Scientific Reports

https://doi.org/10.1038/s41598-026-47313-z

Article in Press

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Received: 19 January 2026

Accepted: 31 March 2026

Published online: 03 April 2026

Cite this article as: Suhaimi N., Shamsol Anuar N.S., Higashi M. *et al.* Potential of sustainable, ecofriendly sterol derivatives as additives for water and oil repellency. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-47313-z

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Potential of Sustainable, Ecofriendly Sterol Derivatives as Additives for Water and Oil Repellency

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Abstract

An increasing global demand for sustainable packaging and protective materials has led to significant interest in developing effective water-resistant and oil-repellent materials from natural resources. This is because some of the current synthetic water-resistant and oil-repellent materials are made from organofluorine compounds. This study explored the potential of isoprenoid compounds, such as sterols, as biobased replacements for surface coating or packaging applications. The hydrophobicity and oleophobicity of various sterols and related structural compounds coated on silicon wafers and annealed at 60°C and 140°C were evaluated. As the annealing temperature increased, the hydrophobicity of sterol groups, such as cholesterol, ergosterol, β-sitosterol, and stigmasterol, increased, with their contact angles ranging from 100.7±0.5° to 105.7±1.1° when annealed at 140°C. Moreover, cholesterol myristate and stearyl glycyrrhetinate exhibited good hydrophobicity and oleophobicity characteristics when annealed at 60°C, with water contact angles of 106.4±0.2° and 100.9±0.7° and hexadecane contact angles of 46.1±1.1° and 47.6±5.0°, respectively. Based on the findings of this study, isoprenoids have potential as sustainable alternatives to water and oil repellents, particularly in applications where hydrophobicity is essential. Furthermore, this study offers valuable insights into the significant role of the annealing temperature in enhancing coating properties.

Keywords: Hydrophobicity, Oleophobicity, Isoprenoids, Sterol derivatives, Sustainable additives

Introduction

Currently, the market for water and oil-repellent materials used in industrial applications is dominated by synthetic materials, primarily fluorinated compounds. These materials typically possess a rare combination of hydrophobic and oleophobic characteristics [1]. Materials with low surface energy properties are widely adopted across various sectors [2], including food packaging, electronics, textiles, and paper products [1,3], as they serve crucial functions in protecting contents, extending shelf life, and maintaining product integrity [4]. Efforts to improve environmental sustainability have led to intensive research on developing innovative biobased materials that exhibit water and oil repellency [5].

Recent developments in bioresource utilization have opened promising avenues for creating sustainable alternatives. Among emerging candidates, isoprenoids are promising as natural amphiphiles, offering inherent water-repellent properties while maintaining biodegradability [6]. Thus, isoprenoids can be used as alternatives to food packaging materials. Isoprenoid compounds, such as sterols, are primarily naturally occurring hydrocarbons found in various organisms, and they can be developed as sustainable hydrophobic materials [7,8]. These compounds have a unique structure made up of repeating five-carbon isoprene units, which are crucial for imparting their predominantly hydrophobic character [9,10].

Although some isoprenoids contain functional groups that introduce slight polarity, the presence of a nonpolar hydrocarbon backbone governs their overall water-repellent nature [11]. This hydrophobic trait is essential to diverse biological roles that isoprenoids play—from forming the structural core of cell membranes to acting as signaling molecules and contributing to the rich array of natural products found in plants and animals [12]. In addition to their inherent hydrophobic properties [13–15] these compounds have various structures and functionalities that can be utilized for various applications, such as pharmaceuticals, cosmetics, and biofuels [16]. Furthermore, the presence of both hydrophobic and hydrophilic regions in some isoprenoids enables them to act as surfactants and emulsifiers [17,18].

However, the hydrophobic interaction is weaker compared to fluorinated compounds, for example, Polytetrafluoroethylene (PTFE) or Perfluorooctane Sulfonate (PFOS), which possessed C-F bond that has a stronger attraction due to a larger dipole moment but also exhibit low polarizability [19]. This results in exceptionally low surface energy, allowing the material to exhibit both hydrophobic and oleophobic characteristics [20]. Even so, isoprenoid compounds with their biobased origin and biodegradability by specialized microbes [21–23],

may potentially offer more significant environmental benefits [24–26] compared to the artificial compounds.

