Next Generation Chemical Preservatives: Protecting People, Products, and our Planet

Heather L. Buckley, Adam P. Byrne, William M. Hart-Cooper and Jiawen Liao
University of California, Berkeley – Greener Solutions
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1. Executive Summary

This report provides chemical recommendations for greener, safer, and more sustainable preservative antimicrobials for use in home and personal care products. With the help of our partner organizations at Seventh Generation and Beautycounter, we initially explored the relevant product space where these preservatives could be used, which informed us as to what physical and chemical properties (e.g. water solubility, volatility) are necessary for a potential drop-in antimicrobial (Section 2).

Having explored the relevant product space, we investigated four classes of naturally-derived chemicals (terpenes, peptides, flavonoids, lipids) that are already represented in the products of our partner organizations (Sections 3-6). Individual chemicals were assessed for antimicrobial efficacy by reported minimum inhibitory concentration (MIC). In all chemical classes, antimicrobial candidate chemicals were identified that exhibit MICs that are comparable or superior to current industry standards. These compounds are effective against a broad spectrum of microbes, including Gram-positive and Gram-negative bacteria, yeast, and mold. Once identified, hazard assessments were completed that categorized these chemical classes by 18 health endpoints (Section 7). Data gaps and possible areas for concern are noted. Collectively, viable antimicrobial candidates were identified that conform to current health and efficacy standards.

Formulation aspects are discussed and several strategies are proposed to maximize the efficacy of selected antimicrobials and minimize negative aesthetic and hazard properties (Section 8). A summary and discussion of future areas of investigation is provided (Section 9).

2. Introduction

Home and personal care products are important parts of daily life in North America, providing a standard of cleanliness, skin protection, and food safety that we take for granted. However, many of the products that are used to clean and protect our clothing, our dishes, and ourselves are water based mixtures. Combined with the functional organic compounds that make up the non-water residual of these mixtures, a rich environment for microbial growth is present.

Yeast, mold, and bacteria grow opportunistically in any available medium. Their presence in home and personal care products can be problematic for several reasons. They can change the scent or texture of a product, which can be a problem for public perception and marketing. They can render a product ineffective if they consume the active ingredients. In the worst case, microbial growth can be pathogenic, resulting in harm to the end user.

The risk associated with microbial growth in home and personal care products necessitates the inclusion of preservatives that are either microbicidal (kill microbes) or microbiostatic (prevent growth of any microbes that come to be present). The development of suitable antimicrobials has been achieved with a range of broad-spectrum preservatives that include quaternary ammonium salts, chelators, formaldehyde releasers, parabens and isothiazolinones.

While these solutions have been effective, insufficient consideration has been given to the ubiquitous introduction of potentially hazardous chemicals in these classes. This proliferation is due in part to limited public scientific evidence of the health effects of those chemicals on human beings. Furthermore, the focus on product performance often supersedes attention to the potential health risk of certain ingredients, which undermines the health effects of the whole product. Recently, isothiazolinone preservatives have been shown to exhibit tissue sensitizing effects, leading to concerns over their safety as components in consumer products.

Many preservatives used in existing products, including paraben esters, formaldehyde (or formaldehyde releasers), isothiazolinones, dichlorophene, and iodopropynyl butylcarbamates,[1–3] have been linked to health hazards and ecological threats. This is unsurprising given that the role of a preservative is to be bioactive. Examples of hazards include skin irritation, respiratory sensitization, and tumor promoting effects demonstrated in humans. Triclosan and parabens have exhibited affinity to the human estrogen receptor and can enable consequent endocrine disruption.[4,5] Beyond potential human health hazard, some preservatives in home and personal care products cannot be fully degraded in sewage treatment plants. Ultimately, these compounds may pollute soil and water and show toxicity to aquatic and terrestial organisms.[6] Additionally, most clinical antibiotics, some of which are used in consumer products, encourage evolutionary adaptation by the microbial growth they target and eventually lead to problems with resistance.

