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Cholesterol Content and Free Fatty Acids in Edible Oils and Health Effects: A review

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Abstract: ***Cholesterol plays a major role in human heart health and high cholesterol is a leading risk factor for human cardiovascular diseases such as coronary heart disease and stroke. Not only cholesterol content but also cholesterol oxidation (favoured during thermal-processing of food) can be potentially harmful to human health. A person with the level of 240 mg/dl or above has more than twice the risk of heart disease as someone whose cholesterol is below 200 mg/dl. Most literatures showed that there is no cholesterol free oil in the market as shown on the vegetable oil brand labels and companies producing and advertising vegetable oils are enjoined to desist from misleading the public by labeling their products as "cholesterol free". Hence this review explains the way of determining the cholesterol content in edible oils and call attention to write or label the amount of cholesterol present in the oil, no matter how small quantity may be. In addition, this review describes acid value, iodine value, and saponification value and oil rancidity.***
Key words: Lipoprotein; Cholesterol; Rancidity; Liebermann-Burchard reaction

I. INTRODUCTION

Many vegetable oils are consumed directly or used as ingredients in food. Reports show that approximately 75% of the World's production of oils and fats come from plant sources. Although many plant parts yield oil, in actual marketable practices, oil is extracted primarily from seeds of oilseed plants such as soybean, cotton, palm, rape and groundnut (Okpuzor *et al*., 2009). Oils have always been an integral part of human foods, being fundamental for health. Industrially, they play an important role in the development of different areas of chemical products, pharmaceutical, cosmetics, paints and most importantly, food. Oils are naturally occurring esters of long straight-chain carboxylic acids. They belong to the saponifiable group (contain an ester groups) of lipids. Lipids are biologically produced materials that are relatively insoluble in water but soluble in polar and non-polar organic solvents. Edible oils are constituted of triacylglycerol molecules, mainly formed by unsaturated (oleic, linoleic, linolenic acids etc.) and saturated fatty acids (myristic, palmitic, stearic acids etc.) esterified to glycerol units (Andersson *et al*., 2010). They can be formed from a single fatty acid that could be esterified up to three times into glycerol backbone, or at least by three different ones. Almost every adult at present times develops some degrees of atherosclerosis, commonly known as "hardening of the arteries". Atherosclerosis leads to strokes, heart attacks and other serious health problems. High cholesterol, smoking and high blood pressure are the principal risk factors linked to heart disease (Dimberu and Belete, 2011).

Cholesterol is a waxy substance made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. It is needed in the body to insulate nerves, make cell membranes and produce certain steroid hormones, and for the biosynthesis of bile and bile acid salts. Bile is the major excretion route of cholesterol from the body, predominantly as unesterified cholesterol. In the adult human, approximately 400 mg of cholesterol per day are converted to bile acids and only approximately 50 mg are converted to hormones (Valenzuela *et al*., 2003). Sterol plays also an important role in embryonic development and an important lipid in some membranes. However, the body makes enough cholesterol, so any dietary cholesterol isn't needed (Ma, 2006).

Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. LDL cholesterol is considered as "bad cholesterol" responsible for allowing fatty plaques to develop in the lumen of arteries, leading to their narrowing. If this narrowing develops in coronary arteries (supplying blood to the heart), the person can develop coronary artery disease (CAD) and can lead to heart attacks (Mishra and Manchanda, 2012).

HDL cholesterol is desirable as it is a means of transporting cholesterol from parts of the body where there is too much of it to the liver where it can be disposed of. High cholesterol in serum is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke - America's number one killer. Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely. When there is too much LDL cholesterol in the blood, it is deposited inside the blood vessels, where it can build up to hard deposits and causes

atherosclerosis, the disease process that underlies heart attacks (Ma, 2006).

Consuming cholesterol in our diet increases the level of low density lipoproteins (LDL) cholesterol, which has been positively associated with cardiovascular disease (CVD) risk. There are so many different varieties of vegetable oil brands in our markets and all of them claim to be cholesterol free. Most findings have led to worldwide recommendations to decrease the consumption of saturated fat to decrease the risk of CVD (Hoenselaar, 2012). Due to increasing awareness on the health implications of high cholesterol in the diets, most people now prefer to purchase cholesterol free vegetable oils.

