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Effective Methods of Reducing Cholesterol in Food: An Updated Review

Mandana Dousti [1], Asma Ghaani [1], Parastoo Abdulahi Fard [1], Aylar Shiri [1], Khadije Abdolmaleki [2,3*]

[1] Department of Food Science and Technology, School of Nutrition Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Research Center of Oils and Fats, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[3] Nutrition Sciences and Food Technology Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

The increasing prevalence of chronic diseases associated with dietary factors has elevated the importance of food safety science in disease prevention. Cardiovascular diseases (CVDs) remain the leading cause of mortality, particularly in developed nations, with prolonged high cholesterol consumption being a significant risk factor. Since many animal-derived food products contain cholesterol and are widely consumed, developing low-cholesterol alternatives represents a crucial step in CVD prevention. Various biological, chemical, and physical processes have been designed to remove or reduce cholesterol in animal-derived foods. This study examines several cholesterol reduction methods, including probiotics, supercritical fluid extraction, beta-cyclodextrin complexation, enzymatic degradation, and cryogel-based techniques. The efficacy of adsorbents, focusing on beta-cyclodextrin, and their recovery rates are discussed. Additionally, the potential of combining these methods is explored.

1. Introduction

The shift in lifestyle and dietary patterns, societal industrialization, and increased demand for processed foods with extended shelf life have led to a rise in various diseases (1, 2). Globally growing health issues such as cancers, gastrointestinal infections, diabetes, and allergies are often associated with the consumption of unhealthy foods. Various natural and added compounds in food have contributed to numerous health problems and diseases. Cholesterol, a compound detrimental to human health, is implicated in several diseases, including hypertension, type 2 diabetes, dyslipidemia, and coronary heart disease. The latter remains a leading cause of mortality in many countries, with an increasing prevalence. Individuals with elevated blood cholesterol levels face a higher risk of myocardial infarction compared to those with normal levels (3). Cholesterol is a biomolecule and steroid in lipids, classified as a sterol. This 27-carbon molecule possesses a four-ring structure comprising three hexagonal rings and one pentagonal ring. It is an essential component of animal cell membranes. It serves as a precursor for various biomolecules, including steroid hormones, bile acids, and vitamin D. The primary function of cholesterol is to provide strength and flexibility to cell membranes (4). Cholesterol is predominantly found in animal-derived foods such as meat, dairy products, poultry, eggs, and shellfish, with minimal amounts in plant membranes. Dietary cholesterol is often associated with foods that are significant sources of saturated fatty acids. A correlation exists between dietary cholesterol consumption and blood LDL cholesterol concentration, with elevated LDL levels increasing the risk of cardiovascular diseases. Consequently, reducing high LDL-C levels is a crucial public health objective (4, 5). Given this context, strategies for cholesterol reduction in foods should be developed. Animal-derived foods typically contain high levels of LDL cholesterol. While avoiding these foods can reduce cholesterol intake, this approach is limited due to the significant nutrients present in animal diets. Therefore, physical, chemical, and biological methods should be employed to reduce cholesterol content. These methods

*Corresponding author: Nutrition Sciences and Food Technology Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.

E-mail address: khadije.abdolmaleki@gmail.com (Khadije Abdolmaleki).

include organic solvents, adsorbents (e.g., beta-cyclodextrin, titanium, aluminum), centrifugation, molecular distillation, and probiotics (6). However, many of these methods are costly and may result in losing desirable properties such as aroma, natural taste, nutrients, and alterations in rheological properties (7). Adsorbents represent the most common method of cholesterol reduction, with beta-cyclodextrin being particularly effective due to its selective removal of cholesterol while minimally affecting other food components and flavors (8). This approach is preferable to different techniques, offering higher efficiency and the ability to remove up to 98% of the cholesterol in milk and other dairy products. Additionally, cyclodextrins are natural, non-toxic, edible, chemically stable, and easily separated after forming cholesterol-cyclodextrin complexes (9, 10). This study investigates various methods of cholesterol reduction, with a focus on the removal method utilizing beta-cyclodextrin. The recovery of this substance and its use in combination with other methods are also examined.