Given these safety challenges, several companies in the home and personal care product space have recognized the importance of developing alternatives. However, barriers exist in finding alternative compounds, such as limited access to proprietary or subscription-based scientific studies on preservative toxicity. To overcome these barriers, we have partnered with Beautycounter and Seventh Generation to identify safe and high-performing preservatives for their

cosmetics and home care products. We have taken an iterative approach to evaluating consumer- and environment-friendly alternatives to current preservatives (Figure 2.1).

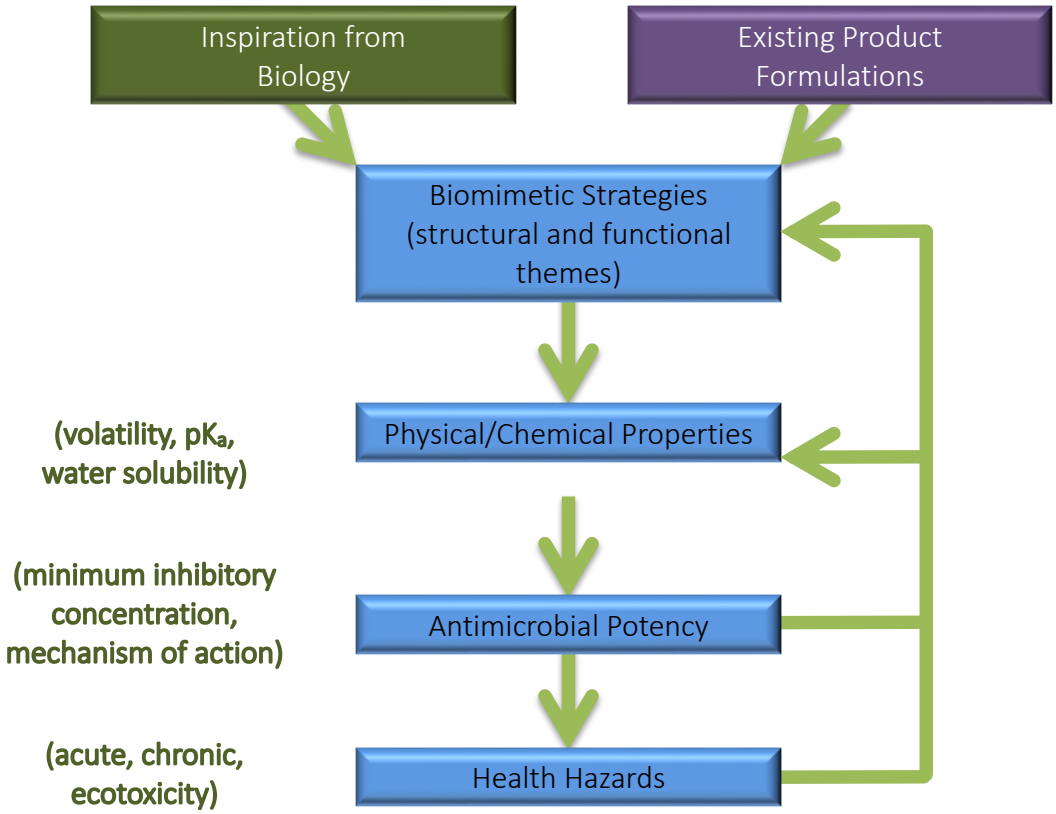

Figure 2.1. Identifying alternative preservatives through an iterative approach.

Our work included an initial compilation of potential strategies based on a review of relevant scientific literature and biomimetic alternatives conducted with the support of Biomimicry 3.8. The results of this compilation are briefly discussed below, and the biological precedent for each of our strategies is discussed in detail as they appear in the report. Our synthesis of the biological literature was followed by close collaboration with Beautycounter and Seventh Generation to understand the constraints of their existing product formulations. These constraints vary for different classes of products and are outlined below. Finally, we iteratively analyzed the physical and chemical properties, antimicrobial effectiveness, and health hazards of the major classes of compounds we selected to study. The details of this analysis are found in Sections 3-6.

2.1. Biomimetic Strategies

Several major strategies are used by biological systems to control or inhibit microbial growth.

