly. However, the partially hydrogenated vegetable oil used in bakery products is rich in TFA. The replacement of vansapathi with oils reasonably be expected to coronary heart disease risks. Thus the specific effect on cardiovascular risk is dependent on the original content of TFA in the food product[17].

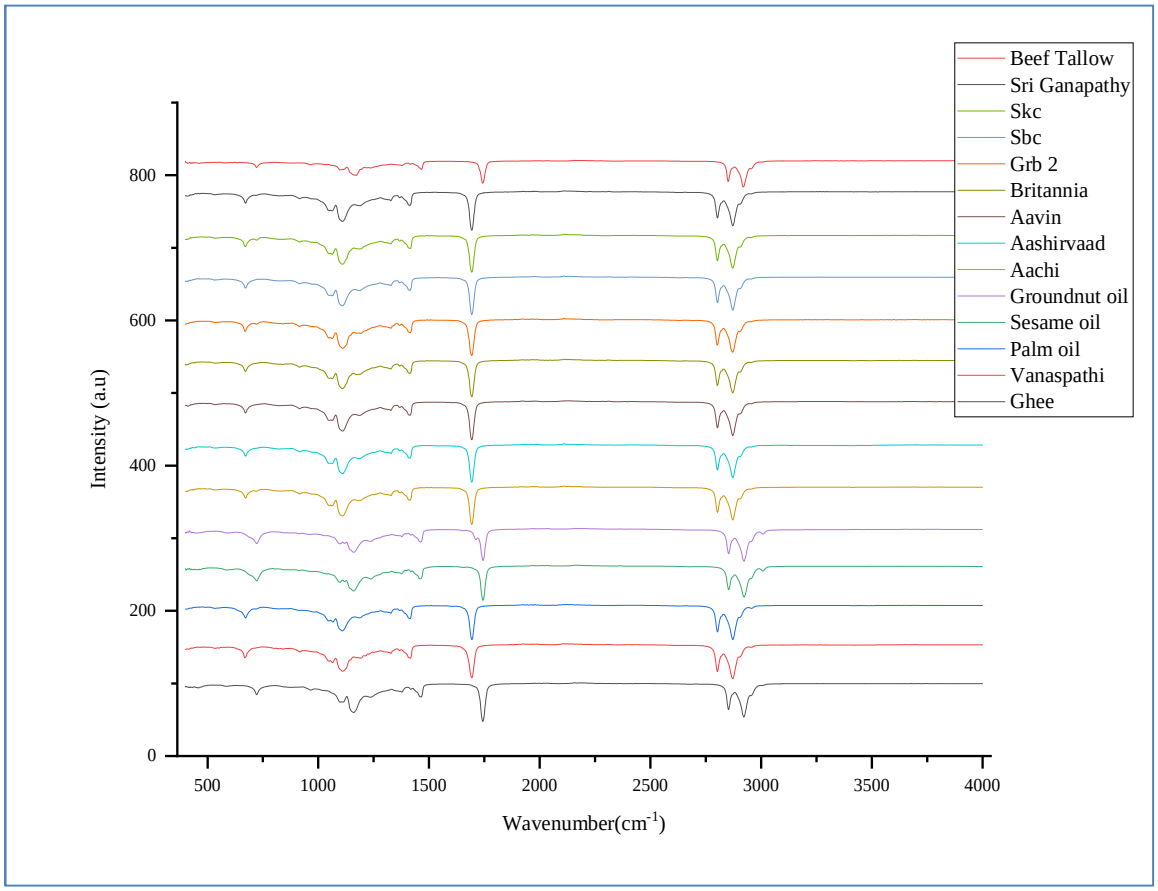


Fig1 FTIR spectrum of home based ghee, brands of ghee, Vanaspathi, Beef fat,sesame oil, Ground nut oil, Palm oil.