2. Effective methods for cholesterol reduction

The effective methods for reducing food cholesterol are illustrated in Fig.1. The following sections examine these methods and their application in food to reduce cholesterol. Examples are presented in Table 1, along with the concentrations used and the extent of cholesterol reduction achieved.

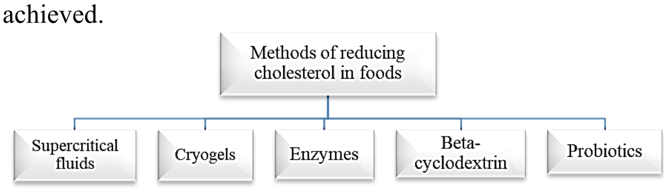

Fig. 1. Effective methods of reducing cholesterol in foods.

2.1. Probiotics

Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer health benefits to the host (11). These microorganisms are common ingredients in functional foods. Probiotic microbes produce various antimicrobial compounds, including bacteriocins, siderophores, lysozymes, proteases, and hydrogen peroxide, inhibiting pathogenic microorganisms' growth (12). Probiotics have been associated with numerous health benefits, including reduced lactose intolerance, enhanced immune responses, alleviation of diarrhea, and potential cancer prevention. Moreover, they may play a role in the prevention and treatment of inflammatory bowel disorders such as Crohn's disease and atopic disorders in children. Recent studies investigating the effects of probiotic consumption on serum lipid profiles, cholesterol levels, blood pressure, and glucose regulation have suggested potential benefits in these areas as well (13). One of the most significant applications of probiotics is their ability to reduce cholesterol levels in foods (14). Fig.2 illustrates the probiotics effective in cholesterol reduction.

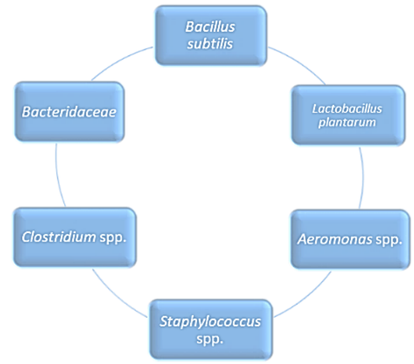

Fig. 2. The effects of probiotics in reducing cholesterol (12).

Coronary heart disease remains the primary cause of mortality in many countries worldwide, with the incidence of cardiovascular diseases continuing to rise. Probiotics have been shown to reduce human blood cholesterol levels and decrease cholesterol content in foods. Foods containing probiotics have been reported to reduce the risk of coronary heart disease; therefore, the characteristics of the active ingredients and the probiotics' type and quantity are crucial factors to consider. Lactic acid bacteria (LAB), particularly *Lactobacillus* and *Bifidobacterium* strains and certain Bacillus species, represent potential strategies for reducing cholesterol in foods. Specific strains like *Lactobacillus casei*, *L. plantarum*, *L. paracasei*, *L. lactis*, and *Enterococcus faecium* have demonstrated cholesterol-reducing capabilities. Albano et al. (3) investigated lactic acid bacteria with cholesterol-lowering properties in dairy products. Their study evaluated 58 potential probiotic lactic acid bacteria for their ability to reduce cholesterol in a medium containing cholesterol and bile acids. The most effective strains, including *Lactobacillus casei* VC199, *Lactobacillus paracasei* SE160 and VC213, *Lactobacillus plantarum* VS166 and VS513, *Enterococcus faecium* VC223, and *Enterococcus lactis* BT161, achieved a 42-55% reduction in cholesterol levels. Furthermore, when tested in cheese production, all cheeses exhibited decreased cholesterol levels during ripening. Kim et al. (15) evaluated the cholesterol absorption ability of lactic acid bacteria (LAB) isolated from kimchi, a traditional Korean fermented cabbage. The isolated strain, identified as *Pediococcus acidilactici* LRCC5307, exhibited 30.5% cholesterol absorption when cultured in a modified MRS medium. The effects of bile salt types and concentrations on enhancing the cholesterol absorption ability of the LRCC5307 strain were investigated, with a 74.5% decrease in cholesterol observed after adding 0.2% bile salt. Additionally, the production of low-cholesterol butter using LRCC5307 was examined. After fermentation with 8.74 log CFU/g of live cells at pH 5.43, an 11% decrease in cholesterol was observed. These results suggest that LRCC5307 may contribute to producing healthier butter by reducing cholesterol content.

