

Review

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Review

A General Review of Methodologies Used in the Determination of Cholesterol Levels in Foods

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Abstract: Cholesterol is a lipid-derived substance found in lipoproteins and cell membranes. It is also one of the main sources for the production of bile acids, vitamin D, and steroid hormones. Today, foods are evaluated by consumers not only according to their taste and nutritional content but also according to their effects on consumer health. For example, many consumers choose foods according to their cholesterol level. The cholesterol in the food can directly affect the blood cholesterol level when consumed, which can lead to cardiovascular diseases. High levels of cholesterol can lead to diet-related human diseases such as cardiac arrest, paralysis, type II diabetes cerebral hemorrhage. In societies with high living standards, interest in and consumption of foods that lower or have low cholesterol levels have increased recently. Accordingly, efforts to increase the variety of foods with reduced cholesterol levels are on the rise. This has indirectly insulted in accurate measurement of cholesterol levels in blood and food being of great importance. Classical chemical, enzymatic, colorimetric, polarographic, chromatographic, spectrophotometric methods, and enzymatic, nonenzymatic, and electrochemical sensors and biosensors are used for the determination of cholesterol in foods. The purpose of this review is to reveal and explore current and future trends in cholesterol detection methods in foods. The review will summarize the most appropriate and standard methods for measuring cholesterol in biological components and foods.

Keywords: cholesterol; foods; determination methods; disease

1. Introduction

Cholesterol represents the main part of milk fat sterols. It is a mono-olefinic, secondary, high molecular, and complex alcohol [1]. Cholesterol is an alcohol-like steroid found in human and animal organisms' cells and cell membranes and carried in the blood plasma. It is located in the structure of the cell wall and is known as one of the most essential sterols because it contributes to intercellular signal transmission (Figure 1). Cholesterol has a vital importance in the human metabolism. It was first discovered in gallstones, in 1754, hence its name was derived from the Greek words chole-(bile) and stereos (solid) and the suffix-ol in chemistry [2].

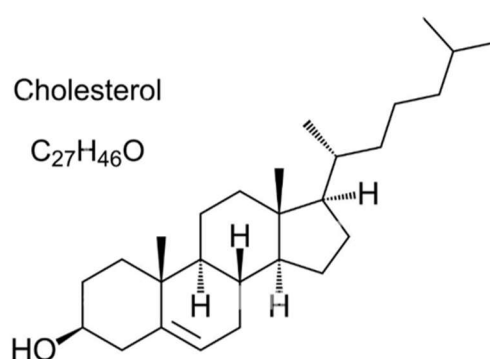


Figure 1. The general chemical structure of cholesterol.

Humans obtain cholesterol mainly from animal foods. However, a very small part of it in the body is obtained from food, and the rest of it is synthesized within the body. Although it is found in every cell of the body, its density is higher in organs and tissues where it is synthesized, especially in the liver, spinal cord, brain, and arteries [1–4]. Its function is to modulate dietary fat absorption in the small intestine (as well as bile acids). It is one of the basic components of nerves and cell membranes. It is also a precursor for steroid hormones produced in the adrenal cortex, female and male sex hormones, and vitamin D, and is an essential substance for the development and growth of mammals [1]. Apart from animals, cholesterol is an essential compound also found in plant and fungal organisms. It plays an important role in structure of the cell membranes, blood lipoproteins, and the biosynthesis of steroid hormones, vitamins, and bile acids. Its determination is very important not only for clinical examination but also for food quality control [7,8].

The presence of high levels of cholesterol in the blood, results in its builds up on the walls of blood vessels and causing hardening and narrowing of the arteries, a medical condition called atherosclerosis. There are generally two types of cholesterol, defined by their carrier molecules. High levels of cholesterol carried by low-density lipoprotein (LDL), mostly referred to as the 'bad cholesterol' is the most harmful. The other type, carried by high-density lipoprotein (HDL) is called 'good cholesterol'. HDL is commonly found in the brain synapses, playing a key role in the immune system and protection against cancer [4]. Higher cholesterol concentrations in the blood, especially the ones associated with LDL, cause some health problems including arteriosclerosis, hypertension, and coronary heart disease [9–13]. On the other hand, low concentration of cholesterol can cause anemia and fatigue syndrome. In addition, cholesterol sometimes combines with bile pigments and leads to the complications of gallstones [6,14–16].

The normal level of cholesterol in a normal adult human body ranges from 150-200 mg/dL. Significant amounts of cholesterol are synthesized by the body per day to compensate for the loss occurring in excretion and other ways [17–19]. A daily intake of cholesterol of about 300mg for adults is recommended by food safety authorities [20,21].

To control cholesterol levels in the body, it is necessary to pay attention to several factors. These include but are not limited to food and nutrition as well as lifestyle such as habits that control blood pressure and body weight including smoking habits and increasing physical activity. This lowers plasma cholesterol concentrations and plasma lipids [22].

Dietary habits have a significant effect on the amount of cholesterol in the blood. Dietary intake of high-animal-fat foods instead of plant-based intake of fruits, vegetables and dairy products causes an increase in the level of cholesterol in the blood [23]. Although it has been stated that dietary fat intake has positive effects on human health, it is known to have a very significant effect on coronary heart disease. Determining total cholesterol in serum is important for clinical measurements. The high amount of cholesterol accumulates on the walls of various vessels and causes closure of the

vessels, and as a result of the thyroid gland working a little, it causes thyroid disorder, diabetes, and jaundice [24]. The amounts of cholesterol in different foods are given in Table 1.

Table 1. Cholesterol amounts in different foods.

Foods	Cholesterol (mg/100g)
Brain	2353
Egg yolk	1260
Kidney	803
Egg	396
Liver	360
Butter	240
Cheese	160
Cream	109
Veal	100
Chicken meat	98
Beef	60
Breast milk	25
Cow's milk	12.3
Yogurt	12.2
Skim milk	3

Accurate measurement of cholesterol in the body system is important in the diagnosis and treatment of cholesterol-based conditions. Modern amperometric and voltammetric measurement techniques are increasingly used in the measurement of cholesterol. Basic research on biological materials and chemicals has been the driving force in the development and application of analytical technologies for the determination of cholesterol [25].

Measuring the presence of cholesterol in foods has been the subject of extensive research. In general, the production of electrodes for cholesterol detection is of great importance in terms of clinical tests. Additionally, monitoring the cholesterol level in blood and food is a critical parameter for diagnosing and preventing many diseases [26]. Researchers have thus far developed different methods to monitor cholesterol, including colorimetry [4,27]. These are chromatographic [28–30], fluorometric [31,32] and chemiluminescent methods [33]. In general, many of these mentioned methods require expensive instrumentation, complex preparation procedures to precipitate lipoproteins, or lack accepted sensitivity and selectivity.

Various colorimetric, polarographic, chromatographic, spectrophotometric, and biosensor methods are used in the determination of cholesterol. However, these are generally time-consuming and expensive systems. Measurements made by gas and liquid chromatography are the most suitable for cholesterol detection in terms of separating cholesterol from other similar compounds as well as determining its quantity [34]. Therefore, it is of great importance to develop systems that can determine the amount of cholesterol more accurately, in a shorter time, and at a lower cost. Generally, conventional methods such as colorimetry, spectrophotometry, fluorimetry, polarography, thin layer chromatography, gas chromatography, and high-performance liquid chromatography are used to measure cholesterol levels in the sample under investigation [35,36]. The majority of current sensors used are capable of adequately detecting free or esterified cholesterol. However, their originality is poor, complex, expensive, labor-intensive, and time-consuming [18]. In the last decade, enzymatic, non-enzymatic, and redox mediator-based sensors have been developed for cholesterol detection in enzymatic systems, enzymes such as cholesterol oxidase (ChOx) or cholesterol esterase (CE) catalyze the hydrolysis of cholesterol ester, resulting in the formation of fatty acids and free cholesterol. Because of the complexity of the matrix, chromatographic measurements with different types of detectors are the most common methods for cholesterol determination [37,38]. The main advantages of chromatographic methods are high selectivity, low LOD (depending on the detection used), and

high accuracy. On the other hand, these techniques traditionally have disadvantages such as high cost and high personnel requirements [39,40].