In this context, this study focuses on investigating the hydrophobicity and oleophobicity characteristics of selected isoprenoid compounds and assessing their potential as biobased materials, for example, for food wrapping or paper coating applications. An oleophobic surface repels oil molecules, while a hydrophobic surface repels water [27]. The number of chemical compounds that possess both of these characteristics, specifically chemical compounds with characteristics that match those of organofluorine compounds, is quite limited. Other than fluorinated compounds, Janus membrane is also a type of material that has both characteristics, however, with opposing properties to each other in a spongy form [28]. Thus, this research aims to leverage the natural abundance and renewability of isoprenoids with both properties to offer valuable insights for designing environmentally friendly, high-performance alternatives for various industrial applications.

Results and Discussion

Contact angle measurement of sterol. The hydrophobic and oleophobic properties of various sterols and related structural compounds were analyzed based on contact angle measurements against the coated silicon wafer annealed at different temperatures. Silicon wafer is used because it is widely known as a standard substrate for hydrophobicity and oleophobicity analysis, due to its precise wettability characteristics, making it easier to control the variability [29–31]. Since the aim is to investigate the potential of selected compounds for packaging application material, the contact angle of > 100° against a water droplet is considered good hydrophobicity, and a contact angle of > 40° against a hexadecane droplet is defined as moderate oleophobicity [32–35]. Annealing temperatures were chosen based on the fact that low temperatures (60°C) enable controlled molecular reorganization, while high temperatures (140°C) provide sufficient energy for major structural changes [36]. Additionally, comparing the hydrophobic and oleophobic properties between annealing temperatures of 60°C and 140°C enables the evaluation of the stability of the compounds and the homogeneity of the surface structure during the molding of wrapping papers or coating of materials. The data obtained from these measurements are summarized in Table 1 (actual images of contact angle measurements are provided in Supplemental Figure 1).

As shown in Table 1, the contact angles of the stearyl acrylic polymer (positive control) when annealed at 60°C were 109.7±0.3° (water) and 41.8±0.1° (hexadecane), indicating reliable hydrophobic and oleophobic characteristics, respectively. This is a significant improvement compared to the contact angles measured for the polymer when annealed at 140°C. Relative to the positive control, cholesterol showed sufficient hydrophobicity and oleophobicity, with contact angles of 100.7±0.5° and 30.6±1.4° when annealed at 140°C, respectively. The rigid structure of the ring and flexible hydrocarbon tail may contribute to the hydrophobic characteristic [37] (Table 2). However, contact angles for water and hexadecane droplets significantly decreased to 93.8±1.0° and 26.1±0.6°, respectively, when annealed at 60°C. Owing to these variations, other sterol compounds were also investigated.

Ergosterol (with a double bond and an additional methyl group at the side chain), β-sitosterol (with an extra ethyl group at the side chain), and stigmasterol (with a double bond and an additional ethyl group at the side chain) were tested. These compounds were chosen because they enable the analysis of the effect of an additional methyl or ethyl group and a double bond on the side chain on the contact angle. These compounds exhibited higher water contact angles of 101.1±1.9°, 104.3±0.2°, and 105.7±1.1° when annealed at 140°C. These values were better than the result of cholesterol (100.7±0.5°). Their hydrophobic characteristics lie in their key structural order; ergosterol, is known to have tight molecular packing [38], β-sitosterol has high crystalline order [39], and stigmasterol can self-assemble and form compact crystalline order [40]. However, when these compounds were annealed at 60°C, the contact angles decreased to 96.7±2.0°, 93.1±1.6°, and 97.6±2.7°.