2.2. Supercritical fluids

Supercritical fluid extraction (SFE) development represents one of the most significant advancements in food extraction technology over the past two decades. SFE is a rapid, environmentally friendly, and highly selective method for cholesterol extraction from food matrices (16, 17). Increasing environmental concerns associated with organic solvent use have propelled the food industry towards adopting "green chemistry" principles. In this context, utilizing supercritical fluids, particularly CO_2 presents an excellent alternative to conventional chemical solvents. Supercritical fluid extraction garnered significant research attention in the 1980s, with some publications referring to it as a "magical" technology. A fluid becomes supercritical when its temperature and pressure exceed its critical point. The boundary between gas and liquid phases disappears in the supercritical region, resulting in a homogeneous fluid (18). Supercritical fluids exhibit properties of both gases and liquids in terms of density and permeability. Unlike liquids, the density of these fluids is highly responsive to changes in temperature and pressure; a slight increase in pressure can lead to a substantial increase in fluid density. This characteristic confers a significant advantage in terms of selectivity. By modulating pressure or temperature, the dissolving power of the fluid can be altered, enabling the extraction of complex compounds (19). Carbon dioxide has emerged as the most effective supercritical fluid among various compounds. Supercritical CO_2 is inert, cost-effective, pure, and non-toxic. This method is particularly suitable for heat-sensitive materials due to the relatively lower critical temperatures and pressures of fluids such as CO_2 and N_2O. Using CO2 as a supercritical solvent offers additional advantages, including environmental friendliness, non-flammability, low cost, non-toxicity, and its natural abundance (20). Despite its numerous advantages, SFE is not without limitations. The primary challenge associated with supercritical CO_2 is its low affinity for highly polar components. Consequently, incorporating an organic solvent as a modifier is often necessary to enhance extraction efficiency. However, these modifiers must be separated in the subsequent purification step, complicating the process. Additionally, the use of absorbents can make the collection stage more challenging. The required specialized equipment and expertise to perform SFE processes represent another significant barrier. Although SFE is a rapid extraction system, the initial capital investment for equipment acquisition and operational costs are substantial (21). SFE has found diverse applications in food processing, including the decaffeination of green coffee beans, production of hop extracts, recovery of aroma and flavor compounds from herbs and spices, extraction and fractionation of edible oils, and removal of contaminants. The application of supercritical fluids has expanded to new areas, such as formulation processes and specific chemical reactions (22). Recently, SFE has gained attention as an alternative to organic solvent extraction for cholesterol removal from various food products, including beef, fish muscle, egg yolk, and milk fat (23). Pourortazavi et al. (22) investigated the use of supercritical fluids for cholesterol extraction from food matrices. Their findings indicated that this method allows efficient extraction using reduced solvent volumes and shorter processing times. SFE can effectively extract cholesterol from muscle tissues, cellular structures, egg yolk, and milk fat. However, achieving high cholesterol extraction efficiency requires precise control of supercritical fluid parameters, including temperature, pressure, density, and flow rate. Under optimized conditions, cholesterol extraction efficiencies of up to 98% can be achieved. The authors concluded that this technique is potentially the most effective cholesterol extraction based on the results from several studies. Higuera-Ciapara et al. (24) investigated the production of low-cholesterol shrimp using supercritical extraction. Their process involved freeze-drying, cholesterol extraction, and rehydration. The study examined three variables (pressure, volume, and temperature) at five levels each. Post-extraction, various hydration and cooking conditions were applied to obtain a processed product with characteristics similar to natural shrimp. Two sensory analyses were conducted: one comparing the characteristics of fresh shrimp with freeze-dried and rehydrated products and another evaluating the acceptability of fresh and low-cholesterol shrimp after freeze-drying, supercritical extraction, and rehydration. The optimal conditions for producing low-cholesterol shrimp with acceptable organoleptic properties were 310 bar pressure, 1875 L of carbon dioxide, and a temperature of 37°C (25).