Industrial processing especially catalytic hydrogenation of vegetable oils affects their fatty acid composition (Gur and Harwood, 1991). Processing increases saturated fatty acids component of oils. Saturated fatty acid rich diets have been found to increase the level of cholesterol. Thus, we are concerned by the fact that Ethiopian markets are flooded with assorted processed *vegetable oils from different parts of the world all labeled to be cholesterol free.*

A. Dietary Cholesterol

Blood cholesterol is a fatty substance found in the blood and is often referred to as *blood fat or blood lipid* (the medical term). High total blood cholesterol is a major risk factor for heart disease. The higher your total blood cholesterol level, the higher your risk of heart disease. High total blood cholesterol can gradually clog the blood vessels that supply blood to your heart and other parts of your body. If your blood vessels become clogged, it can reduce the blood flow to your heart and lead to symptoms such as angina (a condition marked by severe pain in the chest, arising from an inadequate blood supply to the heart). If a blood clot forms in the narrowed blood vessel and completely blocks the blood supply to part of your heart, it can cause a life-threatening heart attack (National Heart Foundation of Australia, 2009).

Generally, most part of the cholesterol in the human organism is produced endogenously, and approximately 30% is obtained by dietary intake (exogenous cholesterol) coming from foods of animal origin, such as eggs, meats, milk, oils and whole-fat dairy products. The amount of cholesterol obtained by dietary intake depends on the habitual diet and, an elevated intake of animal fats leads to high levels of cholesterol intake and, consequently, to high blood levels of cholesterol. Consequently, dietary intake of cholesterol must be controlled both at the individual and the population levels (Bauer *et al*., 2014).

B. Chemical Structure of Cholesterol

Cholesterol is the most common steroid in animals and the precursor for all other animal steroids. The numbering system for cholesterol applies to all such molecules. Many steroids contain methyl groups at positions 10 and 13 and an 8- to 10-carbon alkyl side chain at position 17. The polyprenyl nature of this compound is particularly evident in the side chain. Many steroids contain oxygen at C-3, either a hydroxyl group in sterols or a carbonyl group in other steroids. Significantly, the carbons at positions 10 and 13 and the alkyl group at position 17 are nearly always oriented on the same side of the steroid nucleus, the β-orientation. Alkyl groups that extend from the other side of the steroid backbone are in α-orientation (Garrett and Grisham, 2012).

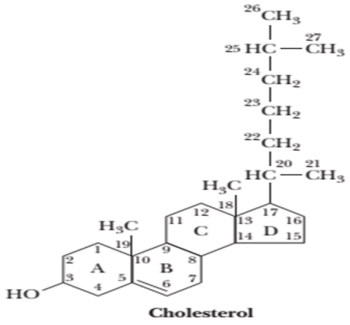

Figure 2: The structure of cholesterol and its steroid ring designations and carbon numbering.

C. Good Cholesterol and Bad Cholesterol

Cholesterol is a sterol, which is necessary in the body for producing cell membranes, some hormones and vitamin D. Our bodies would not normally be dependent on food for cholesterol, as the liver produces its own. However, cholesterol in the blood must be transported from cells and to cells by special carriers called lipoproteins. Cholesterol is carried in the bloodstream by high-density lipoproteins (HDL) and low-density lipoproteins (LDL). A lipoprotein is a combination of fat and protein as it moves through the body from your liver to other tissues. HDL cholesterol (HDL-C) is called “good” cholesterol. It helps to prevent cholesterol buildup on the walls of your arteries. A high reading of HDL-C in your blood helps protect your heart and arteries.

LDL cholesterol (LDL-C) is called "bad" cholesterol. It helps cholesterol stick to the inner walls of your arteries, which, together with other substances, eventually restricts the flow of blood. LDL-C readings in your blood should be low to help protect your heart and arteries (American Heart association, 2004). These are the form in which cholesterol travels in the blood. LDLs have little protein and high levels of cholesterol and HDL has a lot of Protein and very little cholesterol. LDL is the main source of artery clogging plaque. HDL actually works to clear cholesterol from the blood (Ma, 2006; Nelson and Cox, 2005). Diets high in trans fatty acids correlate with increased blood levels of LDL (bad cholesterol) and decreased HDL (good cholesterol), it is generally recommended that one avoid large amounts of these fatty acids (Nelson and Cox, 2005).

Table 1: Initial classification based on total cholesterol, HDL, LDL and Triglyceride (Ma, 2006).