On the other hand, no specific trend was observed in the hexadecane contact angles of these sterol compounds. Changes in annealing temperature did not significantly affect the contact angle of hexadecane against these compounds, as shown in Table 1. For ergosterol and β-sitosterol, albeit insignificantly, the contact angles of the hexadecane droplet increased from 30.0±2.9° to 33.2±3.0° and from 28.8±3.7° to 31.1±1.4°, respectively, when the annealing temperature decreased from 140°C to 60°C.

Statistical analysis, as shown in Supplementary Table S1, confirmed that the contact angles of β-sitosterol and stigmasterol annealed at 140°C were statistically significant compared to that of cholesterol ($p < 0.05$), indicating an improvement in surface hydrophobicity. However, the comparison between cholesterol and ergosterol was not statistically significant, which can be attributed to a large variance in the ergosterol dataset. Based on these results, it is safe to suggest that the addition of an ethyl chain to the

cholesterol by structure, as observed on β-sitosterol and stigmasterol, improves hydrophobicity [41,42].

To support these findings, laser microscopy analysis was performed to examine the uniformity of sterol compound films on the silicon wafer and subsequently analyze the efficacy of coating at an annealing temperature of 140°C, as all compounds showed good hydrophobicity (contact angles ≥100°) at this temperature. Supplemental Fig. 2 and Supplemental Fig. 3 illustrate the laser microscopy images of cholesterol, ergosterol, β-sitosterol, and stigmasterol films on a silicon wafer. These images (left panels) facilitate the qualitative visualization of the surface morphology, where bright spots indicate surface irregularities or particulate features. The corresponding 2D surface-height maps (right panels) represent the topographical distribution of coating. Furthermore, warm colors (red/yellow) denote elevated regions, and cool colors (blue/green) indicate lower areas.

However, Supplemental Fig. 2 reveals that the cholesterol and β-sitosterol films formed a uniform coating on the silicon wafer. The figures also show images of the laser observation and surface height of cholesterol and β-sitosterol, where the surface appears smooth. This indicates their good spreading, especially when magnified at 20×. These results are likely related to the heat response and crystallization tendencies of sterol compounds during annealing [43].

Annealing is a treatment in which a coated material is heated at a certain temperature to improve its adhesion, remove solvents or unwanted residues, and enhance its structural or functional properties [44]. An optimal annealing temperature enhances molecular mobility and promotes film spreading [45,46]. Because cholesterol and β-sitosterol have a similar melting point range of 140°C–151°C (Table 1), uniform coating formation strongly depends on the optimum annealing temperature relative to the melting point of sterols. This uniformity is directly linked to improved hydrophobic properties, as surface defects or aggregates disrupt water repellency [46,47].

Contrary to the cholesterol and β-sitosterol films, neither ergosterol nor stigmasterol formed a perfectly uniform coating. In the laser microscopy results (Supplemental Fig. 3), a cloudy appearance is shown in both the optical view and surface height images. This indicates microscale inhomogeneity rather than smoothness. Previous reports suggested that the melting points of ergosterol and stigmasterol ranged from 156°C to 170°C (Table 1), thereby supporting the hypothesis that the selection of an optimum annealing temperature is intrinsically linked to the melting point of each compound.

Based on these observations, it is safe to suggest that, while addition of alkyl chain helps to enhance hydrophobicity, the homogeneity of coating also plays an important role in ensuring a reliable hydrophobicity reading. Therefore, to combine both factors, only β-sitosterol showed a significant hydrophobic characteristic when compared to cholesterol.

Effects of alkyl chains on the contact angles of cholesterol derivatives. With reference to the above-mentioned results, several cholesterol derivatives with alkyl chain attachments were investigated. The selected compounds were cholesterol benzoate, cholesterol butyrate, cholesterol decanoate, cholesterol myristate, and stearyl glycyrrhetinate (a compound with a related structure). These compounds are known to have highly rigid molecular order due to the alkyl chain attachments, and thus, they may exhibit better hydrophobic characteristics [48,49]. Cholesterol benzoate has a benzoate group that forms an ester bond with the hydroxyl group (-OH) located at carbon number 3 of cholesterol. At an annealing temperature of 140°C, this compound exhibited weak hydrophobicity and no oleophobic characteristics. However, these characteristics improved when it was annealed at 60°C, with contact angles improving from 80.3±5.0° and 22.4±5.0° to 100.5±1.4° and 24.3±4.9°, respectively (Table 1).