2.3. Enzymes

Cholesterol oxidase, an enzyme of high commercial value, has garnered significant attention due to its utility in determining cholesterol levels in various clinical and food samples and its novel applications in biosensor technology (26). This enzyme can be produced by isolating it from diverse organisms, primarily fungi, and bacteria, with bacteria being the predominant producers. Bacteria can synthesize cholesterol oxidase in three forms: intracellular, extracellular, and membrane-bound. The enzyme exhibits substrate inhibition properties at varying enzyme concentrations, making it a valuable reference for cholesterol concentration determination (27). Perdani et al. (28) investigated the oxidation of cholesterol extracted from egg yolk, chicken liver, chicken meat, and beef using cholesterol oxidase enzyme derived from Streptomyces sp. Cholesterol was extracted by mixing lipid-rich foods with a non-polar solvent. The study found that crude cholesterol extracts from egg yolk, chicken liver, and chicken meat were degraded by up to 20% after a 3-hour reaction with 2 mg/ml cholesterol oxidase. In contrast, beef crude extract cholesterol was oxidized by 10% under similar conditions.

2.4. Adsorbent materials

2.4.1. Cryogels

Cryogel beads with uniform particle sizes have demonstrated potential in adsorption and catalytic reaction processes as packing materials. One of the most promising applications of molecularly imprinted cryogel beads is in food analysis. These materials also show potential in sensor applications, medical diagnostics, environmental analysis, drug delivery, and chromatographic separation. Studies have indicated that molecularly imprinted polymers can help mitigate complex food matrix effects, enhance analyte recovery, and improve detection limits by providing high selectivity and loading capacity (29). A significant approach to reducing cholesterol levels involves the production of specific inhibitors that prevent cholesterol absorption from foods. From a clinical perspective, another strategy is the development of selective cholesterol sorbents that are easily accessible and effective for the absorption and removal of cholesterol to achieve recommended blood cholesterol concentrations. Cryogels are sponge-like, elastic, and soft materials with pore sizes ranging from a few microns to several hundred microns. They are versatile materials with diverse applications. In recent years, various integrated cryogels have been prepared by cryopolymerization under freezing conditions. However, scientific reports on fabricating cryogel beads suitable for bioseparation applications remain limited (30). Kartal et al. (29) developed molecularly imprinted cryogel beads for cholesterol removal from milk samples. With a narrow particle size distribution, these beads were prepared by combining microfluidic flow-focusing with copolymerization. Cholesterol-imprinted cryogel beads (CHO-MIPs) were synthesized to remove cholesterol from milk effectively. The molecularly imprinted polymer (MIP) approach enhanced selectivity and accuracy. CHO-MIPs exhibited high selectivity due to their molecular imprinting. The cryogels also demonstrated distinctive properties such as high flow rates, controllable pore structures, large surface areas, and mechanical and chemical stability in biological matrices. Furthermore, their simple and cost-effective preparation makes them excellent adsorbents for milk processing. The cholesterol adsorption capacity of the CHO-MIP beads was determined to be 288.72 mg/g at a cholesterol concentration of 0.3 mg/ml in solution. After eight adsorption-desorption cycles, the adsorption capacity of the CHO-MIP beads decreased by 9.21%, demonstrating good reusability.

2-4-2- Beta-cyclodextrin

Beta-cyclodextrin has emerged as the predominant approach for cholesterol removal in food applications due to its numerous advantageous properties. It is edible, cost-effective, non-toxic, non-hygroscopic, and chemically stable. Its facile separation from the cholesterol complex renders it an attractive food additive (31, 32). Numerous studies have demonstrated its efficacy as a non-toxic and indigestible molecule for removing cholesterol from dairy and animal products, enhancing their nutritional profiles (33). Recent research has elucidated that beta-cyclodextrin can effectively extract cholesterol from milk, cream, cheese, pork, and egg yolk, making it one of the most promising tools for extracting cholesterol from milk fat and preparing low-cholesterol butter (34, 35).