- Membrane disruption: either the complete dispersion of cell membranes by surfactants or the creation of pores that disrupt proton or other ion gradients. Many classes of molecules are used for this approach, including fatty acids, terpenes, and cationic polypeptides.

- Nutrient limitation: sequestering of essential nutrients to prevent growth of microbial colonies. This approach often relies on highly specific binding of metals, and we did not pursue it further in this project due to conflicting evidence about both the applicability and hazard risks of introducing selective chelators into the environment.
- Multifunctionality: many antimicrobial molecules also serve other roles in their biological environments. Additionally, many antimicrobial molecules are not highly specific, but can be active against a range of potential pathogens. This strategy will be discussed further in Section 8.
- Synergistic formulation: working together, two or more ingredients can be more effective than the sum of each acting individually. This strategy will be discussed in more detail in Section 8.

Each of these strategies could be used to develop preservatives in home and personal care products. To better understand their utility and constraints, we summarized the functions essential to the formulation of home and personal care products, as well as what molecules or classes of molecules performed these.

2.2. Current Formulations: Beautycounter

From a regulatory perspective, personal care products face minimal restriction. They are regulated by the Food and Drug Administration (FDA) and the Federal Food, Drug, and Cosmetics Act (FDCA), a very minimal piece of legislation crafted in the 1930s.[7] Beautycounter places a top priority on the safety of their ingredients and formulations and competes as a premium brand of personal care products and color cosmetics. From a practical perspective, it is worth noting that personal care products are typically formulated at a slightly acidic pH, and replacement preservatives that can either be dissolved or suspended/emulsified in water without changing the user experience will be most readily adopted.

Beautycounter prioritizes preservatives that are safe for use by children and when applied to skin. We have chosen three representative products that fit one or both of these criteria: “All Over Sunscreen” (used by adults and children), Children’s “Nice Do Shampoo” and “Every Day AM Hydrating Cream” (used by adults).

For each of these products, we have identified the major functions served by the ingredients that they contain. (A spreadsheet found in Appendix C lists all of the ingredients of each product along with their chemical formula and functions as identified by Beautycounter or from a search of other cosmetics websites.) Most of these functions are common to all of the products; a few (i.e. sunscreen) are product specific. All of these factors are listed in the Table 2.1, defined based on our understanding and grouped where they are similar.

Table 2.1. Functions of ingredients found in Beautycounter formulations.

Broad Function	**Specific Function**	**Definition/Explanation**
Control viscosity	Viscosity control	Controls "thickness" of liquid product, how easily it flows
	Texture control	Controls "smoothness" – (may not be distinct from viscosity?)
Solvent	Solvent	Provides a bulk material in which all other ingredients are dissolved, emulsified, or otherwise suspended
Control Smell	Scent	Smells nice at concentration used – volatile
	Deodorizer (cream only)	Prevents bad smells – in this case appears to be through antimicrobial activity; this could also be accomplished by binding to bad-smelling volatile compounds
Surfactant	Non-ionic surfactant	Molecule with no charge that has a hydrophobic and hydrophilic region (amphiphilic)
	Anionic surfactant	Molecule with a negative charge that is amphiphilic
	Emulsifier, Binder, Stabilizer	Similar to a surfactant, however polyhydroxystearic acid is listed as a nonsurfactant suspending agent
	Amphiphilic Surfactant	Molecule with no net charge but cationic and anionic sites
	Antistatic	Dissipate charge buildup (not quite clear application in cosmetics)
	Skin Absorption	Penetrate surface layer of skin
Moisturize	Moisturizer	Defined as combination of humectant+emollient+occlusive agent
	Humectant	Pulls water out of the air, keeping the relative humidity high near the skin
	Emollient	Smoothes skin by filling cracks with lipids
	Occlusive Agent (not listed)	Forms oil film on skin to prevent moisture loss
	Conditioner (shampoo only)	Coats strand of hair to trap moisture
Prevent Irritation	Antihistamine	Prevent mild skin irritation reactions
Prevent Decomp.	Antioxidant	Reacts with oxidizers that form (i.e. peroxide) to prevent them from reacting with critical ingredients
Prevent Growth	**Antimicrobial**	**Biostatic or biocidal against one or more of yeast, mold, and bacteria**
Other	Anticaking (sunscreen only)	Silica – possibly keeps zinc oxide from caking by breaking up dehydrated surface films?
	Sunscreen (sunscreen only)	Zinc oxide – absorbs and scatters or reflects UV light to prevent sunburn