Sensors that can only measure cations and anions with the classical electrochemistry system have enabled the determination of many substances with the inclusion of biomaterial in the system. Biosensors finds usage areas in applications such as bacteria and virus diagnosis, agriculture, veterinary, biomedical sector, toxic gas analysis in mining enterprises, food production and analysis, drug analysis, military applications, process control, environmental protection and pollution control, clinical diagnosis, bioreactor control, agriculture veterinary, and industrial waste. With the developing technology, biosensors, especially enzymatic biosensors, are used in hospitals and the food field. Biosensors can be used for complex parameters such as the detection of foreign substances in foods, freshness, and aroma control. In addition to this, biosensors can be used in the fight against drugs and preventing the misuse of drugs [41]. Although biosensors have high specificity and speed, they require the development of an enzyme stabilization method. This is one of the main disadvantages of this type of system. The instability of the enzyme poses a problem in obtaining accurate and precise results [42]. Proper orientation and high surface biocompatibility of the enzyme play an important role in facilitating electron transfer between the enzyme and the electrode surface. In these sensors, random orientation may lead to a decrease in the concentration of active enzymes on the electrode surface, which may result in a decrease in the sensitivity of the biosensor used [43]. In this regard, it is clear that non-enzymatic sensors that provide direct cholesterol signals have some advantages compared to biosensors, such as more advanced methodological features, simple structure, low cost, and long shelf life.

This study aims to reveal the methods applied for the determination of cholesterol in food matrices and to reveal simple, fast, and sensitive methods by scanning different literature and using the data obtained in these studies.

2. Cholesterol Determination Methods in Foods

Recently, many new methods have been developed to detect the amount of cholesterol in various materials. Cholesterol can be detected by a variety of analytical methods, such as gravimetry, colorimetry, fluorimetry, chromatography, and enzymatic and non-enzymatic sensors. These methods can be examined in four main categories: 1) Classic chemical tests, 2) Colorimetric and fluorometric enzymatic tests frequently used in test kits and automatic plate readers, 3) Analytical methods such as gas and liquid chromatography or mass spectrometry, and 4) Enzymatic and non-enzymatic sensors.

Classical chemical methods are relatively simple and inexpensive to apply compared to others but require multi-step procedures. Enzymatic assays involve the use of expensive enzymes, but their limits of detection (LOD) are generally low. Methods such as chromatography and mass spectrometry are the most accurate and sensitive methods. However, they require expensive equipment and long sample preparation preprocessing [44].

Details of these methods are explained comprehensively in the following subsections.

2.1. Detection of Cholesterol Using Enzymatic and Non-Enzymatic Methods

A wide variety of methods have been reported to monitor cholesterol in biological fluids and foods [45–48]. Enzymatic and non-enzymatic electrochemical determination methods to determine cholesterol have also been recently introduced [20].

Cholesterol was thought to be electrochemically inactive until the late 1980s [49]. In 2005, methods that first provided direct oxidation of cholesterol and then indirect cholesterol oxidation methods (using an electron mediator) were developed [50,51].

Cholesterol oxidase, less commonly cholesterol esterase, and cholesterol modified with nanomaterials have been widely evaluated as sensing materials in electrochemical sensors [43]. Each of these approaches varies significantly and their general use is quite limited because cholesterol oxidation products must be identified separately for each chosen electrolysis environment. Many

new methods have been introduced for the characterization of direct oxidation products of cholesterol in non-aqueous media [49,51,52].

2.2. Determination of Cholesterol in Foods by Enzymatic Methods

This type of analysis relies on the presence of an enzymatic reaction to determine total cholesterol levels in food or other materials. The first enzymatic test capable of detecting cholesterol in serum was introduced in 1974 [53]. Enzymatic tests have been widely used since then, and more recently test kits and automated analyzers are now widely used [54]. In these methods, esterified cholesterol is first hydrolyzed to free cholesterol by cholesterol esterase. The resulting free cholesterol is then oxidized to cholesta-4-en-3-one by the cholesterol oxidase enzyme. Hydrogen peroxide is produced as a byproduct in this reaction and can be easily detected using high-sensitivity colorimetric or fluorometric probes [53,55,56].

2.3. Detection of Cholesterol in Foods by HPLC

2.3.1. Detection of Cholesterol in Foods by HPLC

The most common techniques for nonenzymatic removal of cholesterol from foods are HPLC and other techniques such as GC-MS and LC-MS [30,57].

These mentioned methods are extremely sensitive and selective; however, they require complex sample preparation procedures and expensive equipment. Measurement of total cholesterol is generally made using chromatographic or enzymatic methods. The most commonly used method is gas chromatography, although some HPLC methods are also popular [58,59]. However, the determination of cholesterol by HPLC has received less attention than the measurement by GC. The application of HPLC with UV detection to determine cholesterol has been limited in a complex sample environment, as the poor absorption of cholesterol at low wavelengths poses a problem in spectrophotometric detection [60]. It has been determined that gas chromatography (GC) is more sensitive, especially in determining cholesterol in food matrices [61–66]. These drawbacks encountered in HPLC measurements can be easily overcome by using high-performance liquid chromatography (HPLC) and especially reversed-phase HPLC. Additionally, the main advantage of HPLC over GC is that it is performed at low temperatures and prevents cholesterol oxidation [67].

Compared to HPLC, cholesterol determination by GC is more laborious. For example, it has disadvantages such as derivatization of cholesterol compounds, checking the reliability of measurement using internal standards before use, sample preparation being time-consuming (involving steps such as saponification and extraction, and a chromatographic run takes approximately 25 minutes), and a costly method. In addition, the GC instrument is operated at a higher temperature than HPLC, which induces the formation of cholesterol oxides [30,67].

UHPLC and HPLC may be ideal analytical techniques for measuring cholesterol in food matrices because they are more sensitive, cost-effective, and less time-consuming than other traditional methods.

The most common detector used for cholesterol detection in HPLC is the diode array detector (DAD). Studies have found that the most suitable HPLC detector to measure cholesterol levels in foods is a Diode array detector (DAD), and other detectors such as ultraviolet (UV), fluorescence detection, evaporative light scattering detection, infrared detection, and electrochemical detection have also been reported to be suitable for measurement [30,61–63,66,68,69]. Reverse-phase HPLC combined with a UV or DAD detector is the most common technique. In the literature, it has been reported that columns with a particle size of 5 microns can be used in most analytical methods, but it has been stated that the use of columns with a particle size of 3 or 4 microns may be appropriate [70].

The most convenient and often used method for sample preparation in liquid chromatography is direct saponification of the sample followed by extraction of the unsaponifiable residue into a nonpolar solvent [71]. In this method, direct saponification is preferred because of the possibility of converting non-polar fatty acid esters to polar products by effectively removing them by multiple

extractions with n-hexane [30,71,72]. Different options for extraction include single-stage extraction with toluene [68] or three-stage extraction with diethyl ether. It is also recommended to use a mixture of polar and non-polar solvents to enable more efficient extraction of cholesterol from food matrices where cholesterol is often bound by many other biological compounds such as proteins, lipoproteins, and phospholipids [68].

Saponification and extraction processes are very important in determining the cholesterol ratio by HPLC. The saponification process is one of the most important steps in obtaining cholesterol purified from other components. In this process, potassium hydroxide is the most common solvent used to separate cholesterol from fatty acids [61,68,73]. The mixture is then washed with ultrapure water to remove these compounds, and the resulting cholesterol remains in the extracted solution layer for analysis [61]. Hexane is the most common reagent used for the extraction of cholesterol in complex food matrices such as egg yolks, due to its low polarity compared to toluene, which provides emulsion.

2.3.2. Detection of Cholesterol in Foods with GC-MS

Gas chromatography (GC) is also one of the most commonly used analytical techniques for the quantification of cholesterol and other sterols. However, although GC columns are a very effective method for the separation of cholesterol, sometimes problems may arise in the separation of cholesterol due to its similarities with other sterols [68,73].