2-4-2-1- Characteristics of beta-cyclodextrin

Cyclodextrins are cyclic oligosaccharides comprising seven glucopyranose units linked by α-(1,4) glycosidic bonds. Three types exist: α-, β-, and γ-cyclodextrins, with β-cyclodextrin being the most commonly employed due to its cost-effectiveness and broad applicability. Beta-cyclodextrin is synthesized from starch using the enzyme cyclodextrin glycosyltransferase, which cleaves the polysaccharide chain to form cyclic molecules. It possesses a toroidal structure with a central hydrophobic cavity sufficiently large to accommodate a cholesterol molecule, forming an insoluble complex that can be isolated via centrifugation. Cyclodextrin effectively absorbs cholesterol at temperatures below 4°C, aiding in maintaining milk quality during removal (36, 37). In addition to natural cyclodextrins, numerous synthesized derivatives have been developed through amination, esterification, or etherification of cyclodextrins' primary and secondary hydroxyl groups. These derivatives often exhibit different solubility from their parent compounds, contingent upon the attached functional groups. Almost all derivatives retain hydrophobic cavities, which can enhance their solubility and stability against light and oxygen (38). Cyclodextrins and their derivatives offer several advantages, including enhanced stability under various conditions, function as dietary fiber, stabilization of volatile substances, removal of unpleasant tastes and odors, control of chemical reactions, selective purification of molecules, stabilization of light- and oxygen-sensitive substances, and color coating of pigments and materials. These properties render cyclodextrins suitable for applications in analytical chemistry, agriculture, pharmaceuticals, and food industries (38, 39). They are cost-effective, safe for human consumption, and approved by the Joint Expert Committee on Food Additives (JECFA) of the World Food and Agriculture Organization for use in many countries (36). In Japan, cyclodextrins have been authorized as "modified starch" for food use for over two decades. Meanwhile, some European countries, such as Hungary, have approved cyclodextrin for specific applications due to its minimal toxicity. Cyclodextrin can enhance the flavor and stability of artificial sweeteners like aspartame and mitigate the unpleasant taste of alternative sweeteners such as stevioside, glycyrrhizin, and rubusoside (40). The applications of cyclodextrins in the food industry are diverse and include their use as a promising new sweetener, removal of bitter components in citrus juice processing, cholesterol reduction in foods (up to 80% reduction), enhancement of frying characteristics, removal of phenolic compounds causing enzymatic browning in fruit and vegetable juices, complexation with flavonoids and terpenoids to improve their solubility and taste, improvement of dough elasticity and flexibility in flour-based products, development of antimicrobial food preservatives, creation of controlled-release powder flavors and confectionery products, and extended flavor retention in chewing gums. These applications demonstrate the versatility and potential of cyclodextrins in food processing and product development, highlighting their significant role in advancing food science and technology (41-43).