Each of these products contains an average of 27 ingredients. For the sunscreen, we grouped these products according to type of molecule and found that each class of molecules had multiple functions. We noted that the function listed on the Beautycounter website was at times different for two very similar molecules (particularly in the case of esters and triglycerides). Table 2.2 lists classes of molecules that we identified along with their various functions.

Table 2.2. Breakdown of molecule classes found in Beautycounter's "All-Over Sunscreen," outlining their potential functions in the formulation.

Molecule Class	**Functions**	**General Properties**
Esters	Surfactant Moisturizing Viscosity control	Non-volatile Water-emulsifying
Triglycerides	Surfactant Moisturizing Viscosity control	Non-volatile Water-emulsifying
Fatty Acids	Surfactant	Non-volatile Water-emulsifying
Lecithin	Surfactant (amphiphilic)	Non-volatile Water-emulsifying
Terpenes	Scent Antioxidant Antihistamine	Volatile Water-insoluble
Glycols (1-2-diols)	Moisturizing **Antimicrobial** Solvent (glycerol)	Non-volatile Water soluble
Flavanols	Antioxidant **Antimicrobial**	Non-volatile Water-insoluble?
Steroidals	Antihistamine?	Non-volatile Water-insoluble?
Phenoxyethanol	**Antimicrobial**	Non-volatile Moderately water soluble
Water	Solvent	N/A
Inorganics (various)	Viscosity control Anticaking Sunscreen	Non-volatile Varied solubility

2.3. Current Formulations: Seventh Generation

In contrast to personal care products, home care products face considerably greater legislative restriction from the Environmental Protection Agency (EPA) and the Federal Insecticide, Fungicide, and Rotenticide Act (FIFRA). However, this legislation does not require a level of safety that meets current consumer standards. Seventh Generation's priorities for preservative replacement are their products that currently contain methylisothiazolinone (MIT) and

benzisothiazolinone (BIT). These products include dish liquid, auto dish gel, laundry detergent, fabric stain remover, and fabric softener. In general, these products function at a slightly basic pH, and preservative alternatives that can be dissolved or suspended/emulsified in water will again be the most actionable alternatives. Seventh Generation places highest priority on choosing ingredients that are safe and naturally sourced.

Many of the ingredients and the functions they serve are fairly consistent across this subset of Seventh Generation's products. In general, each product contains one or more active cleaning agents (a surfactant or enzyme) in conjunction with organic and inorganic ingredients that achieve the desired properties and performance of the final product mixture. Table 2.3 provides an overview of the functions that ingredients serve (as defined by Seventh Generation) in their laundry and dish products. Additional information on specific ingredients and their function is available in Appendix C.

Table 2.3. Functions of ingredients found in Seventh Generation formulations.

Broad Function	**Specific Function**	**Description**
Cleaning	Cleaning agent	Surfactants that solubilize hydrophobic dirt and oil by forming micelles
	Enzyme soil remover	Enzymes that break down compounds to increase their water solubility
	Fabric softener	Binds to clothing fibers to prevent static cling and make fabric feel softer
Control physical properties	Viscosity modifier	Controls how easily a liquid product flows
	Thickener	Increases the viscosity of a liquid product
Support product performance	Foam stabilizer/anti-foaming agent	Prevents excessive foaming of surfactants
	Water softener	Complexes divalent cations (Ca^{2+}, Mg^{2+}) to promote proper function of cleaning agents
	Alkalinity builder	Increases pH buffering capacity
	Enzyme stabilizer	Maintains enzyme activity over product shelf life
	pH adjuster	Achieves desired final pH in product mixture
	Preservatives	**Prevents growth of bacteria, yeast, and mold**
Aesthetics	Fragrance	Gives the product a pleasant aroma

Similar to our analysis of Beautycounter's products, we grouped ingredients by molecule class to identify trends between important functional groups and the functions ingredients serve in the product. Most molecule classes are associated with only one primary function as defined by Seventh Generation. However, some types of molecules may have additional uses not explicitly identified on the product label and our investigation involved identifying relevant multifunctional ingredients. Table 2.4 provides a summary of the common molecule types, their associated functions, and general properties.