Cholesterol	Cholesterol Level	Category
Total Cholesterol	Less than200 mg/dl	Desirable level.
	200 to239 mg/dl	Borderline highfor heartdisease.
	240 mg/dl and above	Highbloodcholesterol. A personwiththis level has more thantwice the risk of heartdiseaseas someone whosecholesterolis below 200 mg/dl.
HDL Cholesterol	Less than 40mg/dl	Low HDL cholesterol. A major risk factor for heart disease.
	40to 59mg/dl	he higher HDL level, the better.
	60mg/dl and above	High HDL cholesterol. An HDL of 60mg/dland aboveis considered protective against heart disease.
LDL Cholesterol	Less than100 mg/dl	Optimal
	100 to 129 mg/dl	Nearorabove optimal
	130 to 159 mg/dl	Borderlinehigh
	160 to 189 mg/dl	High
	190 mg/dland above	Very high
Triglyceride	Less than150 mg/dl	Normal
	150-199mg/dl	Borderline high
	200-499 mg/dl	High
	500 mg/dland above	Very high

Too much cholesterol in your blood together with other substances can contribute to a gradual buildup of plaque, which slows the flow of blood to the heart or the brain. Eating foods that contain cholesterol can raise your blood cholesterol. Eating meals (made from whole grains, fruits and vegetables) that are low in cholesterol and saturated fat helps to reduce the level of cholesterol in your blood (American Heart Association, 2004).

The standard test of cholesterol is done after a 9-12 hours fast without food, liquids or pills. It gives information about total cholesterol, LDL, HDL and triglycerides (blood fats). The cholesterol content in blood is the key data for the health information of cholesterol related (Table 1). A person's LDL cholesterol goal depends on how many other risk factors he/she has: (1) If he/she does not have coronary heart disease or diabetes and have one or no risk factors, his/her LDL goal is less than160 mg/dl. (2) If he/she does not have coronary heart disease or diabetes and have two or more risk factors, his/her LDL goal is less than130mg/dl. (3) If he/she has coronary heart disease or diabetes, his/her LDL goal is less than 100 mg/dl (Ma, 2006).

D. Causes and Factors of cholesterol accumulation

Diet: Foods high in saturated fats and cholesterol can increase your levels of LDL and total cholesterol.

Overweight: Excess weight can raise your LDL, total cholesterol and triglycerides.

Smoking: Tobacco smoke contributes to high triglycerides and low HDL levels.

Exercise: Physical activity can raise HDL, and the lack of physical exercise contributes to being overweight.

Genes: If your family members, especially your parents, have high LDL, high total cholesterol, high triglycerides or low HDL, you may inherit these conditions (American Heart Association, 2004).

II. WAYS OF DETERMINING CHOLESTEROL CONTENT, IODINE, ACID, SAPONIFICATION VALUE AND TEST FOR PRESENCE OF RANCIDITY

A. Liebermann- Burchard method

Cholesterol content can be estimated using Liebermann-Burchard reaction method (Attarde et al., 2010). The **Liebermann-Burchard reaction method** is a colorimetric method in which cholesterol is treated with chloroform, acetic anhydride and concentrated sulfuric acid to produce a green colour which is measured spectrophotometrically. The **Liebermann–Burchard** or **acetic anhydride test** is used for the detection of cholesterol. The formation of a green or green-blue colour after a few minutes is positive. Lieberman–Burchard is a reagent used in a colourimetric test to detect cholesterol, which gives a deep green colour. This colour begins as a purplish, pink colour and progresses through to a light green then very dark green colour. The colour is due to the hydroxyl group (-OH) of cholesterol reacting with the reagents and increasing the conjugation of the unsaturation in the adjacent fused ring. Since this test uses acetic anhydride and sulfuric acid as reagents, caution must be exercised so as not to receive severe burns (http://en.wikipedia.org/wiki/Liebermann%E2%80%93Burchard_test).

According to Dimberu and Belete (2011), oil samples that are contained cholesterol include cottonseed oil, 80% cottonseed + 20% rapeseed oil, rapeseed both branded and non-branded oils, olive oil, palm oil and sunflower oil. Among these, rapeseed oil has significantly maximum (257.10 ± 0.42 mg/L) cholesterol content and palm oil has significantly low (88.8 ± 0.85 mg/L) cholesterol content. Surprisingly, the Niger seed oils commonly sold in Ethiopia contained zero cholesterol content.

B. Iodine value

This method is frequently used to measure fat stability. The iodine value estimates the degree of unsaturation present in the fat or oil (Baião and Lara, 2005). Iodine is used to halogenate the double bonds present in unsaturated fatty acids (Stauffer, 2005). High content of unsaturated fatty acids indicates high iodine values (Okpuzor *et al.*, 2009).

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of the oil/fat, when determined by using Wijs solution. The oil/fat sample taken in carbon-tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulfate solution (I.S.I. Handbook of Food Analysis, 1984).

$$\text{Iodine value} = \frac{12.69\ (B - S)\ N}{W}$$

Where, B = volume in ml of standard sodium thiosulphate solution required for the blank.
S = volume in ml of standard sodium thiosulphate solution required for the sample.
N = normality of the standard sodium thiosulphate solution.
W = weight in g of the sample.