It is widely accepted that this method is more reliable, sensitive, and accurate than other methods. In chromatographic columns, additional selectivity can be added to separate cholesterol from inhibitory sterols. Additionally, another advantage of this method is that a low volume of sample (tens of mL) is required for analysis [74].

The AOAC has published 994.10, an official method for the analysis of cholesterol in foods by GC-flame ionization detector after saponification and derivatization with trimethylchlorosilane [75]. In general, procedures applied in GC require extraction of total lipids, separation of solvents, saponification of cholesterol esters, detailed solvent extraction of unsaponifiable material, repeated washing concentration of the analyte, and appropriate derivatization before GC analysis [75]. These process steps are quite burdensome in terms of both labor and materials and require increasingly expensive supply, recovery, and disposal costs. Although newer methods based on direct saponification of the sample have been developed and some steps have been eliminated, this method remains laborious and costly [76]. Although the lipid extraction and saponification steps are laborious, the derivatization reagents are unstable, and cholesterol is thermally decomposed in the GC column, the method is based on sound scientific principles. This method is still considered one of the reliable methods.

2.3.3. Electrospray Ionization Tandem Mass Spectrometer (ESI)

ESI is not an effective method for ionization to measure neutral-free sterol molecules such as cholesterol. The molecular ions of the sterol molecules are easily fragmented in the $[M+H]^+$ ion source. For that reason, they are very difficult to detect in the matrix with this method. In contrast, cholesterol ester species tend to form more stable ammonium adduct ions $[M+NH_4]^+$, which can be detected successfully [77].

2.3.4. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI)

MALDI is an effective ionization method for neutral-free sterol molecules. Recent studies have provided increasing evidence that MALDI-MS can be used for cholesterol measurements. In a study conducted by Hidaka et al. [78], MALDI-TOF (time-of-flight) MS was used to analyze cholesterol in human serum lipoproteins.

2.3.5. Ambient Ionization Mass Spectrometer

In recent years, a method known as ambient ionization mass spectrometry (AIMS) has been developed for the chemical analysis of cholesterol in biological systems [79]. The ambient ionization technique operates at atmospheric pressure, is real-time, and requires minimal sample pretreatment for rapid mass spectrometric analysis. Desorption electrospray ionization (DESI) can be used directly for real-time direct analysis (DART) and quantitative cholesterol measurements. It is also a twice as fast technology that is more effective than direct ESI approaches for cholesterol ionization [80].

2.3.6. Removal of cholesterol from foods by nonenzymatic methods

Cholesterol replacement therapy is required for the treatment of some inborn errors of metabolism. Therefore, most cholesterol determination techniques target blood and blood serum. Determination of cholesterol in foods is extremely important for health. Over the last decade, a wide variety of cholesterol sensors have been developed, including enzymatic, non-enzymatic, and redox mediator-based sensors.

In enzymatic systems, the hydrolysis of cholesterol ester is catalyzed by using enzymes such as cholesterol oxidase (ChOx) or cholesterol esterase (CE), resulting in the formation of fatty acids and free cholesterol. Enzymatic sensors demonstrate high sensitivity and selectivity. However, the enzymes have a short lifespan and are easily denatured during immobilization. Additionally, their activities are easily affected by temperature, pH value, and toxic chemical [81,82]. Non-enzymatic cholesterol sensors have eliminated the limitations and problems caused by enzymatic ones. Electrode surfaces modified with metals, metal oxides, or composites have electrocatalyst functions. The most important feature of non-enzymatic electrodes is the use of nanomaterials with high surface-to-volume ratios that provide good interaction with external reagents, high conductivity, and excellent biocompatibility. Therefore, they are interesting components for bringing electrochemical devices to the nanoscale [83]. In this regard, it is very important to understand the latest developments in nanomaterial-based electrochemical cholesterol sensors and to follow future developments. The main feature of non-enzymatic electrodes is the use of high surface-volume nanomaterials that interact well with external reagents, have high conductivity and good biocompatibility. Electrochemical and nanomaterial-based sensors represent an important alternative to traditional methods in terms of their low cost, portability, high sensitivity, stability, and repeatability [84].

In recent years, electrochemical sensors have been accepted as a good alternative to traditional methods due to their low cost, reduced size, portability, high sensitivity, and shorter detection time [70,85]. Due to these properties, it may be very useful to develop an electrochemical sensor that can detect cholesterol in food and clinical samples. The most important consideration when developing a sensor system for any material is sensitivity. Various redox nanoparticles, including metallic nanoparticles and nanowires magnetic nanoparticles, carbon nanomaterials, conductive polymers, or their hybrids are used to increase sensitivity due to rapid electron transfer, which facilitates the conversion of an irreversible oxidation process into a reversible one [6,70,86–90].

2.4. Electrochemical Sensors

Electrochemical sensors are preferred because they are simple, require low cost, have high accuracy, and have high and fast sensitivity levels. Carbon-based electrodes are widely used in electrochemical analysis because of their advantages such as ease of surface modification and low background current [91,92].

The electron transfer rates of electrochemical sensors are generally lower than electrodes made of noble metal [93]. The electrochemical activity of carbon-based electrodes against some analytes can be increased by anodic oxidation of the surfaces, resulting in the formation of new oxidized functional groups. In bare electrodes, high voltage is required for cholesterol oxidation [49]. Overvoltage occurring in chemically modified electrodes (CMEs), popular in electro-analytical chemistry, can be significantly reduced using electrochemical sensors [92–95] reported that they obtained a new sensor

for rapid cholesterol determination by electrochemical deposition of 1,4-diacetylglycoluril (DPADGU) diphosphonic acid onto the carbon-containing electrode surface. The DPADGU used here catalyzes the oxidation of cholesterol. The electrochemical oxidation step is a complex process and is controlled by both diffusion and adsorption. However, the prepared sensor proved to be highly selective when tested in the presence of complex substances such as cholecalciferol, retinyl acetate, tocopherol acetate, albumin, lactose, uric acid, lactic acid, and ascorbic acid. Under optimum operating conditions (phosphate buffer pH 6.8), the developed sensor demonstrated reliable linear DPV response from 1 to 200 $\mu\text{mol L}^{-1}$, high sensitivity of 20 $\mu\text{A mol}^{-1} \text{cm}^{-2}$, low detection limit of 1.53 μM , and reasonable selectivity. This sensor, which has good performance, simple manufacturing, and low cost, is very promising for the food industry [93,94].

2.4.1. Nanomaterial-Based Electrochemical Sensors

Electrochemical sensors are very attractive methods for detecting glucose, cholesterol, cancer, and infectious diseases due to their high sensitivity, low cost, functionality, easy microfabrication to nanosize, low energy requirements, and easy control. The development of nano biosensors is fraught with enormous challenges, such as ensuring longevity and native biocompatibility with monitoring of analytes. Additionally, random signals resulting from nonspecific adsorption events can cause significant problems, especially in diagnostic analyses. In nanotechnological sensors, it is necessary to create and design structures with a size below 100 nm. Changes can be made in the optical, magnetic, and electrical properties, sizes, shapes, and compositions of nanostructures. These attributes can facilitate improvements in biocompatibility, sensitivity, and specific binding of biomaterials [93,94].

Electrochemical biosensors (EBS) have recently received great attention for the management of cholesterol (CHO) in healthcare. Quantitative analysis of cholesterol is performed using amperometric data of the linear calibration chart. Determining the cholesterol in the blood is very important to control the health problems that may occur when it reaches abnormal levels. Electrochemical sensors have been recognized as highly selective methods for cholesterol detection due to their simplicity, low cost, wide working range, and high sensitivity. These sensors are analytical device that combines the specificity of biomolecules with an electronics-based physicochemical transducer to convert a biochemical signal into a measurable electrical signal. A conventional EBS contains three electrodes, a saturated Ag/AgCl electrode, a platinum wire, and a glassy carbon electrode or metal plate electrode, which serve as a reference and counter electrode, respectively. In achieving EBS, cholesterol oxidase is widely used as a biosensing material immobilized by a different type of electroactive material. Metal nanoparticles were effectively immobilized with cholesterol oxidase due to their high conductivity properties with different binders and were used in the surface modification of working electrodes to detect cholesterol. Metal nanoparticles have been reported to be used in various combinations with conductive polymers, graphene, chitosan, and graphene oxide carbon nanotubes with high electrical conductivity, effective surface area, and rapidity to improve EBS performance [96].

