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Evaluation of Microbial & Allergen Content (Gluten) In Wheat Samples

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Abstract: Wheat flour is most widely used all over the world. The present study investigates the quality of the different brands of wheat flour. Microbiologically, total plate count, coliforms, yeast and mould count, Staphylococusaures spp, E.coli, salmonella spp, Listeria monocytogenes are also determined. The Indian standard which is identical with ISO (International Organization for Standardization) helps to identify the different species in the food samples. The use of wheat flour and gluten food stuff is extremely common because of their heat stability and useful effects on the texture, moisture retention and flavor. Gluten is a combination of proteins such as prolamine and glutelin present in wheat flour. Number of coliforms are also identified in different brands of wheat flour samples. This study shows different gluten content in different wheat flour samples. Different brands of wheat samples of two brands (195 ppm), (130 ppm) and local brand (745 ppm)
Key words*: wheat flour, quality, gluten, microbiologically*

I. INTRODUCTION

The packaged wheat flour market in India started breaking the old age tradition of grinding of wheat at local chakkimills. Wheat flour market is largely dominated by local chakki mills in India. The packaged wheat flour market in India remained nearly at 1.85 kg during the fiscal 2014-15. However, urban market leads in per consumption of packaged wheat flour with almost 5.5 kg, making the packaged wheat flour an urban phenomenon. The major consumers of the packaged wheat flour in India is north central region. The Indian packaged wheat flour market consists of plenty of brands. Each brand is trying to promote itself by emphasising on the origin of wheat, manufacturing process, quality, taste, textures and price to attract customers. Besides leading brands, there are more than 500 regional brands in India. Brand 2 is the clear market leader among the national players in branded packaged wheat flour market in India. Various factors are driving consumers for purchase of packaged wheat flour fulfill basic nutrition needs, convenience and time saving, lack of storage of wheat in bulk and perceived high quality of packaged wheat flour. More than 70%, particularly health and quality conscious consumers prefer to buy specific brands of wheat flour samples. Wheat flour is a powder made for human consumption. Wheat varieties are categorised as soft or weak and hard or strong. If gluten content is low, it is called soft wheat flour and if gluten content is high, it is called hard wheat flour. Hard wheat flour has a 12 to 14% of gluten content. Atta is an Indian flour used to make most south Asian flatbreads, such as chapatti, roti, naan and puri. Hard wheat has a high gluten content and it provides elasticity. So dough made out of atta flour is strong and can be rolled out thinly

II. MATERIALS AND METHODS

Sample selection : A total of three fresh wheat flour samples were collected from local area of Hosur (Tamilnadu), India.

1) Brand 1
2) Brand 2
3) One local brand.

These three different types of samples were stored in ambient conditions in a microbiology laboratory.

A. Media

Different media are used in the microbiological methods such as plate count agar, chloramphenicol agar, violet red bile agar, baird parker agar, macconkeyagar, eosin methylene blue agar, brilliant green agar, xylose lysine deoxyxholate agar, Fraser broth, PALCAM agar, oxford agar. These are the different types of media used in microbiological method

B. List of organism used

organisms	Medium	Incubation condition
Staphylococcus aureus	Baird Parker agar (BPA)	37°c for 24 -48 hrs
E.coli	Macconkey agar (MA), Eosin methylene blue agar (EMBA)	37°c over night
salmonella	Brilliant green agar (BGA), xylose lysine deoxycholate agar (XLDA)	37°c for 24 -48 hrs
Listeria monocytogenes	Oxford agar, PALCAM agar	37°c for 48 hrs

C. Microbiological test

For microbial analysis, 10 ml of weighed flour samples were suspended in 90 ml of buffer peptone water, 1 ml of each of the following dilution was pipetted in duplicate sterile petri dishes (IS5402:2002). Serial dilution was made and plated in each specific medium, each dilution was used to spread and pour plate method. To enumerate total bacteria count (TPC), plate count agar was used and incubated at 30°C for 72 hrs.