2-4-2-2- Metabolism, pharmacokinetics and toxicity

In nature, cyclodextrin glycosyltransferase is the primary enzyme responsible for converting starch to cyclodextrins, although other enzymes may be utilized in industrial production. Various enzymes and mechanisms can degrade cyclodextrins into glucose-related compounds within the human body. Upon ingestion, salivary α-amylase can rapidly break down dextrins in the oral cavity; however, their swift passage to the stomach results in minimal degradation (44). Alpha and beta cyclodextrins exhibit stability towards α-amylase, whereas gamma cyclodextrins are readily digestible. In the stomach, pH-dependent non-specific degradation may occur. Subsequently, in the small intestine, pancreatic amylase hydrolyzes cyclodextrins at neutral pH. Colonic bacteria metabolize alpha and beta-cyclodextrins, with alpha-cyclodextrin being digested more rapidly than beta-cyclodextrin. Gamma cyclodextrin is almost entirely degraded within the digestive tract. Undigested cyclodextrins are ultimately metabolized by microbiota in the lower gastrointestinal tract, where they are nearly completely degraded, a characteristic that has led to their use as prebiotics (45). Generally, the bioavailability of natural and conventional cyclodextrin derivatives is very low, contributing to their safety when administered orally. Dextrin can also be administered via injection. Urinary clearance of linear dextrin and cyclodextrin decreases with increasing molecular weight. Molecules under 15 kDa are almost completely (>90%) excreted unchanged in the urine. Regarding toxicity, most research has focused on the medical applications of cyclodextrins. Large oral doses of cyclodextrin can lead to diarrhea and cecal enlargement and may affect the bioavailability of certain substances. In response to these concerns, the European Commission has released guidelines for drug development researchers to mitigate these effects (46). Beta-cyclodextrin toxicology has been studied more extensively, with particular attention to its substitution rate due to its classical use as a medical supplement. Recent studies suggest that lower substitution levels are optimal for reducing toxicity. However, further research in this field is warranted. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended a maximum daily intake of 5 mg/kg body weight for beta-cyclodextrin in foods. Toxicity studies consistently indicate that oral beta-cyclodextrins are virtually non-toxic due to their lack of absorption in the gastrointestinal tract (47).

2-4-2-3- Removal of cholesterol by beta-cyclodextrin

Beta-cyclodextrin has proven to be an effective method for reducing and eliminating cholesterol in various food products. Multiple studies have demonstrated its efficacy across different concentrations and applications (48). Alonso et al. (49) tested three concentrations (0.5%, 1%, and 1.5%) of beta-cyclodextrin and found that a 1% concentration successfully eliminated >90% of cholesterol. However, efficiency slightly decreased at 1.5%, suggesting that excessive beta-cyclodextrin may reduce cholesterol removal efficiency due to intermolecular reactions. The same researchers reported that beta-cyclodextrin molecules are safe for consumption and non-toxic, making them suitable for cholesterol removal in Manchego cheese without compromising nutritional qualities. Yen and Chen (48) demonstrated that beta-cyclodextrin reduced pork meat's cholesterol and saturated fatty acid content. Alonso et al. (36) achieved up to 95% cholesterol removal in raw milk processing by mixing with beta-cyclodextrin at 4°C, a technique suitable for industrial production due to its efficiency at lower temperatures. Extending the application, Alonso et al. (49) investigated beta-cyclodextrin's efficacy in removing cholesterol from natural eggs, powdered eggs, and duck liver pâté, demonstrating its potential to create low-cholesterol meals while maintaining fatty acid composition. Raju et al. (50) observed a 95% reduction in cholesterol levels, an 8.99% increase in polyunsaturated fatty acids (PUFA), and an 86.27% increase in astaxanthin when using beta-cyclodextrin. The complexation of beta-cyclodextrin with cholesterol is illustrated in Fig. 3 (51).

2-4-2-4- The effect of beta-cyclodextrin on organoleptic, technological, and physicochemical properties

Studies have demonstrated that beta-cyclodextrin not only effectively removes cholesterol but also impacts food products' organoleptic, technological, and physicochemical properties. Bhatia et al. (52) investigated the effects of cholesterol removal on beef milk fat's composition and physical properties. They found that beta-cyclodextrin could reduce cholesterol levels by over 90% without altering physical properties. While chemical properties remained largely consistent, vitamin D levels decreased by 75%, though triglyceride levels were unaffected. Kumar et al. (34) examined the impact of beta-cyclodextrin on the physical and chemical properties of butter and animal fat. Their findings showed that the physicochemical properties of low-cholesterol butter and animal fat remained largely unchanged compared to control samples, with notable variations in carotene, vitamin A, and E levels. However, a significant decline in vitamin D levels was observed in low-cholesterol fat from cow and buffalo sources. Jeon et al. (53) investigated cream cheese made with cholesterol-removed milk and cream using beta-cyclodextrin. Over a 4-week storage period at 7°C, they analyzed sensory, textural, and flavor attributes. The analysis of gas chromatography-mass spectrometry (GC-MS) identified 11 acids, 2 ketones, 1 amine, 1 alcohol, 2 lactones, and 1 alkene among the experimental flavor compounds. No significant differences were observed in texture profile (hardness, cohesiveness, stickiness) or sensory characteristics (appearance, taste, texture characteristics) between the low-cholesterol and control cheese samples ($p>0.05$). Maskooki et al. (33) studied the effects of beta-cyclodextrin on milk components during cholesterol removal. They found that the process decreased fat, density, solid non-fat (SNF), and lactose levels in milk, possibly due to bonding with beta-cyclodextrin or separation processes such as filtration and centrifugation when isolating the beta-cyclodextrin-cholesterol complex.