Table 1

Peaks Intensity Of Home Based Ghee, Brands Of Ghee, Vanaspathi, Beef Fat,Sesame Oil, Ground Nut Oil, Palm Oil

Peak	Ghee	Aavin	Aashirvaad	Aachi	Brittania	GRB	SKC	SBC	Sri Ganapathy	Vansapathi	Beef fat	Sesame oil	Groundnut oil	Palm oil
721.38	84.35	84.26	84.46	84.73	84.48	83.79	84.6	84.64	84.29	81.81	77.54	79.15	79.46	82.33
966.34	-	-	-	-	-	-	-	90.82	-	90.09	-	-	-	-
1031.92	-	-	-	-	75.17	-	-	-	-	89.9	-	88.79	-	-
1097.5	74.23	74.03	74.49	74.72	74.65	74.79	74.75	-	73.94	77.18	-	77.14	79.28	78.38
1114.86	74.38	73.94	74.5	74.39		73.87	74.03	74.26	73.75	75.27		78.3	79.74	76.8
1159.22	59.97	59.33	60.47	60.27	61	60.38	60.25	60.38	59.18	63.46	63.11	65.34	67.6	64.76
1220.94	-	-	-	-	80.47	81.24	81.58	-	-	81.81	80.85	-	-	-
1226.73	81.02	-	-	-	80.51	81.32	-	-	80.82	82.19	-	-	-	-
1242.16	-	80.95	81.19	81.38	-	81.07	81.29	81.45	-	81.45	-	81.8	82.68	82.45
1265.3	-	-	-	-	-	84.68	-	-	-	84.59	-	-	-	-
1278.81	-	-	-	-	-	86.81	-	-	-	86.8	-	-	-	-
1300.02	89.34	-	-	-	-	89.2	89.46	-	-	89.39	-	-	-	-
1377.17	87.87	87.75	87.99	88.2	87.99	87.99	88.16	-	87.7	88.05	86.21	88.81	88.97	88.82
1465.9	81.26	81.19	81.68	81.9	81.99	81.9	81.97	81.67	81.17	81.31	75.85	82.16	81.92	81.66
1743.65	47.61	47.41	48.72	48.67	49.74	50.72	49.41	48.37	47.11	54.34	51.62	52.38	56.28	52.69
2852.72	64.09	63.82	65.36	65.23	65.2	64.72	65.06	64.8	63.85	63.14	53.22	67.18	65.61	63.25
2922.16	53.65	53.01	55.02	54.85	55.11	55.05	54.94	54.45	53.11	52.94	43.91	57.03	55.31	52.73
2954.95	83.16	82.8	83.75	83.78	83.73	83.48	83.67	83	82.8	83	82.74	82.35	82.27	83.18

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Table 2

Area Percentage Of Home Based Ghee, Brands Of Ghee, Vanaspathi, Beef Fat, Sesame Oil, Ground Nut Oil, Palm Oil