Coliforms was enumerated on violet red bile agar (VRBA) were incubated at 37°c for 24 hrs (IS 5401- 2002). Yeast and mould counts were enumerated on chloramphenicol yeast glucose agar (IS: 5403:1999), incubated at 25°C for 5 days.

To enumerate *E.coli* species : (25 g flour samples each) were suspended in 200 ml of 0.1% peptone water. Then it was transferred into 1ml in 10 ml of single strength MacConkey broth and 10 ml in 10 ml of double strength MacConkey broth were used, incubated at 37°c for overnight. There after we observed for the growth of with fermentation of lactose in the Macconkey broth tubes (IS: 5887 (Pt-1) 1995). After 24 hrs, if there is a metallic green (EMB) formation, it indicates that E.coli is present in the sample.

To enumerate the *salmonella* spp : flour samples (25 g each) were suspended in 225 ml of buffer peptone water. Then, incubated at 35 (or) 37°c for 16 to 20 hrs. Concurrently, 0.1 ml of the pre-enriched culture was transferred into 10 ml of Rappaport fusillades medium and 10 ml of pre-enriched culture was transferred into 100 ml of fluid selenite cysteine broth (37°c for 48 hrs). Then, the sample was streaked on suitable media (BGA / XDLA) and incubated at 37°c for 24 to 48 hrs (IS: 5887 (pt-3) 1999). After incubation, red to pink colour (black center) formation indicates the presence of this species.

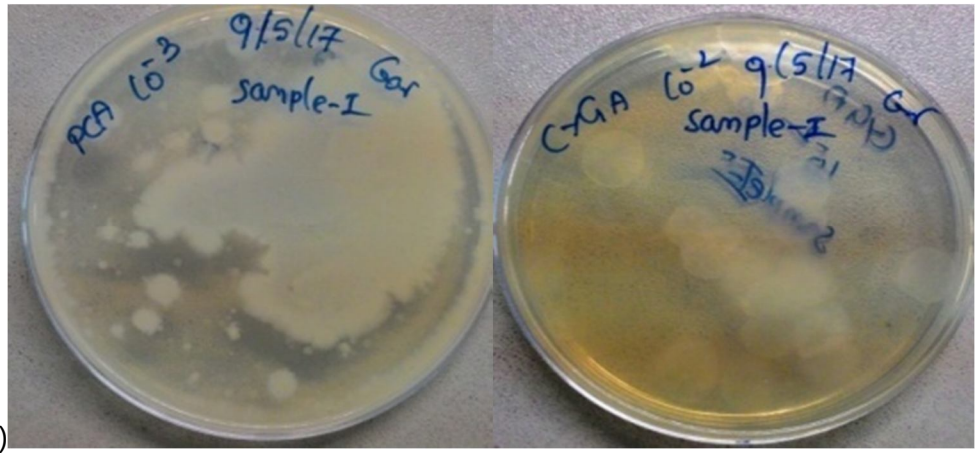
To enumerate the *listeria monocytogenes :* flour samples (25 kg each) were suspended in 225 ml of Fraser broth (primary enrichment medium) and then incubated at 30°c for 24 ± 2 hrs. There after 0.1 ml of pre-enriched culture was transferred to 10 ml of secondary enrichment medium, then incubated at 37°c for 48 ± 2 hrs. Typical colonies listeria species were grown on oxford agar for 24 hrs. After 48 hrs this colony become darker (2mm) with black halus, PALCAM agar exposed after incubation changed from pink to purple (IS :14988 (P*-1) 2001).