While beta-cyclodextrin effectively removes cholesterol from milk and dairy products, its impact on nutritional components such as proteins, lactose, and milk fat require further investigation. Although beta-cyclodextrin molecules are considered safe for consumption and non-toxic, the comprehensive effects of this cholesterol removal process on milk composition and nutritional value remain crucial areas of study.

2-4-2-5- Recovery of beta-cyclodextrin

The reuse or recycling of beta-cyclodextrin is crucial for cost-effectiveness and sustainability in cholesterol removal processes. While information on treatments to obtain recycled beta-cyclodextrin of comparable quality to original pure beta-cyclodextrin is limited, several studies have explored the recovery, reusability, and stability of beta-cyclodextrin used in cholesterol removal. Raju et al. (37) investigated the recovery and reusability of beta-cyclodextrin in removing cholesterol from shrimp fat. They processed beta-cyclodextrin combined with cholesterol (β-CD-CL) using ethanol and ultrasound to obtain cholesterol-free beta-cyclodextrin (R-β-CD). The recycled beta-cyclodextrin retained the ability to eliminate 94% of cholesterol from shrimp fat. However, increased fatty acid levels concurrent with decreased cholesterol suggested slight alterations in beta-cyclodextrin during recycling. The researchers concluded that while beta-cyclodextrin can be effectively recycled using appropriate processing methods, continuous use may decrease cholesterol removal effectiveness. Kwak et al. (54) presented a method using stabilized beta-cyclodextrin on glass beads for cholesterol removal in milk. They optimized the process by analyzing three factors: mixing time, mixing temperature, and tube size. The study found that glass beads (1 mm diameter) treated with 20 mM 3-isocyanatopropyltriethoxysilane and 30 mM beta-cyclodextrin without a base had the most effective cholesterol-removal rate (41%). After optimizing conditions (6-hour mixing time in a 7-mm-diameter tube at 10°C), they achieved a 40.2% reduction in cholesterol using immobilized beta-cyclodextrin glass beads. To assess the recycling efficiency, the researchers immersed the used glass beads in an acetic acid:butanol (3:1 v/v) solution for 24 hours at room temperature, then dried them under normal conditions before reuse. The recycling experiment demonstrated that while the cholesterol removal rate using beta-cyclodextrin-stabilized glass beads remained at approximately 40%, the recycling efficiency was nearly 100%. These studies demonstrate the potential for recycling beta-cyclodextrin, which could significantly reduce costs associated with cholesterol removal processes in food production. However, further research is needed to optimize recycling methods and maintain consistent cholesterol removal efficiency over multiple use cycles.

Table 1. Application of different methods in reducing cholesterol in food.

Methods	Product type	Concentration	Percentage reduction (%)	References
Beta cyclodextrin	cream cheese	10 %	91.12	55
Beta cyclodextrin	Homogenized milk	0.1-0.6 %	33-89	56
Beta cyclodextrin	butter	12 %	96/7	8
Lactic acid bacteria	cheese	10^7 CFU	42-55	3
	egg yolk		39.79	
Bacillus coagulans	chicken liver	10^7 CFU	45.44	57
	butter		49.51	
Lactic acid bacteria	milk	10^7 CFU	10.98	1
Lactic acid bacteria	Cholesterol in the culture medium	10^7 CFU	20	2
Supercritical Co_2	Turtle egg yolk powder fish	10 ml/min	70	58
Supercritical Co_2	cream	5 L/min	39	21
	egg yolk	2 mg/ml	10	
Cholesterol oxidase	chicken liver	2 mg/ml	10	28
	chicken meat	2 mg/ml	10	
Cholesterol oxidase	egg	0.13 U	26.8	4
Molecularly Imprinted Cryogel	Homogenized milk	-	80	30
Microfluidization	Cow fat	150 MPa	39.37	59