Notably, cholesterol butyrate, cholesterol decanoate, and cholesterol myristate share a common core structural feature in which the -OH at the carbon number 3 position of cholesterol is esterified to a fatty acid chain with differing lengths: butyrate (C4), decanoate (C10), and myristate (C14). Hence, an increase in the water contact angle was expected with an increase in the number of alkyl chains. Instead, contact angles were inconsistent when they were annealed at 140°C. Cholesterol decanoate exhibited weaker hydrophobicity (91.8±0.6°), while the contact angles of cholesterol butyrate and myristate were 103.6±1.9° and 105.5±7.5°, respectively. This may be due to cholesterol decanoate experiencing conformational entropy at high temperatures [49,50]. Interestingly, only cholesterol myristate exhibited excellent oleophobic characteristics at both annealing temperatures, with the hexadecane contact angles of 35.8±1.4° (140°C) and 46.1±1.1° (60°C). This may be due to the ability of cholesterol myristate to have reversible smectic and solid phase properties, which contribute to these findings [51].

On the other hand, the hexadecane contact angles of the other three compounds were below 30° and no significant changes were observed between the two annealing temperatures, 140°C and 60°C (Table 1; Supplementary Table S2).

Additionally, significant hydrophobicity and oleophobicity characteristics were observed for the stearyl glycyrrhetinate coating. Because its parent structure is glycyrrhetinic acid, this compound is not classified as a cholesterol derivative. Nevertheless, it was included in this study because it exhibits structural and functional similarities to ester-type derivatives of bioactive molecules. As shown in Table 1, a reduction in the annealing temperature significantly increased the water droplet contact angle against stearyl glycyrrhetinate from 58.1±8.7° to 100.9±0.7° (Supplementary Table S1). Most importantly, this substantial temperature reduction significantly increased the contact angle against the hexadecane droplet, reaching 47.6±5.0° from 28.8±5.5° (Supplementary Table S2). This implies an enhancement in the oleophobicity characteristics, as needed.

Similar to the results presented above, the observed variations in contact angles with different annealing temperatures highlight the importance of optimizing the conditions for each compound. Sterol groups have higher melting points; therefore, the annealing temperature of 140°C potentially helps create a more uniform coating, subsequently contributing to a higher water contact angle. By contrast, stearyl glycyrrhetinate and cholesterol myristate have lower melting points (~75°C–84°C). Consequently, annealing at a lower temperature (60°C) is more effective for stearyl glycyrrhetinate and produces a pronounced change in the oleophobicity of cholesterol myristate (Supplementary Table S2). The contrasting results at 140°C and 60°C may occur when a compound undergoes thermal degradation or structural alteration at elevated temperatures [52,53]. However, this has yet to be confirmed with Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), or chemical analysis.

Compared with the water contact angle of cholesterol (Supplementary Table S1), those of cholesterol derivatives and stearyl glycyrrhetinate significantly differed ($p < 0.05$) at 60°C. In addition, cholesterol myristate showed consistent and significant oleophobicity values at both temperatures. Meanwhile, the hydrophobic results for cholesterol butyrate and cholesterol myristate at 140°C, despite showing a higher contact angle, did not reveal a significant difference ($p > 0.05$). This is because of a high variability in their measurements, as indicated by the large standard deviation value (Supplementary Table S1). Based on these findings, it can be concluded that attachment of C14 to the parent compound (cholesterol) improves the hydrophobicity and oleophobicity characteristics at an annealing temperature of 60°C, despite the variability [54].