To enumerate the staphylococcus spp : (25 g of each) were suspended in 225 ml of peptone salt solution. There after transfer, by means of a sterile pipette spread 0.1 ml of the initial suspension ($10^{-}1$) to each of two Baird parker agar base (BPA) plates. Repeat the procedure for the $10^{-}2$ dilutions and for the further decimal dilution if necessary. Then were allowed the plates to incubate at 37°c for 24 to 48 hrs (IS: 5887 (pt-8/sec-1) 2002). Black colony formation confirms the presence of this species

III. RESULTS AND DISCUSSION

Physiological composition of different wheat flour samples was collected from local area were analyzed for different microbiological parameters. Total plate count, yeast and mould and total coliforms count were analyzed in wheat flour samples. The present study shows different brands of wheat flour samples analyzed by using ISO methods. E.coli, salmonella, staphylococcus

aureus, listeria monocytogenes was not detected in our different brands of wheat flour samples. Some naturally occurring species are only analyzed in wheat flour samples. Specified microorganisms are not detected or their presence is minimal.

(a) 

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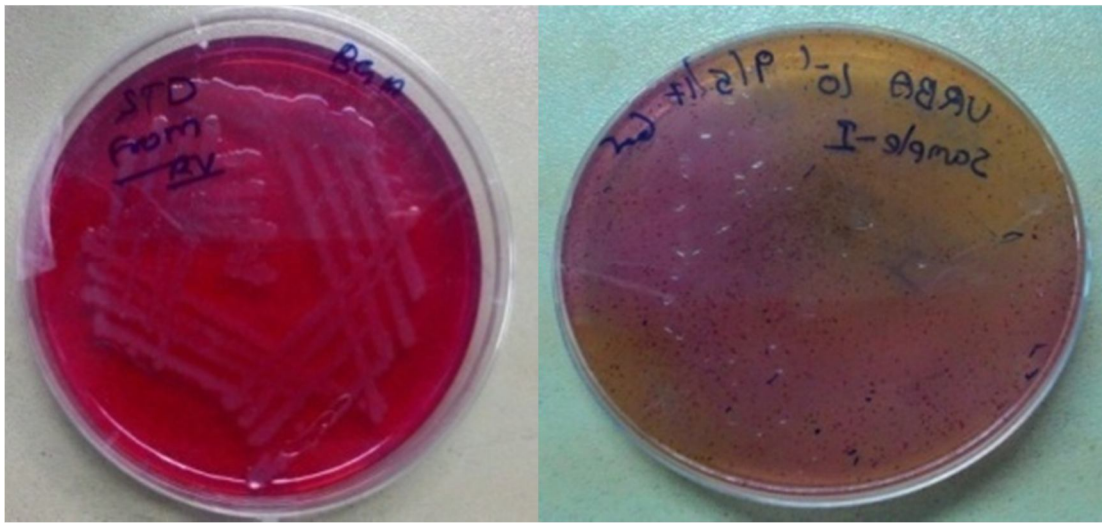
(c) 

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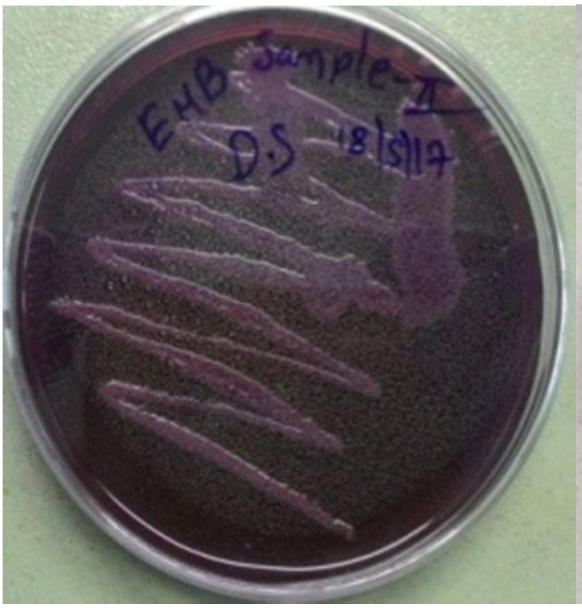
(e) 