Table 2.4. Breakdown of molecule classes found in Seventh Generation's laundry and dish detergents, outlining their function in the formulation.

Compound Class	**Functions**	**General Properties**
Alkyl sulfate	Surfactant/cleaning agent	Amphiphilic Non-volatile Anionic
Polyol	Foam stabilizer Enzyme stabilizer	Water soluble Non-volatile
Polycarboxylic acids	Water softener pH adjuster	Water soluble Non-volatile Anionic (at neutral pH)
Heterocyclic molecules (MIT and BIT)	**Preservative**	Limited volatility Biocidal
Terpenes	Fragrance	Varying water solubility Volatile
Alcohol ethoxylates	Surfactant/cleaning agent	Amphiphilic Non-volatile Nonionic
Enzymes	Soil remover	Water soluble Non-volatile
Polysaccharide	Thickener	Non-volatile Varying water solubility
Fatty acid	Anti-foaming agent	Amphiphilic Non-volatile (if long chain)
Aldehydes	Fragrance	Volatile Varying water solubility
Esters	Fragrance	Volatile Varying water solubility
Mineral salts/inorganics	Viscosity modifier Alkalinity builder Enzyme stabilizer pH adjuster	Ionic Water soluble Non-volatile

Based on our understanding of biological strategies and the constraints of product formulation, we chose four major classes of compounds for further research as "drop-in" preservative replacements. Terpenes, polypeptides, flavonoids, and fatty acids are explored for their physical properties, efficacy, safety, and commercial availability in the following four sections. Following this, Section 7 provides a comparison between the four classes of molecules, and Section 8 moves beyond the idea of existing single molecule solutions and provides recommendations of alternative formulation strategies to be pursued both by Beautycounter and Seventh Generation and by the home and personal care industries as a whole.

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3. Alternative Recommendations: Antimicrobial Terpenes

3.1. Overview

Terpenes are a ubiquitous family of molecules, accounting for between one quarter and one half of all known small molecule natural products.[1,2] Due to their high stereochemical and functional diversity, terpenes are used by all organisms for an enormous range of functions, from vital nutrients to hormones and antimicrobials. The latter category is widely believed to be the most common function of terpenes and includes a diverse set of simple compounds produced abundantly and cheaply by plants.[3] Due to their natural sourcing, many common antimicrobial terpenes are generally safe, being major components of herbs which humans have cultivated for thousands of years. Many of these antimicrobials operate in a synergistic manner, allowing for low inhibitory concentrations to be achieved under certain compound ratios.[4,5] Terpenes often exhibit a pleasant aroma. For this reason, these compounds find abundant use in home and personal care products and are good candidates for consideration as drop-in antimicrobials.

3.1.1. Natural Sources

Although terpenes are produced by all organisms, the majority of practical antimicrobial candidates are produced by plants to repel microbial invasion. Antimicrobial terpenes can be used in pure form or as a component of a crude plant extract or essential oil. Carvacrol, for example, exhibits broad spectrum antimicrobial activity when used as a pure compound or oregano essential oil, of which it constitutes up to 90% by mass.[6] Terpene hydrocarbons, such as α-pinene, likewise contribute to antimicrobial properties of pine sap.[3] More exotic antimicrobial terpenes, such as (+)-totarol, imbue the wood of *P. totora* with an unusual ability to resist rot. Antimicrobial terpenes, whether pure, part of a crude plant extract or essential oil, are often affordably obtained on account of their natural sourcing from plants on the ton scale.