Peak	Ghee	Aavin	Aashirvaad	Aachi	Brittania	GRB	SKC	SBC	Sri ganapathy	vansapathi	Beef fat	sesame oil	Ground nut oil	Palm oil
721.38	738.89	885.44	882.94	680.61	826.23	714.901	653.198	697.559	731.014	921.024	688.962	1505.39	1427.919	947.07
966.34	-	-	-	-	444.94	1055.203	-	437.38	-	401.279	-	-	-	-
1031.92	-	-	-	-	-	540.784	-	-	-	499.163	-	556.03	-	-
1097.5	1627.68	1239.489	1628.8	1076.24	1231.33	2432.66	1075.01	-	1562.95	994.932	-	1022.14	979.61	936.58
1114.86	489.58	548.82	490.782	538.521	531.6	245.383	541.01	540.08	5551.72	519.203	-	360.97	336.51	494.41
1159.22	2445.66	2509.86	2418.367	2425.587	2342.76	143.67	2408.85	2441.94	2485.62	2297.686	1928.56	2276.25	2177.64	2236.457
1220.94	-	-	-	-	291.96	542.808	207.682	--	-	237.8	356.35	-	-	-
1226.73	-	-	-	-	1110	193.939	-	-	-	102.944	-	-	-	-
1242.16	1297.63	1591.11	1597.06	1587.698	-	231.537	1113.74	1534.117	1630.66	468.844	305.58	1302.23	1442.268	1442.52
1265.3	-	-	-	-	-	301.953	-	-	-	251.619	328.46	-	-	-
1278.81	332.36	-	-	-	-	727.693	-	-	-	231.134	-	-	-	-
1300.02	-	-	-	-	-	301.953	312.686	-	-	337.652	-	-	-	-
1377.17	679.37	642.377	632.54	630.95	693.09	727.693	657.341	-	642.151	477.53	-	555.8	315.857	335.508
1465.9	701.52	712.15	698.61	657.732	688.87	666.74	659.787	284.59	699.509	711.464	-	688.182	717.864	686.89
1743.65	1666.64	1694.75	1560.62	1561.87	1584.32	1581.184	1582.501	1608.41	1691.647	1565.658	1499.228	1499.05	1270.83	1459.02
2852.72	1128.09	1129.48	1085.26	1074.40	1085.36	1098.121	1101.078	1085.67	1120.749	1161.621	1367.796	1110.16	1157.22	1134.196
2922.16	1909.64	1936.71	1848.48	1852.15	1855.59	1889.592	1839.075	1857.39	1932.764	2009.352	2180.091	1872.44	1936.27	1977.785
2954.95	394.491	1666.64	378.77	373.89	407.01	411.718	402.775	72.18	400.62	376.099	425.168	400.42	410.23	373.01

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Table 3
Common Peaks And Its Functional Group Of Home Based Ghee, Brands Of Ghee, Vanaspathi, Beef Fat,Sesame Oil, Ground Nut Oil, Palm Oil

S.No	Peak	Wavenumber (cm^{-1})	Functional group assignment	Chemical constituents
1	721.38	500-738	C-Cl	Halogen compound (Chlorocompound)
2	966.34	650-1000	PO3 stretch	Phosphate ion, alkene bends
3	1031.92	1100-1000	C-O stretch	Ether
4	1097.5	1100-1000	C-O stretch	Ether
5	1114.86	1020-1250	C-N stretch	Aliphatic amines
6	1159.22	1000-1200	C-N stretch	Vibration of proteins
7	1220.94	1200-1280	C-H stretch	Aromatics
8	1226.73	1200-1280	C-H stretch	Aromatics
9	1242.16	1200-1280	C-H stretch	Aromatics
10	1265.3	1200-1280	C-H stretch	Aromatics
11	1278.81	1200-1280	C-H stretch	Aromatics
12	1300.02	1000-1320	C-O stretch	Alcohol, carboxylic acids, esters, ethers
13	1377.17	1410-1310	O-H bend	Phenol or tertiary alcohol
14	1465.9	1432-1621	C-H bend	Aliphatic group
15	1743.65	1640-1800	-C=O stretch	Carbonyl / carboxylic group
16	2852.72	2852	C-H stretch	Alkanes
17	2922.16	2922-2923	C-H stretch	Alkanes
18	2954.95	2850-2960	C-H stretch	Alkanes /alkyl groups

From this experiment, we found that so many variations were observed in the active functional group of vegetable oils from the liquid to hydrogenated form at room temperature. In addition, we identified a transfat functional group, and the area intensity was high compared to other oils[18]. Beef fat enunciates the peak 1743.65 peaks corresponding carbonyl groups. There are similarities in fats and oils spectrum, there is no significant difference found in the peaks to detect the adulterants.

IV. CONCLUSION

This work concluded that homemade ghee responds the peak area and intensity variation determining the adulteration using the FTIR spectroscopic method. The vegetable oils such as palm oil, groundnut oil, sesame oil, vanaspathi, beef fat and commercial brands of ghee were observed with relative intensities of the peak and characteristic area percentage of peaks. It is seen that trans fatty acids at the peak of 966.34cm^{-1} at strong C=C bending vibration in the region of 980-960cm^{-1} due to the presence of alkene disubstituted (trans) observed in the hydrogenated form of vegetable oil. Apart from that there is no significant marker identified in commercial ghee to detect the adulterants. Hence these commercial samples are not adulterated with the cheapest fats.

V. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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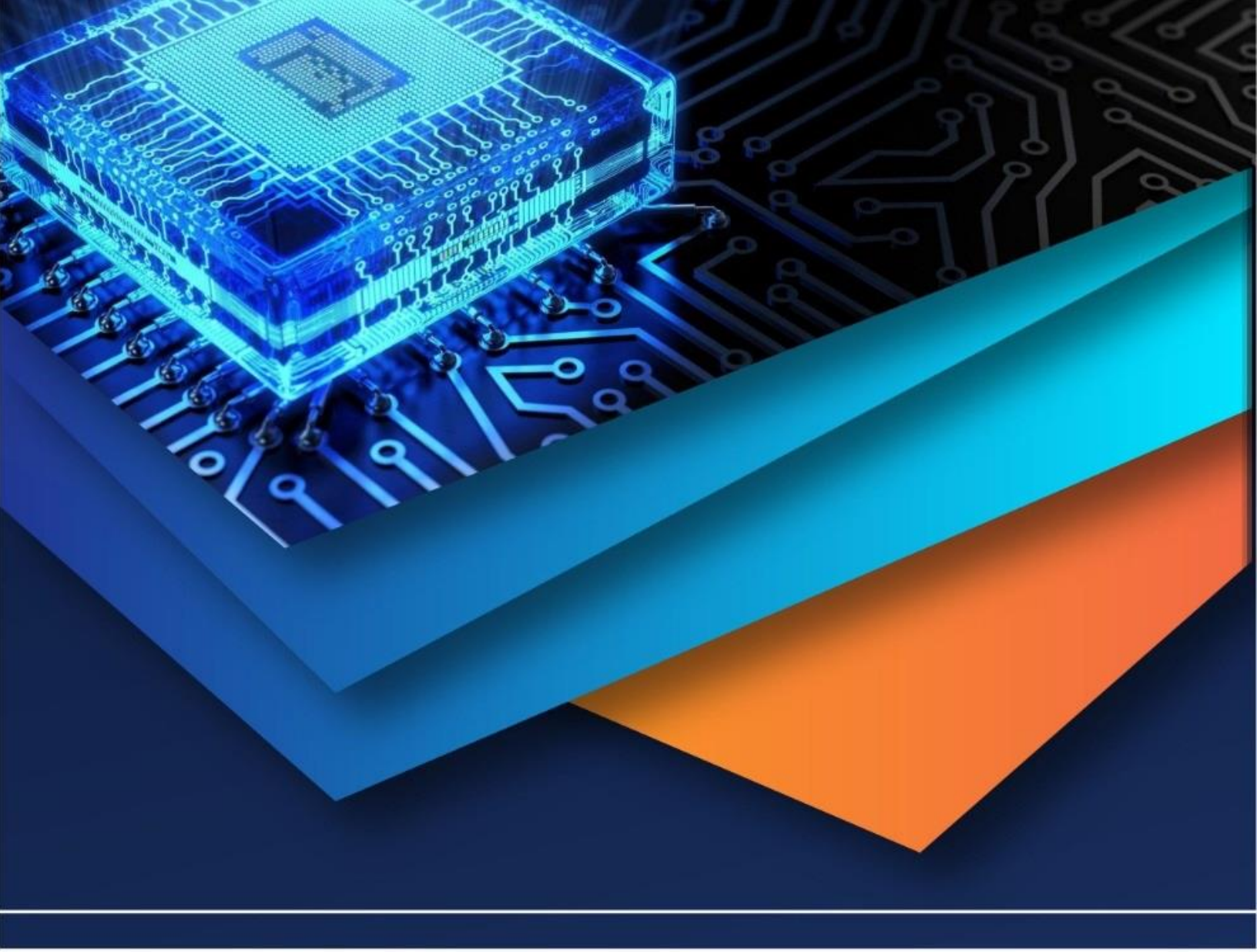
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