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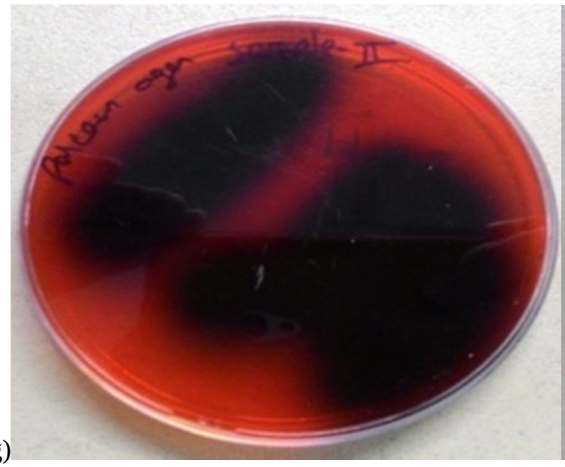
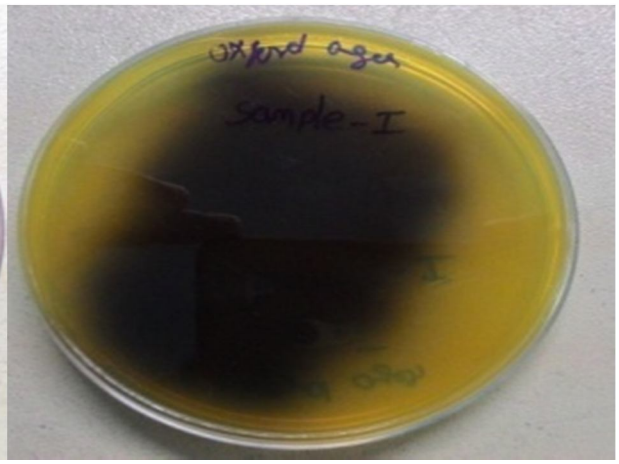
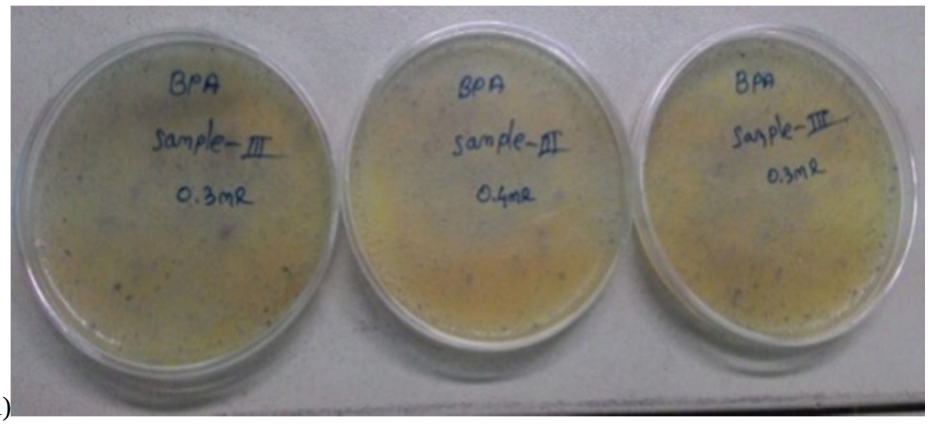
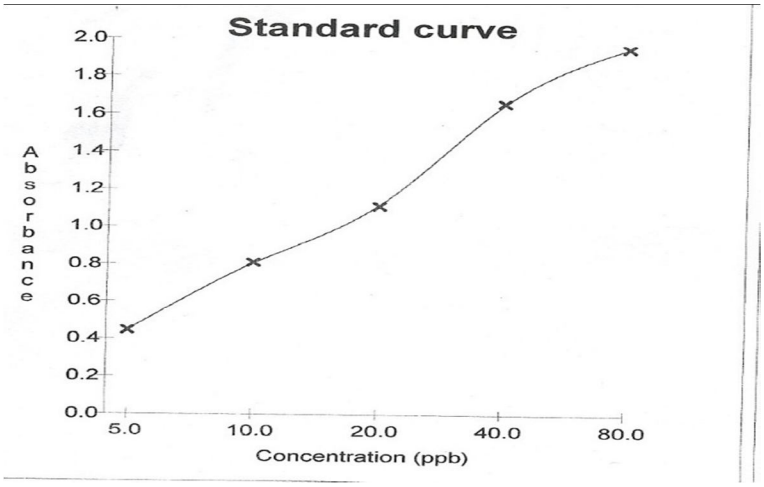
(g)   (h)