The effectiveness and efficiency of EBS generally depend on the modification of electrode surfaces working with different electroactive coating materials. The morphology of the produced working electrode is characterized by scanning electron microscopy, transmission electron microscopy, atomic force microscopy, X-ray diffraction, and energy-dispersive X-ray spectroscopy. The performance of the produced electrodes for determining the cholesterol detection amount is investigated by square wave voltammetry and differential pulse voltammetry methods [93,94,96].

Functional nanomaterials have attracted significant attention in various research fields (especially in the healthcare system) due to their easy control, high chemical and environmental stability, biocompatibility, and unique optoelectronic and sensing properties. The recognition properties of nanomaterials can be used to identify biomolecules such as cholesterol. In the last decade, significant advances have been made in the production of cholesterol biosensors, the main component of which is nanomaterials [43,84].

2.5. Possibilities of using biosensors in measuring cholesterol in foods

Among the various existing methods used to detect cholesterol, biosensors are a relatively simpler, faster, sensitive, and specific method [47,97]. Biosensors are analytical devices that consist of a transducer and a biological element. Bioelements such as enzymes, antibodies, nucleic acids, receptors, organelles, or microorganisms interact with the analyte under test, and the concentration of substances or other biological response parameters are converted into an electrical signal [98].

In biosensor production, immobilization of enzymes on electrodes is important. The high performance of the amperometric biosensor simply immobilizes the enzyme on the electrode and stimulates electron transfer in sensor fabrication using mediators, promoters, or other special materials. The majority of cholesterol biosensors have been developed based on the electrochemical reduction of hydrogen peroxide (H₂O₂) due to their simplicity and specificity [9].

Biosensors can be used in the fabrication of biosensing devices due to their biocompatibility, high viscosity, non-toxicity, and solubility in acidic aqueous environments. Redox-active enzymes are often preferred to test the electron activity and catalytic abilities of cholesterol [99]. Electrochemical biosensors fabricated based on immobilized enzymes have found wide applications for the detection of large masses of biological substrates. Newer systems do not use intermediaries but directly monitor the redox behavior of the enzyme at the electrode surface. This behavior produces or consumes electrons and can increase signal conduction at the working electrode. Gholivand and Khodadadian immobilized ChOx and catalase (CAT) on a graphene/ionic liquid-modified glassy carbon electrode (GR-IL/GCE) [100].

In a study conducted by Ferri et al. [101], the behavior of a multi-enzymatic electrodetetic system for the detection of glucose and choline based on horseradish peroxidase (HRP) was investigated. The electrodic system captured by horseradish peroxidase quickly detected the presence of these analytes, which are involved in the reactions where hydrogen peroxide is produced, even without the need for additional mediators [102].

Enzymatic sensors exhibit high sensitivity and selectivity. On the other hand, enzymes have a short lifespan and are easily denatured during immobilization. Moreover; Their activities are affected by temperature, pH value, and toxic chemicals [81,82].

2.5.1. Metal Nanoparticle (Mnp) Based Cholesterol Biosensors

MNP has very good electronic properties and for this reason, its use in the production of various biosensors has been investigated [103]. Electrodes coated with up to 0.5 nM Pt/Graphene and Pt/CNT have been developed for amperometric measurement used to detect cholesterol. It has been reported that cholesterol can be detected as specific molecules down to the nano level by digitalonin gold nanoparticles [104]. These results allowed us to elucidate various aspects of redox, pointing to a process involving the biosystems under study and the potential of these polymers for the construction of next-generation biosensors. The excellent sensitivity of cholesterol was obtained from the biosensor based on the enzyme-immobilized microtubular ZnO/ZnS heterostructure [105].

The electrochemical photoelectrochemical cholesterol biosensor based on graphene-embedded titanium nanowires has also been reported to have an outstanding sensitivity of 6 μ M [106]. Cholesterol biosensors with graphene oxide and Pd nanostructure have been successfully used for the analysis of total cholesterol in human serum and milk fat [107]. Wang et al. [108] used a nanocomposite consisting of molybdenum disulfide nanoparticles for ultra-measurement of cholesterol. Current developments in electrochemical biosensors are characterized by an application-oriented approach, and studies have led to the formation of miniaturized biosensors by transforming electrode surfaces.

3. Conclusions

This review gives detailed information about the methods used to determine cholesterol in foods. In addition, the strengths and weaknesses of the mentioned methods are revealed. Comparisons were also made about the sensitivity levels of the Classical chemical, enzymatic,

colorimetric, chromatographic, and spectrophotometric methods, and also enzymatic, nonenzymatic, and electrochemical sensors and biosensors.

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References

1. Süt Teknolojisi Sütün Bileşimi Ve İşlenmesi - Mustafa Metin | Nadir Kitap Available online: <https://www.nadirkıtap.com/sut-teknolojisi-sutun-bilesimi-ve-islenmesi-mustafa-metin-kitap26632213.html> (accessed on 20 October 2023).
2. Wang, H.H.; Li, T.; Portincasa, P.; Ford, D.A.; Neuschwander-Tetri, B.A.; Tso, P.; Wang, D.Q.-H. New Insights into the Role of Lith Genes in the Formation of Cholesterol-Supersaturated Bile. *Liver Res* **2017**, *1*, 42–53, doi:10.1016/j.livres.2017.05.005.
3. Ahmadalinezhad, A.; Chen, A. High-Performance Electrochemical Biosensor for the Detection of Total Cholesterol. *Biosensors and Bioelectronics* **2011**, *26*, 4508–4513, doi:10.1016/j.bios.2011.05.011.
4. Hayat, A.; Haider, W.; Raza, Y.; Marty, J.L. Colorimetric Cholesterol Sensor Based on Peroxidase like Activity of Zinc Oxide Nanoparticles Incorporated Carbon Nanotubes. *Talanta* **2015**, *143*, 157–161, doi:10.1016/j.talanta.2015.05.051.
5. Alexander, S.; Baraneedharan, P.; Balasubrahmanyam, S.; Ramaprabhu, S. Modified Graphene Based Molecular Imprinted Polymer for Electrochemical Non-Enzymatic Cholesterol Biosensor. *European Polymer Journal* **2017**, *86*, 106–116, doi:10.1016/j.eurpolymj.2016.11.024.
6. Huang, Y.; Cui, L.; Xue, Y.; Zhang, S.; Zhu, N.; Liang, J.; Li, G. Ultrasensitive Cholesterol Biosensor Based on Enzymatic Silver Deposition on Gold Nanoparticles Modified Screen-Printed Carbon Electrode. *Materials Science and Engineering: C* **2017**, *77*, 1–8, doi:10.1016/j.msec.2017.03.253.
7. Derina, K.V.; Korotkova, E.I.; Dorozhko, E.V.; Voronova, O.A. Voltammetric Determination of Cholesterol in Human Blood Serum. *J Anal Chem* **2017**, *72*, 904–910, doi:10.1134/S1061934817080068.
8. Derina, K.; Korotkova, E.; Barek, J. Non-Enzymatic Electrochemical Approaches to Cholesterol Determination. *Journal of Pharmaceutical and Biomedical Analysis* **2020**, *191*, 113538, doi:10.1016/j.jpba.2020.113538.
9. Aravamudhan, S.; Ramgir, N.S.; Bhansali, S. Electrochemical Biosensor for Targeted Detection in Blood Using Aligned Au Nanowires. *Sensors and Actuators B: Chemical* **2007**, *127*, 29–35, doi:10.1016/j.snb.2007.07.008.
10. Shen, J.; Liu, C.-C. Development of a Screen-Printed Cholesterol Biosensor: Comparing the Performance of Gold and Platinum as the Working Electrode Material and Fabrication Using a Self-Assembly Approach. *Sensors and Actuators B: Chemical* **2007**, *120*, 417–425, doi:10.1016/j.snb.2006.02.035.
11. Saxena, U.; Chakraborty, M.; Goswami, P. Covalent Immobilization of Cholesterol Oxidase on Self-Assembled Gold Nanoparticles for Highly Sensitive Amperometric Detection of Cholesterol in Real Samples. *Biosensors and Bioelectronics* **2011**, *26*, 3037–3043, doi:10.1016/j.bios.2010.12.009.
12. Hong, L.; Liu, A.-L.; Li, G.-W.; Chen, W.; Lin, X.-H. Chemiluminescent Cholesterol Sensor Based on Peroxidase-like Activity of Cupric Oxide Nanoparticles. *Biosensors and Bioelectronics* **2013**, *43*, 1–5, doi:10.1016/j.bios.2012.11.031.
13. Front Matter. In *Clinical Veterinary Advisor*; Mayer, J., Donnelly, T.M., Eds.; W.B. Saunders: Saint Louis, 2013; pp. i–iii ISBN 978-1-4160-3969-3.
14. Gylling, H. Cholesterol Metabolism and Its Implications for Therapeutic Interventions in Patients with Hypercholesterolaemia. *International Journal of Clinical Practice* **2004**, *58*, 859–866, doi:10.1111/j.1742-1241.2004.00351.x.
15. Lee, A.; Griffin, B. Dietary Cholesterol, Eggs and Coronary Heart Disease Risk in Perspective. *Nutrition Bulletin* **2006**, *31*, 21–27, doi:10.1111/j.1467-3010.2006.00543.x.