C. Acid value

The acid value is a measure of the amount of free fatty acids Present in the oil. It is determined as the amount (in milligrams) of potassium hydroxide (KOH) necessary to neutralize the FFAs in One gram of sample. This value is then converted to the percentage of FFAs in the sample. The quantity of sample needed varies from about 0.1 to 20g, depending on the expected acid value (Stauffer, 2005; http://www.word-to-pdf.abdio.com/). High acid value is the indication of high free fatty acid which in turn translates into decreased oil quality (Okpuzor *et al*., 2009).

The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution (I.S.I. Handbook of Food Analysis, 1984).

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where V = Volume in ml of standard potassium hydroxide or sodium hydroxide used
N = Normality of the potassium hydroxide solution or Sodium hydroxide solution;
W = Weight in g of the sample

D. Saponification value

The saponification value (SV) is a measure of the amount of alkali necessary to saponify all the triglycerides present in the sample. It is expressed as the amount (in milligrams) of potassium hydroxide (KOH) required to saponify one gram of the

sample (Stauffer, 2005).

Higher Saponification value indicates high proportion of lower fatty acids. If the saponification value of oil is lower, it suggests that it contains high molecular weight, long chain fatty acids hence are unsuitable for soap making and also unsuitable for human nutrition (Akinhanmi and Atasie, 2008). Therefore, shorter the average chain length (C4-C12) the higher is the saponification number (Muhammad et al., 2011).

Measurement of saponification value is performed according to the below listed official test methods. Here we test a sample of fatty acid. The sample is first saponified by adding 0.5mol/L potassium hydroxide ethanol, and then the excessive potassium hydroxide is titrated with 0.5mol/L hydrochloric acid until the endpoint is reached. End point is determined by the maximum inflexion point on titration curve (http://www.kyoto-kem.com, TIB-99307 Ver.01).

$$R\,COOH + KOH \rightarrow R\,COOK + H2O$$

$$KOH + HCl \rightarrow KCl + H2O$$

$$\text{Saponification Value} = \frac{56.1 \times (B-S) \times N}{W} \quad \text{(Official method 920.160, 2000)}$$

Where, B = Volume in ml of standard hydrochloric acid required for the blank.
S = Volume in ml of standard hydrochloric acid required for the sample
N = Normality of the standard hydrochloric acid and
W = Weight in gm of the oil/fat taken for the test.

E. Test for presence of Rancidity

In routine work apart from the free fatty acids determination, the analysis should include the determination of peroxide value and ultra-violet absorption at 234 m and 268 nm. to establish rancidity

F. Peroxide value

Peroxide value is an indication of the extent of oxidation suffered by oil. High peroxide value indicates high degree of unsaturation, which in turn responsible for oxidative rancidity (Dimberu and Belete (2011).
The test sample is first dissolved in mixture of chloroform and acetic acid (2:3). By flowing nitrogen gas through the sample to dispel residual oxygen, add potassium iodide, and then titrate free iodine with 0.01mol/L sodium thiosulfate. The endpoint is determined by the maximum inflexion point on titration curve (http://www.kyoto-kem.com, TIB-99309 Ver.01)

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

Peroxide value expressed as milli equivalent of peroxide oxygen per kg sample (meq/kg)

$$\text{Peroxide value} = \frac{titre \times N \times 100}{Weight\ of\ the\ Sample}$$

Where, Titre = ml of Sodium Thiosulphate used (blank corrected)

N = Normality of sodium thiosulphate solution.

Fresh oils usually have peroxide values below 10meq/kg. A rancid taste often begins to be noticeable when the peroxide value is above 20meq/kg (Official Method 965.33 Peroxide Value in Oils and Fats, 2000).

G. Ultra-violet absorption

Oxidised fatty acids containing conjugated double bonds absorb UV strongly between 230 and 375 nm, dienes absorbing at 234 nm and trienes at 268 nm. Conjugated trienes may be formed by industrial processing, e.g. decolorising with bleaching earths. A secondary absorption by trienes occurs at about 278 nm. In the early stages of oxidation the UV absorption increases somewhat proportionately to the uptake of oxygen and the formation of peroxides. The UV absorption curve forms plateau just before the end of the induction period. The magnitude of UV absorbance is not readily related to the amount of oxidation; so the method is best applicable to detecting relative changes in oxidation of oil in comparison experiments or stability tests (Manual Methods of