(i) 

(a) Total plate count (b) Yeast and mould count (c) Total coliform count (d) eosin methylene blue agar for E-coli (e) Brilliant green agar for salmonella (Rappaport vassiliadis medium) (f) Brilliant green agar for salmonella (fluid selenite cysteine broth) (g) PALCAM agar for listeria monocytogenes (h) oxford agar for listeria monocytogenes (I) Baird parker agar for staphylococcus aureus.

A. Gluten content (Gliadin) Assay

The gliadin concentration in mg/kg (ppb) was read from calibration curve (RIDA® SOFT) and then calculated ppb multiplied by using recommended dilution factor of 500. Thereafter, this result was then multiplied by 2 in order to obtain gluten concentration (ppm).



The results show similarities related to previous studies. Different kinds of flour samples shows different gluten content.Flour samples contain <20 ppm labelled as "gluten free" and flour samples contain >20 ppm labelled as very low gluten. Brand 1 shows 145 ppm, Brand 2 shows 130 ppm and also local brand shows 749 ppm. The samples of wheat flour were tested for microbial parameters (total plate count, yeast and mould count, coliformcount, staphylococcus aureus spp, salmonella spp, E.coli spp, listeria monocytogenes spp) and gluten allergen test. The three samples were identified based on their bacterial count. The best sample was identified as Brand 2.

Details of the samples

S. No	ID	Absorbance (Mean) (CV) (%)			Calculated ppb	*	ppb	*	ppm
1	Brand 1	2.222	4.0	114.1	195.26*	500.00	9728.72*	2	195
2	Brand 2	2.100	5.2	107.8	130.28*	500.00	65138.80*	2	130
3	Local brand	2.605	2.9	133.7	749.30*	500.00	374648.05*	2	749

WHEAT PRODUCT	DILUTION	TOTAL PLATE COUNT		YEAST AND MOULD COUNT		TOTAL COLIFORM COUNT	
		CFU/ML P_1+P_2	MEAN CFU/ML	CFU/ML P_1+P_2	MEAN CFU/ML	CFU/ML P_1+P_2	MEAN CFU/ML
Brand 1	10^{-1}	TNC	-	TNC	-	67+78	72.5
	10^{-2}	48+53	50.5	35+38	36.5	15+3	9
	10^{-3}	5+7	6	4+6	5	-	-
$N=\sum C/(n_1+0.1n_2)d$		5200 cfu/g		3800 cfu/g		740 cfu/g	
Brand 2	10^{-1}	23+27	25	11+6	8.5	0	-
	10^{-2}	3+5	4	-	-	0	-
	10^{-3}	-	-	-	-	0	-
$N=\sum C/(n_1+0.1n_2)d$		2700 cfu/g		77.27 cfu/g		<10 cfu/g	
Local brand	10^{-1}	TNC	-	TNC	-	30+40	35
	10^{-2}	TNC	-	20+25	22.5	4+8	6
	10^{-3}	TNC	-	-	-	-	-
	10^{-4}	32+34	33	-	-	-	-
	10^{-5}	4+5	4.5	-	-	-	-
$N=\sum C/(n_1+0.1n_2)d$		35000 cfu/g		2100 cfu/g		400 cfu/g	

IV. CONCLUSION

Thus study concludes that different wheat flour samples which were analysed by using microbiological paramaters. Brand 2 is the one of the best wheat flour samples compared other two. Because no specified species are identified in this sample.International standard for organization methods were used to analyse the above samples.