16. Alderton, B.A.; Ball, J.W.; Barbon, A.R.; Batchelder, M.; Beaufrère, H.; Bingley, M.; Blanco, M.C.; Bays, T.B.; Brown, C.J.; Burgdorf-Moisuk, A.; et al. Contributors. In *Clinical Veterinary Advisor*; Mayer, J., Donnelly, T.M., Eds.; W.B. Saunders: Saint Louis, 2013; pp. ix–xii ISBN 978-1-4160-3969-3.
17. Motonaka, Junko.; Faulkner, L.R. Determination of Cholesterol and Cholesterol Ester with Novel Enzyme Microsensors. *Anal. Chem.* **1993**, *65*, 3258–3261, doi:10.1021/ac00070a015.
18. Sekretaryova, A.N.; Beni, V.; Eriksson, M.; Karyakin, A.A.; Turner, A.P.F.; Vagin, M.Yu. Cholesterol Self-Powered Biosensor. *Anal. Chem.* **2014**, *86*, 9540–9547, doi:10.1021/ac501699p.
19. Şanlıdere Aloglu, H.; Öner, Z.; Demir Özer, E.; Uz, E. Gıda kaynaklı mayaların in vitro da kolesterolü asimile etme özelliklerinin belirlenmesi ve yüksek kolestorel içeren diyabetle sıçarlarda kolesterolü düşürücü ajan olarak kullanılabilirliğinin araştırılması. *undefined* **2013**, 1–73.
20. An Overview on Electrochemical Determination of Cholesterol - Amiri - 2020 - Electroanalysis - Wiley Online Library Available online: <https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/elan.201900669> (accessed on 20 October 2023).
21. Li, Y.; Cai, J.; Liu, F.; Yang, H.; Lin, Y.; Li, S.; Huang, X.; Lin, L. Construction of a Turn Off-on Fluorescent Nanosensor for Cholesterol Based on Fluorescence Resonance Energy Transfer and Competitive Host-Guest Recognition. *Talanta* **2019**, *201*, 82–89, doi:10.1016/j.talanta.2019.03.110.
22. Demirci, M.; Gölbaş, M.; Başoğlu, F. Gıdalardan Kolesterol Azaltılabilir mi? *GIDA* **1996**, 21.
23. Huber, W.; Molero, A.; Pereyra, C.; Martínez de la Ossa, E. Dynamic Supercritical CO₂ Extraction for Removal of Cholesterol from Anhydrous Milk Fat. *International Journal of Food Science & Technology* **1996**, *31*, 143–151, doi:10.1111/j.1365-2621.1996.326-28.x.
24. Lunn, J.; Theobald, H.E. The Health Effects of Dietary Unsaturated Fatty Acids. *Nutrition Bulletin* **2006**, *31*, 178–224, doi:10.1111/j.1467-3010.2006.00571.x.
25. Akinwunmi, I.; Thompson, L. d.; Ramsey, C. b. Marbling, Fat Trim and Doneness Effects on Sensory Attributes, Cooking Loss and Composition of Cooked Beef Steaks. *Journal of Food Science* **1993**, *58*, 242–244, doi:10.1111/j.1365-2621.1993.tb04247.x.
26. Nirala, N.R.; Abraham, S.; Kumar, V.; Bansal, A.; Srivastava, A.; Saxena, P.S. Colorimetric Detection of Cholesterol Based on Highly Efficient Peroxidase Mimetic Activity of Graphene Quantum Dots. *Sensors and Actuators B: Chemical* **2015**, *218*, 42–50, doi:10.1016/j.snb.2015.04.091.
27. Nirala, N.R.; Saxena, P.S.; Srivastava, A. Colorimetric Detection of Cholesterol Based on Enzyme Modified Gold Nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2018**, *190*, 506–512, doi:10.1016/j.saa.2017.09.058.
28. Okazaki, M.; Usui, S.; Nakamura, M.; Yamashita, S. Evaluation of an HPLC Method for LDL-Cholesterol Determination in Patients with Various Lipoprotein Abnormalities in Comparison with Beta-Quantification. *Clinica Chimica Acta* **2008**, *395*, 62–67, doi:10.1016/j.cca.2008.05.008.
29. Wu, W.-F.; Wang, Q.-H.; Zhang, T.; Mi, S.-H.; Liu, Y.; Wang, L.-Y. Gas Chromatography Analysis of Serum Cholesterol Synthesis and Absorption Markers Used to Predict the Efficacy of Simvastatin in Patients with Coronary Heart Disease. *Clinical Biochemistry* **2013**, *46*, 993–998, doi:10.1016/j.clinbiochem.2013.04.003.
30. Albuquerque, T.G.; Oliveira, M.B.P.P.; Sanches-Silva, A.; Costa, H.S. Cholesterol Determination in Foods: Comparison between High Performance and Ultra-High Performance Liquid Chromatography. *Food Chemistry* **2016**, *193*, 18–25, doi:10.1016/j.foodchem.2014.09.109.
31. Sun, Q.; Fang, S.; Fang, Y.; Qian, Z.; Feng, H. Fluorometric Detection of Cholesterol Based on β -Cyclodextrin Functionalized Carbon Quantum Dots via Competitive Host-Guest Recognition. *Talanta* **2017**, *167*, 513–519, doi:10.1016/j.talanta.2017.02.060.
32. Sun, L.; Ding, W.; Yao, Y.; Yang, X.; Yao, C. Fluorescence Detection of Cholesterol Using a Nitrogen-Doped Graphene Quantum Dot/Chromium Picolinate Complex-Based Sensor. *J. Mater. Chem. B* **2017**, *5*, 9006–9014, doi:10.1039/C7TB02037F.
33. Hassanzadeh, J.; Khataee, A. Ultrasensitive Chemiluminescent Biosensor for the Detection of Cholesterol Based on Synergetic Peroxidase-like Activity of MoS₂ and Graphene Quantum Dots. *Talanta* **2018**, *178*, 992–1000, doi:10.1016/j.talanta.2017.08.107.
34. Daneshfar, A.; Khezeli, T.; Lotfi, H.J. Determination of Cholesterol in Food Samples Using Dispersive Liquid-Liquid Microextraction Followed by HPLC-UV. *Journal of Chromatography B* **2009**, *877*, 456–460, doi:10.1016/j.jchromb.2008.12.050.
35. Haeckel, R.; Sonntag, O.; Külpmann, W.R.; Feldmann, U. Comparison of 9 Methods for the Determination of Cholesterol. **1979**, *17*, 553–563, doi:10.1515/cclm.1979.17.8.553.
36. Huang, Y.; Tan, J.; Cui, L.; Zhou, Z.; Zhou, S.; Zhang, Z.; Zheng, R.; Xue, Y.; Zhang, M.; Li, S.; et al. Graphene and Au NPs Co-Mediated Enzymatic Silver Deposition for the Ultrasensitive Electrochemical Detection of Cholesterol. *Biosensors and Bioelectronics* **2018**, *102*, 560–567, doi:10.1016/j.bios.2017.11.037.
37. Kotani, A.; Hakamata, H.; Nakayama, N.; Kusu, F. Picomole Level Determination of Cholesterol by HPLC with Electrochemical Detection Using Boron-Doped Diamond Electrode after Performance Assessment Based on the FUMI Theory. *Electroanalysis* **2011**, *23*, 2709–2715, doi:10.1002/elan.201100223.