Analysis for Adulterants and Contaminants, 1990; Pearson's Composition and Analysis of Foods)

III. CONCLUSION

Cholesterol is a soft, fat-like, waxy substance found in the bloodstream and in all your body cells. As a lipid, or fat-like substance, it's an important part of a healthy body because it's used for building cells. But a high blood cholesterol level is a major risk for coronary heart disease, which can lead to heart attack. It's also a risk factor for stroke. One method of reducing high cholesterol levels is following a healthy diet by limiting foods high in cholesterol and saturated fats and eating more whole grains, fruits, vegetables and lean meats. Besides, It is better to use Niger seed oil among edible oils commonly sold in Ethiopia since it has high content of the essential fatty acid, which has the ability to decrease cholesterol levels and hence its content.

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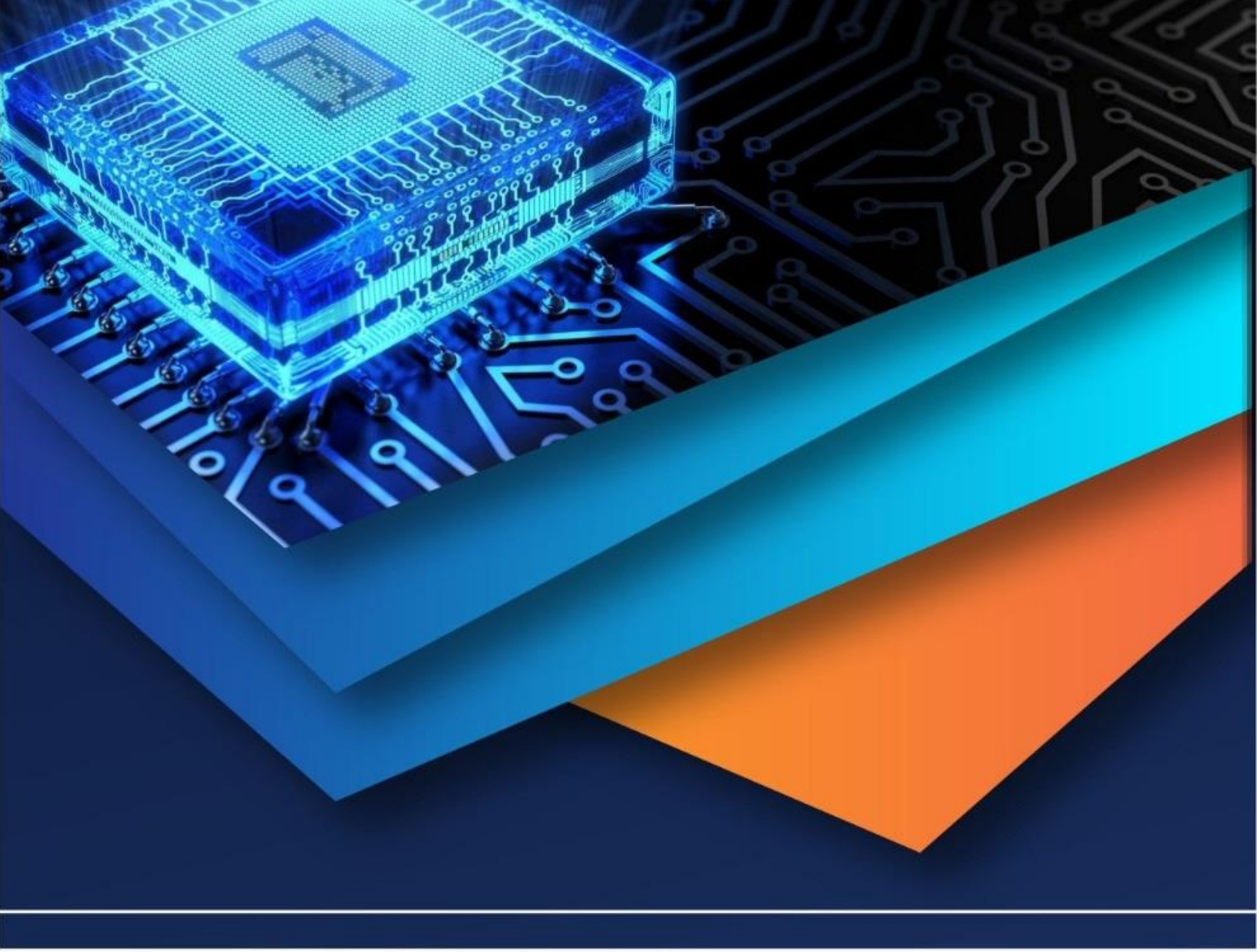

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