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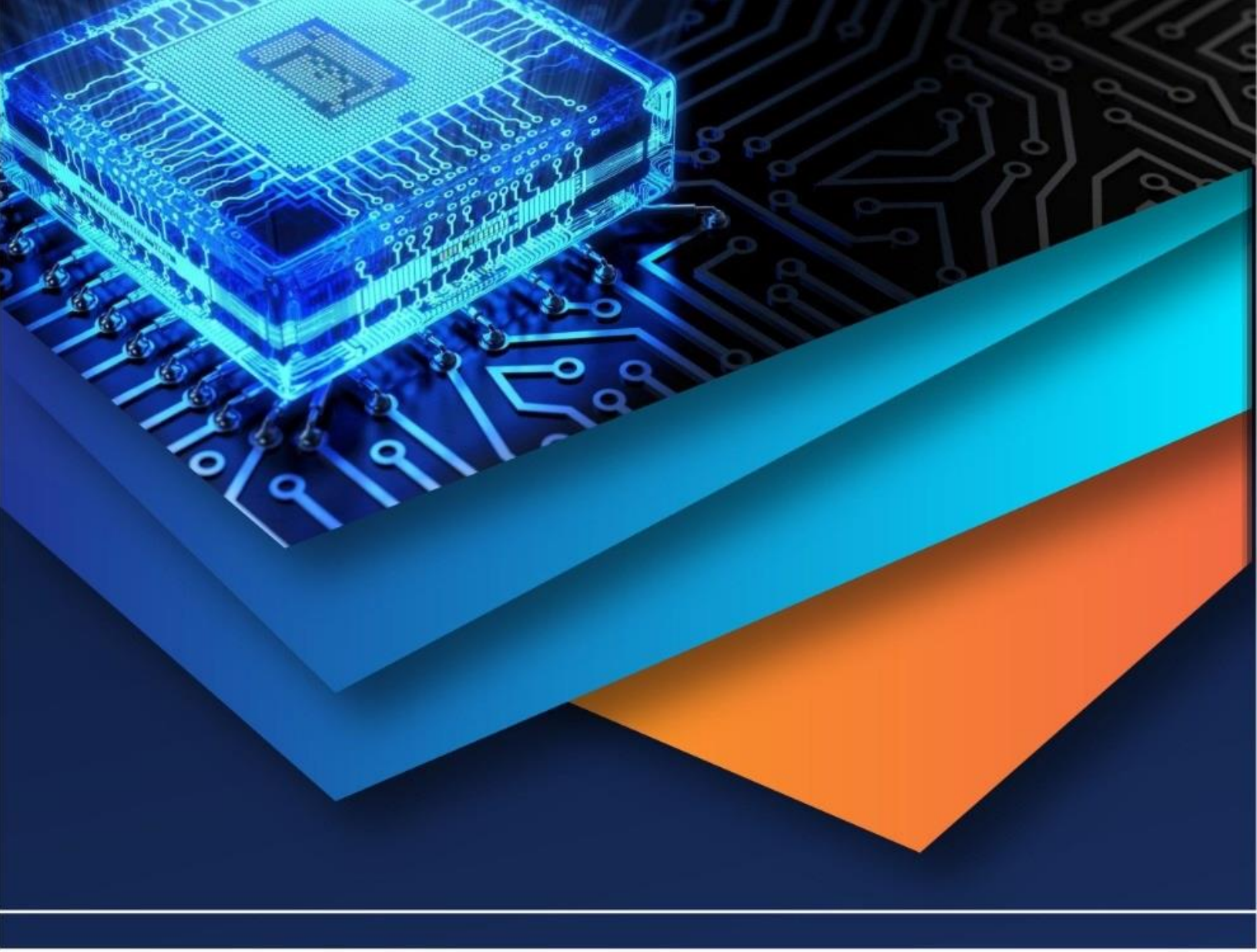

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