38. Becker, S.; Röhnike, S.; Empting, S.; Haas, D.; Mohnike, K.; Beblo, S.; Mütze, U.; Husain, R.A.; Thiery, J.; Ceglarek, U. LC–MS/MS-Based Quantification of Cholesterol and Related Metabolites in Dried Blood for the Screening of Inborn Errors of Sterol Metabolism. *Anal Bioanal Chem* **2015**, *407*, 5227–5233, doi:10.1007/s00216-015-8731-1.
39. Barua, S.; Gogoi, S.; Khan, R. Fluorescence Biosensor Based on Gold-Carbon Dot Probe for Efficient Detection of Cholesterol. *Synthetic Metals* **2018**, *244*, 92–98, doi:10.1016/j.synthmet.2018.07.010.
40. Aryal, K.P.; Ekanayaka, T.K.; Gilbert, S.; Dowben, P.A.; Jeong, H.K. Fluorescent Detection of Cholesterol Using P-Sulfonatocalix[4]Arene Functionalized Carbon Nanotubes and Thermally Reduced Graphite Oxide Composites. *Chemical Physics Letters* **2020**, *738*, 136856, doi:10.1016/j.cplett.2019.136856.
41. Truta, F.; Florea, A.; Cernat, A.; Tertis, M.; Hosu, O.; de Wael, K.; Cristea, C. Tackling the Problem of Sensing Commonly Abused Drugs Through Nanomaterials and (Bio)Recognition Approaches. *Frontiers in Chemistry* **2020**, *8*.
42. Phetsang, S.; Jakmunee, J.; Mungkornasawakul, P.; Laocharoensuk, R.; Ounnunkad, K. Sensitive Amperometric Biosensors for Detection of Glucose and Cholesterol Using a Platinum/Reduced Graphene Oxide/Poly(3-Aminobenzoic Acid) Film-Modified Screen-Printed Carbon Electrode. *Bioelectrochemistry* **2019**, *127*, 125–135, doi:10.1016/j.bioelechem.2019.01.008.
43. Saxena, U.; Das, A.B. Nanomaterials towards Fabrication of Cholesterol Biosensors: Key Roles and Design Approaches. *Biosensors and Bioelectronics* **2016**, *75*, 196–205, doi:10.1016/j.bios.2015.08.042.
44. Cohen, A.; Hertz, H.S.; Mandel, J.; Paule, R.C.; Schaffer, R.; Sniegowski, L.T.; Sun, T.; Welch, M.J.; White, E. Total Serum Cholesterol by Isotope Dilution/Mass Spectrometry: A Candidate Definitive Method. *Clin Chem* **1980**, *26*, 854–860.
45. Miyamoto, T.; Sugiyama, Y.; Suzuki, J.; Oohashi, T.; Takahashi, Y. Determination of Bovine Serum Low-Density Lipoprotein Cholesterol Using the N-Geneous Method. *Vet Res Commun* **2006**, *30*, 467–474, doi:10.1007/s11259-006-3276-6.
46. Srivastava, M.; Srivastava, S.K.; Nirala, N.R.; Prakash, R. A Chitosan-Based Polyaniline–Au Nanocomposite Biosensor for Determination of Cholesterol. *Anal. Methods* **2014**, *6*, 817–824, doi:10.1039/C3AY41812J.
47. Tyagi, M.; Chandran, A.; Joshi, T.; Prakash, J.; Agrawal, V.V.; Biradar, A.M. Self Assembled Monolayer Based Liquid Crystal Biosensor for Free Cholesterol Detection. *Applied Physics Letters* **2014**, *104*, 154104, doi:10.1063/1.4871704.
48. Zhang, J.; Wang, W.; Chen, S.; Ruo, Y.; Zhong, X.; Wu, X. Bi-Pseudoenzyme Synergetic Catalysis to Generate a Coreactant of Peroxydisulfate for an Ultrasensitive Electrochemiluminescence-Based Cholesterol Biosensor. *Biosensors and Bioelectronics* **2014**, *57*, 71–76, doi:10.1016/j.bios.2014.01.046.
49. Morzycki, J.W.; Sobkowiak, A. Electrochemical Oxidation of Cholesterol. *Beilstein J. Org. Chem.* **2015**, *11*, 392–402, doi:10.3762/bjoc.11.45.
50. Steckhan, E. Indirect Electroorganic Syntheses—A Modern Chapter of Organic Electrochemistry [New Synthetic Methods (59)]. *Angewandte Chemie International Edition in English* **1986**, *25*, 683–701, doi:10.1002/anie.198606831.
51. Kowalski, J.; Płoszyńska, J.; Sobkowiak, A.; Morzycki, J.W.; Wilczewska, A.Z. Direct Electrochemical Acetoxylation of Cholesterol at the Allylic Position. *Journal of Electroanalytical Chemistry* **2005**, *585*, 275–280, doi:10.1016/j.jelechem.2005.09.003.
52. Hosokawa, Y.-Y.; Hakamata, H.; Murakami, T.; Aoyagi, S.; Kuroda, M.; Mimaki, Y.; Ito, A.; Morosawa, S.; Kusu, F. Electrochemical Oxidation of Cholesterol in Acetonitrile Leads to the Formation of Cholesta-4,6-Dien-3-One. *Electrochimica Acta* **2009**, *54*, 6412–6416, doi:10.1016/j.electacta.2009.06.005.
53. Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W.; Fu, P.C. Enzymatic Determination of Total Serum Cholesterol. *Clin Chem* **1974**, *20*, 470–475.
54. Owen, W.E.; Thatcher, M.L.; Crabtree, K.J.; Greer, R.W.; Strathmann, F.G.; Straseski, J.A.; Genzen, J.R. Body Fluid Matrix Evaluation on a Roche Cobas 8000 System. *Clinical Biochemistry* **2015**, *48*, 911–914, doi:10.1016/j.clinbiochem.2015.05.012.
55. Amundson, D.M.; Zhou, M. Fluorometric Method for the Enzymatic Determination of Cholesterol. *Journal of Biochemical and Biophysical Methods* **1999**, *38*, 43–52, doi:10.1016/S0165-022X(98)00036-0.
56. Ferreira, C.E. dos S.; França, C.N.; Correr, C.J.; Zucker, M.L.; Andriolo, A.; Scartezini, M. Clinical Correlation between a Point-of-Care Testing System and Laboratory Automation for Lipid Profile. *Clinica Chimica Acta* **2015**, *446*, 263–266, doi:10.1016/j.cca.2015.04.036.
57. Saraiva, D.; Semedo, R.; Castilho, M. da C.; Silva, J.M.; Ramos, F. Selection of the Derivatization Reagent—The Case of Human Blood Cholesterol, Its Precursors and Phytosterols GC–MS Analyses. *Journal of Chromatography B* **2011**, *879*, 3806–3811, doi:10.1016/j.jchromb.2011.10.021.
58. Schneider, C. Chemistry and Biology of Vitamin E. *Molecular Nutrition & Food Research* **2005**, *49*, 7–30, doi:10.1002/mnfr.200400049.