Potential of sterols, cholesterol derivatives, and stearyl glycyrrhetinate as alternative materials from bioresources. The sterol group and cholesterol derivatives demonstrated promising water and oil repellency at a suitable annealing temperature, indicating their potential as alternative biobased materials to organofluorine compounds. As mentioned above, the contact angles of the stearyl acrylate polymer (positive control) were 109.7±0.3° and 41.8±0.1°, while those of the stearyl acrylate monomer were 33.8±3.2° and 28.3±1.8° for water and hexadecane at 60°C, respectively. Using these values as a reference and comparing the results exhibited by compounds listed in Table 1 indicate that sterols and cholesterol derivatives have potential as hydrophobic agents, although further optimization is needed to achieve comparable oleophobicity.

Notably, these compounds demonstrated high contact angles in the monomeric form, in contrast to the results obtained for stearyl acrylate. The ability of organic compounds to display high contact angles on silicon wafer substrates in their monomeric form rather than as polymers offers distinct advantages in surface modification for hydrophobicity and oleophobicity characterizations because this promotes a more organized molecular orientation [55] and less aggregation when in contact with solvents [56]. This enables fine-tuned control over surface wettability [57].

Utilizing these monomers in polymerization or copolymerization strategies, the hydrophobic and oleophobic characteristics of them can be further enhanced, and a range of polymers can be developed for which surface interactions can be fine-tuned [58]. For instance, incorporating hydrophobic comonomers can amplify the water-repellent nature of the resulting polymers, while the strategic addition of hydrophilic comonomers can introduce a balance of hydrophilic and hydrophobic domains, yielding amphiphilic materials with unique self-assembly behaviors. This ability to manipulate surface properties through polymerization expands the potential applications of isoprenoids in various fields, especially as alternatives to organofluorine compounds. However, there is also a need for the comprehensive testing of these alternatives, including testing their persistence, bioaccumulation potential, and toxicity to humans and the environment.

Future perspectives and potential applications. The studied compounds, if explored and developed carefully as mentioned in the previous section, could be a versatile building block for sustainable next-generation functional materials. Sterols and their derivatives were already found to possess anti-fouling activities [59,60]. Subsequently, if superhydrophobic characteristics could be developed with the copolymerization technique,

it could potentially graft both anti-fouling and self-cleaning bio-coating materials [61,62], that prevent bacterial adhesion by entropic repulsion, without the use of harmful biocides.

Furthermore, the integration of these compounds with other biobased materials to form biobased packaging films shows promise in addressing the permeability issues of sustainable plastics, providing a reliable water-vapor barrier [63]. As material science moves toward green chemistry, the biodegradability and structural rigidity of sterol compounds may provide a viable substitute for artificial fluorocarbons in the development of superhydrophobic and self-healing interfaces.

Conclusion. This study reveals that even though more optimizations are needed, sterol groups, cholesterol myristate, and stearyl glycyrrhetinate offer significant advantages over current synthetic materials in terms of environmental impact and biocompatibility, making them promising for use as sustainable alternatives. Although some compounds approached the hydrophobicity of stearyl acrylic polymer, further research is needed to optimize their surface properties and potentially surpass the performance of stearyl acrylate in terms of oleophobicity.

Methods

Chemicals. Stearyl acrylate was used as a positive control. Several sterols and related structural compounds available in the market were selected based on their distinguished chemical structures and their ability to have both hydrophobic and oleophobic characteristics. All sterol compounds (β-sitosterol, cholesterol, ergosterol, and stigmasterol), several cholesterol derivative compounds (such as cholesterol benzoate, cholesterol butyrate, cholesterol decanoate, and cholesterol myristate), and stearyl glycyrrhetinate were purchased from Tokyo Chemical Industry (TCI), Japan.

Positive control preparation. Stearyl acrylate was used in both monomer and polymer form as positive controls. To synthesize the corresponding polymer, 2 g stearyl acrylate was dissolved in 8 g toluene, followed by the addition of 38 mg 2,2'-azobisisobutyronitrile (AIBN). The mixture was heated and stirred at 75°C for 24 hours under a nitrogen atmosphere. The reaction mixture was then purified by reprecipitation with acetone and dried under reduced pressure to obtain the desired stearyl acrylic polymer. Molecular

weight (GPC): Mw 18.8 K, Mn 9.9 K, Purity (^{1}H-NMR, $CDCl_3$): Residual stearyl acrylate content < 1%.