3. Combined Methods with β-cyclodextrin

The process of separating and collecting β-cyclodextrin post-treatment is labor-intensive and time-consuming. Consequently, exploring more efficient and cost-effective techniques to reduce cholesterol levels in food products is imperative. Surface-modified materials are gaining rapid popularity in scientific and technological fields. The utilization of these modified surfaces offers several advantages over conventional chemical and physical methods, including the reduced quantity of reagent required and cost savings due to facile post-process separation. Various materials, such as silicon, glass, quartz, and organic polymers, create functional surfaces. Among these, glass is frequently utilized due to its low cost, high mechanical stability, and well-established surface modification techniques (60). Tahir et al. (61) investigated cholesterol extraction from fat using glass beads and cyclodextrin. β-cyclodextrin was covalently immobilized on the glass surface. X-ray photoelectron spectroscopy (XPS) and elemental analysis were employed to characterize the modified glass surface. The β-cyclodextrin-modified glass surface was utilized for cholesterol removal, achieving a 78.8% reduction within 2 hours at 25°C and 170 rpm. These modified surfaces can be readily separated from cholesterol and reused multiple times without loss of efficiency. The cholesterol removal efficiency remained consistent after 10 cycles of use. The modified glass surface exhibited minimal degradation during repeated use in the cholesterol reduction process. Escobar et al. (56) examined the effects of high-pressure homogenization combined with β-cyclodextrin on producing low-cholesterol whole milk. Whole raw milk containing 3.87% fat was subjected to ultra-high-pressure homogenization (UHPH) treatment at pressures of 0, 100, 200, and 300 MPa, with the addition of 0%, 0.1%, 0.3%, and 0.6% β-cyclodextrin. Higher β-cyclodextrin concentrations and pressures resulted in enhanced cholesterol removal. A 65% cholesterol reduction was achieved at 0.6% β-cyclodextrin concentration and 100 MPa pressure. The combination of 0.6% β-cyclodextrin and 200-300 MPa yielded the highest cholesterol removal, reaching 87-89%. Low-cholesterol milk produced using specific parameters (200 MPa, 0.6% β-cyclodextrin) was as well-received by consumers as commercial pasteurized milk. Köse et al. (62) investigated cholesterol removal through binding to β-cyclodextrin on a poly-nano-composite adsorbent (HEMA-GMA) linked to cellulose nanocrystals (CNC). This novel cyclodextrin-enhanced nanocomposite adsorbent was employed for the first time to eliminate cholesterol and low-density lipoprotein. The adsorption efficiency in a continuous setup for cholesterol and low-density lipoprotein was determined to be approximately 99%, consistent between experimental results and mathematical models. In summary, the findings demonstrated that β-cyclodextrin, combined with other methods, exhibits high potential as an adsorbent for cholesterol removal.

4. Conclusions

Studies have demonstrated the efficacy of the abovementioned techniques in reducing cholesterol levels in food products. However, many of these techniques are cost-prohibitive and result in the loss of desirable characteristics such as aroma, original taste, and nutrients, as well as alterations in the physical properties of the products. The most prevalent approach for cholesterol reduction is adsorbents, with β-cyclodextrin emerging as the most effective and efficient method. This method selectively removes cholesterol while minimally impacting the organoleptic and physicochemical properties of the food. Furthermore, it has been observed that this substance's removal efficiency increases when combined with other methods. Therefore, applying these methods can contribute to reducing cholesterol levels in food products, ultimately mitigating the risk of cardiovascular diseases and stroke.

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