59. Mestre Prates, J.A.; Gonçalves Quaresma, M.A.; Branquinho Bessa, R.J.; Andrade Fontes, C.M.G.; Mateus Alfaia, C.M.P. Simultaneous HPLC Quantification of Total Cholesterol, Tocopherols and β -Carotene in Barrosã-PDO Veal. *Food Chemistry* **2006**, *94*, 469–477, doi:10.1016/j.foodchem.2005.01.021.
60. Hamill, T.W.; Soliman, A.M. Determination of Cholesterol by P-Nitrobenzoate Derivatization and Liquid Chromatography. *Journal of AOAC INTERNATIONAL* **1994**, *77*, 1190–1196, doi:10.1093/jaoac/77.5.1190.
61. Ahn, J.-H.; Jeong, I.-S.; Kwak, B.-M.; Leem, D.; Yoon, T.; Yoon, C.; Jeong, J.; Park, J.-M.; Kim, J.-M. Rapid Determination of Cholesterol in Milk Containing Emulsified Foods. *Food Chemistry* **2012**, *135*, 2411–2417, doi:10.1016/j.foodchem.2012.07.060.
62. Bavisetty, S.C.B.; Narayan, B. An Improved RP-HPLC Method for Simultaneous Analyses of Squalene and Cholesterol Especially in Aquatic Foods. *J Food Sci Technol* **2015**, *52*, 6083–6089, doi:10.1007/s13197-015-1793-4.
63. Lioe, H.; Setianingrum, T.; Anggraeni, R. Method Validation of Cholesterol Analysis in Egg Using HPLC-ELSD. *Jurnal Ilmu Pertanian Indonesia* **2013**, *18*, 178–185.
64. Park, J.; Jeong, I.-S.; Kwak, B.-M.; Ahn, J.-H.; Leem, D.; Jeong, J.; Kim, J.-M. Application of Rapid Sample Preparation Method and Monitoring for Cholesterol Content in Chicken Egg and Egg Powder. *Korean Journal for Food Science of Animal Resources* **2013**, *33*, doi:10.5851/kosfa.2013.33.5.672.
65. Souza, H.A.L.; Mariutti, L.R.B.; Bragagnolo, N. Microwave Assisted Direct Saponification for the Simultaneous Determination of Cholesterol and Cholesterol Oxides in Shrimp. *The Journal of Steroid Biochemistry and Molecular Biology* **2017**, *169*, 88–95, doi:10.1016/j.jsbmb.2016.03.027.
66. Stroher, G.L.; Rodrigues, A.C.; Dias, L.F.; Pedrão, M.R.; Paula, L.N. de; Visentainer, J.V.; Souza, N.E. de Comparative Analysis and Validation Methodologies of GC and HPLC for Analysis of Cholesterol in Meat Products. *American Journal of Analytical Chemistry* **2012**, *3*, 306–311, doi:10.4236/ajac.2012.34042.
67. Ramalho, H.M.M.; Casal, S.; Oliveira, M.B.P.P. Total Cholesterol and Desmosterol Contents in Raw, UHT, Infant Formula Powder and Human Milks Determined by a New Fast Micro-HPLC Method. *Food Anal. Methods* **2011**, *4*, 424–430, doi:10.1007/s12161-010-9182-0.
68. Dinh, T.T.N.; Thompson, L.D.; Galyean, M.L.; Brooks, J.C.; Patterson, K.Y.; Boylan, L.M. Cholesterol Content and Methods for Cholesterol Determination in Meat and Poultry. *Comprehensive Reviews in Food Science and Food Safety* **2011**, *10*, 269–289, doi:10.1111/j.1541-4337.2011.00158.x.
69. Shimada, K.; Mitamura, K.; Higashi, T. Gas Chromatography and High-Performance Liquid Chromatography of Natural Steroids. *Journal of Chromatography A* **2001**, *935*, 141–172, doi:10.1016/S0021-9673(01)00943-8.
70. Lin, X.; Ni, Y.; Kokot, S. Electrochemical Cholesterol Sensor Based on Cholesterol Oxidase and MoS₂-AuNPs Modified Glassy Carbon Electrode. *Sensors and Actuators B: Chemical* **2016**, *233*, 100–106, doi:10.1016/j.snb.2016.04.019.
71. Borkovcová, I.; Janoušková, E.; Dračková, M.; Janštová, B.; Vorlová, L. Determination of Sterols in Dairy Products and Vegetable Fats by HPLC and GC Methods. *Czech Journal of Food Sciences* **2009**, *27*, S217–S219, doi:10.17221/1073-CJFS.
72. Kolarič, L.; Šimko, P. The Comparison of HPLC and Spectrophotometric Method for Cholesterol Determination. *Potravinárstvo Slovak Journal of Food Sciences* **2020**, *14*, 118–124, doi:10.5219/1302.
73. Fenton, M. Chromatographic Separation of Cholesterol in Foods. *Journal of Chromatography A* **1992**, *624*, 369–388, doi:10.1016/0021-9673(92)85689-Q.
74. Kanal, M. Gas Chromatographic Separation of Sterols and Its Clinical Application. *The Journal of Biochemistry* **1964**, *56*, 266–272, doi:10.1093/oxfordjournals.jbchem.a127988.
75. AOAC 994.10-1994(2010), Cholesterol in Foods. Direct Saponificat - \$14.30: AOAC Official Method Available online: http://www.aocofficalmethod.org/index.php?main_page=product_info&products_id=186 (accessed on 20 October 2023).
76. Thompson, R.H.; Merola, G.V. A Simplified Alternative to the AOAC Official Method for Cholesterol in Multicomponent Foods. *J AOAC Int* **1993**, *76*, 1057–1068.
77. Liebisch, G.; Binder, M.; Schifferer, R.; Langmann, T.; Schulz, B.; Schmitz, G. High Throughput Quantification of Cholesterol and Cholesteryl Ester by Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS). *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2006**, *1761*, 121–128, doi:10.1016/j.bbalip.2005.12.007.
78. Hidaka, H.; Hanyu, N.; Sugano, M.; Kawasaki, K.; Yamauchi, K.; Katsuyama, T. Analysis of Human Serum Lipoprotein Lipid Composition Using MALDI-TOF Mass Spectrometry. *Ann Clin Lab Sci* **2007**, *37*, 213–221.
79. Hsu, C.-C.; Dorrestein, P.C. Visualizing Life with Ambient Mass Spectrometry. *Current Opinion in Biotechnology* **2015**, *31*, 24–34, doi:10.1016/j.copbio.2014.07.005.
80. Cody, R.B.; Laramée, J.A.; Durst, H.D. Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Anal. Chem.* **2005**, *77*, 2297–2302, doi:10.1021/ac050162j.