Substrate preparation and coating on silicon wafers. All chemical compounds were prepared and dissolved in chloroform, with a weight/weight concentration of 1%. A silicon wafer (2-960-15; AS ONE Corporation, Japan) was used as the substrate for contact angle measurements. The prepared silicon wafer had a size of 30 mm × 30 mm and was washed with acetone for 30 min ultrasonically. The as-prepared silicon substrates were then dried and kept in a clean glass container. The prepared chemical solution in chloroform was applied to the silicon substrate using a spin coater (Opticoat MS-B150, MIKASA) at 2000 rpm for 25 s. A total of 500 μL of the chemical solution was deposited onto the surface of the silicon substrate until it completely covered the surface. The as-coated silicon substrate was then incubated in a preheated oven at annealing temperatures of 140°C and 60°C for 1 min. The choice of an annealing temperature primarily depends on the melting point of analyzed compounds to avoid their decomposition or degradation and achieve a proper molecular rearrangement [64].

Contact angle measurement. The contact angles of water and hexadecane droplets were measured using a contact angle meter (Drop Master DM-701, Kyowa Interface Science Co., Ltd). A total of 2 μL of water or hexadecane was dropped onto the coated silicon substrate. All measurements were triplicated.

Laser microscopy observation. Surface roughness parameters—Sa (arithmetical mean height) and Sz (maximum height)—were obtained for each sample using a 3D laser scanning microscope (VK-X3000 Series; Keyence, Tokyo, Japan). These two parameters were analyzed at ×5 and ×20 objective and laser light (intensity) over areas of 10 mm × 0.6 mm and 2,500 μm × 1,500 μm, respectively. Three points of measurement were selected on the silicon wafer coated with each substrate.

Statistical analysis. All statistical analyses were conducted using unpaired two-tailed Student's *t*-tests, with significance set at $p < 0.05$.

Author Contribution

NS and HH designed the research; NS performed the experiments; NS, TT and HH wrote the manuscript; and NSSA, MH, YT, YM, KI, YK, KS, NS and YO helped prepare the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgements

The authors wish to thank the University of Tokyo and Daikin Industries, Ltd. for providing the necessary facilities to carry out this research.

Data Availability

All data generated and analyzed in this study are included in this article.

Funding

Support for this research was provided by Daikin Industries, Ltd.

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Table and Figure captions

Table 1. Contact angle measurements of coated sterol as well as cholesterol and its derivatives on a silicon wafer

Table 2. Structural comparison of the tested sterol, as well as cholesterol and its derivatives

Table S1. Contact angle measurements of water droplets and statistical comparisons of cholesterol derivatives and stearyl glycyrrhetinate with cholesterol at different annealing temperatures

Table S2. Contact angle measurements of hexadecane droplets and statistical comparisons of cholesterol derivatives and stearyl glycyrrhetinate with cholesterol at different annealing temperatures

Supplemental Figure 1. Contact angle measurements of cholesterol and its derivatives. The names of compounds and their structures are shown, and actual images of contact angle measurement against a water droplet for estimating hydrophobicity and a hexadecane droplet for determining oleophobicity at two annealing temperatures, together with the contact angle, are presented, similar to Table 1.

Supplemental Figure 2. Laser microscopy observations of silicon wafers coated with (A) cholesterol and (B) β-sitosterol. The annealing temperature was 140℃. Left panels show laser-based surface observations; right panels represent 2D height maps (red/yellow = higher, blue/green = lower regions). The differences between (i) and (ii) denote the magnifications of the measured locations at 5× and 20×.

Supplemental Figure 3. Laser microscopy observations of silicon wafers coated with (A) ergosterol and (B) stigmasterol. The annealing temperature was 140℃. Left panels show laser-based surface observations; right panels represent 2D height maps (red/yellow = higher, blue/green = lower regions). The differences between (i) and (ii) denote the magnifications of the measured locations at 5× and 20×.