81. Lee, W.-C.; Kim, K.-B.; Gurudatt, N.G.; Hussain, K.K.; Choi, C.S.; Park, D.-S.; Shim, Y.-B. Comparison of Enzymatic and Non-Enzymatic Glucose Sensors Based on Hierarchical Au-Ni Alloy with Conductive Polymer. *Biosensors and Bioelectronics* **2019**, *130*, 48–54, doi:10.1016/j.bios.2019.01.028.
82. Saha, M.; Das, S. Fabrication of a Nonenzymatic Cholesterol Biosensor Using Carbon Nanotubes from Coconut Oil. *J Nanostruct Chem* **2014**, *4*, 94, doi:10.1007/s40097-014-0094-1.
83. Lee, Y.-J.; Park, J.-Y. Nonenzymatic Free-Cholesterol Detection via a Modified Highly Sensitive Macroporous Gold Electrode with Platinum Nanoparticles. *Biosensors and Bioelectronics* **2010**, *26*, 1353–1358, doi:10.1016/j.bios.2010.07.048.
84. Nawaz, M.A.H.; Majdinasab, M.; Latif, U.; Nasir, M.; Gokce, G.; Anwar, M.W.; Hayat, A. Development of a Disposable Electrochemical Sensor for Detection of Cholesterol Using Differential Pulse Voltammetry. *Journal of Pharmaceutical and Biomedical Analysis* **2018**, *159*, 398–405, doi:10.1016/j.jpba.2018.07.005.
85. Hayat, A.; Barthelmebs, L.; Sassolas, A.; Marty, J.-L. An Electrochemical Immunosensor Based on Covalent Immobilization of Okadaic Acid onto Screen Printed Carbon Electrode via Diazotization-Coupling Reaction. *Talanta* **2011**, *85*, 513–518, doi:10.1016/j.talanta.2011.04.034.
86. Borisova, B.; Sánchez, A.; Jiménez-Falcão, S.; Martín, M.; Salazar, P.; Parrado, C.; Pingarrón, J.M.; Villalonga, R. Reduced Graphene Oxide-Carboxymethylcellulose Layered with Platinum Nanoparticles/PAMAM Dendrimer/Magnetic Nanoparticles Hybrids. Application to the Preparation of Enzyme Electrochemical Biosensors. *Sensors and Actuators B: Chemical* **2016**, *232*, 84–90, doi:10.1016/j.snb.2016.02.106.
87. Samdani, K.J.; Joh, D.W.; Rath, M.K.; Lee, K.T. Electrochemical Mediatorless Detection of Norepinephrine Based on MoO₃ Nanowires. *Electrochimica Acta* **2017**, *252*, 268–274, doi:10.1016/j.electacta.2017.08.187.
88. Settu, K.; Liu, J.-T.; Chen, C.-J.; Tsai, J.-Z. Development of Carbon-graphene-Based Aptamer Biosensor for EN2 Protein Detection. *Analytical Biochemistry* **2017**, *534*, 99–107, doi:10.1016/j.ab.2017.07.012.
89. David, M.; Barsan, M.M.; Brett, C.M.A.; Florescu, M. Improved Glucose Label-Free Biosensor with Layer-by-Layer Architecture and Conducting Polymer Poly(3,4-Ethylenedioxythiophene). *Sensors and Actuators B: Chemical* **2018**, *255*, 3227–3234, doi:10.1016/j.snb.2017.09.149.
90. Lee, C.-Y.; Wu, L.-P.; Chou, T.-T.; Hsieh, Y.-Z. Functional Magnetic Nanoparticles-Assisted Electrochemical Biosensor for Eosinophil Cationic Protein in Cell Culture. *Sensors and Actuators B: Chemical* **2018**, *257*, 672–677, doi:10.1016/j.snb.2017.11.033.
91. Hernández, D.M.; González, M.A.; Astudillo, P.D.; Hernández, L.S.; González, F.J. Modification of Carbon Electrodes by Anodic Oxidation of Organic Anions. *Procedia Chemistry* **2014**, *12*, 3–8, doi:10.1016/j.proche.2014.12.034.
92. Yao, C.; Sun, H.; Fu, H.-F.; Tan, Z.-C. Sensitive Simultaneous Determination of Nitrophenol Isomers at Poly(p-Aminobenzene Sulfonic Acid) Film Modified Graphite Electrode. *Electrochimica Acta* **2015**, *156*, 163–170, doi:10.1016/j.electacta.2015.01.043.
93. Wang, W.; Bai, H.; Li, H.; Lv, Q.; Zhang, Q.; Bao, N. Carbon Tape Coated with Gold Film as Stickers for Bulk Fabrication of Disposable Gold Electrodes to Detect Cr(VI). *Sensors and Actuators B: Chemical* **2016**, *236*, 218–225, doi:10.1016/j.snb.2016.05.155.
94. Sajid, M.; Nazal, M.K.; Mansha, M.; Alsharaa, A.; Jillani, S.M.S.; Basheer, C. Chemically Modified Electrodes for Electrochemical Detection of Dopamine in the Presence of Uric Acid and Ascorbic Acid: A Review. *TrAC Trends in Analytical Chemistry* **2016**, *76*, 15–29, doi:10.1016/j.trac.2015.09.006.
95. Derina, K.; Korotkova, E.; Taishibekova, Y.; Salkeeva, L.; Kratochvil, B.; Barek, J. Electrochemical Nonenzymatic Sensor for Cholesterol Determination in Food. *Anal Bioanal Chem* **2018**, *410*, 5085–5092, doi:10.1007/s00216-018-1164-x.
96. Mehtab, S.; Zaidi, M.G.H.; Joshi, P. Metal Nanoparticles Based Electrochemical Biosensors for Cholesterol. *Journal of Nanomedicine & Nanotechnology* **2020**, *11*, 1–2, doi:10.35248/2157-7439.19.11.540.
97. Ruecha, N.; Rangkupan, R.; Rodthongkum, N.; Chailapakul, O. Novel Paper-Based Cholesterol Biosensor Using Graphene/Polyvinylpyrrolidone/Polyaniline Nanocomposite. *Biosensors and Bioelectronics* **2014**, *52*, 13–19, doi:10.1016/j.bios.2013.08.018.
98. Singh, J.; Roychoudhury, A.; Srivastava, M.; Solanki, P.R.; Lee, D.W.; Lee, S.H.; Malhotra, B.D. A Dual Enzyme Functionalized Nanostructured Thulium Oxide Based Interface for Biomedical Application. *Nanoscale* **2013**, *6*, 1195–1208, doi:10.1039/C3NR05043B.
99. Souza, T.T.L.; Moraes, M.L.; Ferreira, M. Use of Hemoglobin as Alternative to Peroxidases in Cholesterol Amperometric Biosensors. *Sensors and Actuators B: Chemical* **2013**, *178*, 101–106, doi:10.1016/j.snb.2012.12.005.
100. Gholivand, M.B.; Khodadadian, M. Amperometric Cholesterol Biosensor Based on the Direct Electrochemistry of Cholesterol Oxidase and Catalase on a Graphene/Ionic Liquid-Modified Glassy Carbon Electrode. *Biosensors and Bioelectronics* **2014**, *53*, 472–478, doi:10.1016/j.bios.2013.09.074.
101. Ferri, T.; Poscia, A.; Santucci, R. Direct Electrochemistry of Membrane-Entrapped Horseradish Peroxidase.: Part I. A Voltammetric and Spectroscopic Study. *Bioelectrochemistry and Bioenergetics* **1998**, *44*, 177–181, doi:10.1016/S0302-4598(97)00088-3.

102. Santucci, R.; Bongiovanni, C.; Marini, S.; Del Conte R; Tien, M.; Banci, L.; Coletta, M. Redox Equilibria of Manganese Peroxidase from *Phanerochaete Chrysosporium*: Functional Role of Residues on the Proximal Side of the Haem Pocket. *Biochem J* **2000**, *349*, 85–90.
103. Shrivastava, S.; Jadon, N.; Jain, R. Next-Generation Polymer Nanocomposite-Based Electrochemical Sensors and Biosensors: A Review. *TrAC Trends in Analytical Chemistry* **2016**, *82*, 55–67, doi:10.1016/j.trac.2016.04.005.
104. Raj, V.; Jaime, R.; Astruc, D.; Sreenivasan, K. Detection of Cholesterol by Digitonin Conjugated Gold Nanoparticles. *Biosens Bioelectron* **2011**, *27*, 197–200, doi:10.1016/j.bios.2011.06.015.
105. Giri, A.K.; Charan, C.; Saha, A.; Shahi, V.K.; Panda, A.B. An Amperometric Cholesterol Biosensor with Excellent Sensitivity and Limit of Detection Based on an Enzyme-Immobilized Microtubular ZnO@ZnS Heterostructure. *J. Mater. Chem. A* **2014**, *2*, 16997–17004, doi:10.1039/C4TA03627A.
106. Komathi, S.; Muthuchamy, N.; Lee, K.-P.; Gopalan, A.-I. Fabrication of a Novel Dual Mode Cholesterol Biosensor Using Titanium Dioxide Nanowire Bridged 3D Graphene Nanostacks. *Biosensors and Bioelectronics* **2016**, *84*, 64–71, doi:10.1016/j.bios.2015.11.042.
107. Dey, R.S.; Raj, C.R. Enzyme-Integrated Cholesterol Biosensing Scaffold Based on in Situ Synthesized Reduced Graphene Oxide and Dendritic Pd Nanostructure. *Biosensors and Bioelectronics* **2014**, *62*, 357–364, doi:10.1016/j.bios.2014.06.063.
108. Wang, T.; Du, K.; Liu, W.; Zhang, J.; Li, M. Electrochemical Sensors Based on Molybdenum Disulfide Nanomaterials. *Electroanalysis* **2015**, *27*, 2091–2097, doi:10.1002/elan.201500117.

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