3.1.2. Structure

Antimicrobial terpenes exhibit high structural diversity which influences efficacy against different microbe classes. The presence of polar groups (aldehydes, ketones, alcohols, phenols) occurs with generally higher antimicrobial efficacy. Of these functional groups, terpenes containing alcohols or phenols are generally most effective.[7,8] Acyclic terpene alcohols are effective in a similar manner to fatty acids, where hydrocarbon chain length can be used as a crude predictor of antimicrobial potency against certain organisms.[7] Cyclic terpenes in general are more abundant than acyclic ones to the high structural diversity that may be generated upon cyclization. There are consequently many more examples of cyclic than acyclic terpenes that exhibit antimicrobial activity. Cyclic hydrocarbon terpenes lacking other functionality, while effective against yeasts, are generally less effective against bacteria (Gram-negative and Gram-positive) and molds.[9] Contingent on the mechanism of biocide, lipophilicity correlates with antimicrobial efficacy. Although some degree of lipophilicity is necessary for antimicrobial activity, extremely lipophilic terpenes are often ineffective due to their low water solubility.[10,11] Although the pK_a of most terpene functional groups is several pH units away from physiologically relevant conditions, basic solutions will accelerate the oxidation of phenolic compounds to products whose hazard traits may be poorly understood. The antimicrobial efficacy of phenolic compounds will likewise vary

between neutral and strongly basic pH (Figure 3.1). Overall, while certain structural characteristics of terpenes are generally related to their antimicrobial efficacy, activities are highly dependent on the microbe under consideration and *a priori* predictions should be treated as generalizations in need of continued experimental validation.

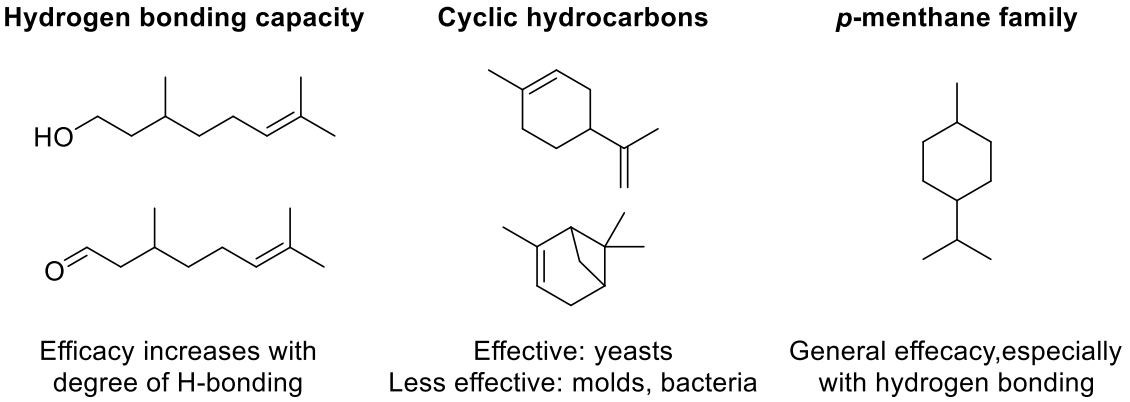


Figure 3.1. General trends between antimicrobial efficacy and terpene structure. Hydrogen bonding capacity often accompanies increased antimicrobial efficacy, while cyclic hydrocarbons are more effective against yeasts than other microbes. The *p*-methane family includes a diverse set of effective antimicrobials.

3.1.3. Mechanism of action

Antimicrobial terpenes most frequently operate through disrupting microbial cell membranes, and are effective against a broad spectrum of species (Figure 3.2).[9,10,12] Phenolic terpenes[13,14] are reported to attenuate the cross-membrane proton gradients of prokaryotes, thereby directly targeting the energy production processes of the cell.[15–17] This mechanism is favorable from a human hazard standpoint, as unlike those of bacteria, eukaryotic proton gradients are localized to the mitochondria and are considerably more resistant to disruption by these compounds.[8] By virtue of their general mechanisms of action (targeting membranes, proton gradients), antimicrobial terpenes exhibit broad spectrum activity to which it is difficult for microbes to develop resistance. This property stands in contrast to many clinical antibiotics that target a particular protein, and whose properties are easier to obviate by adaptive mutation.

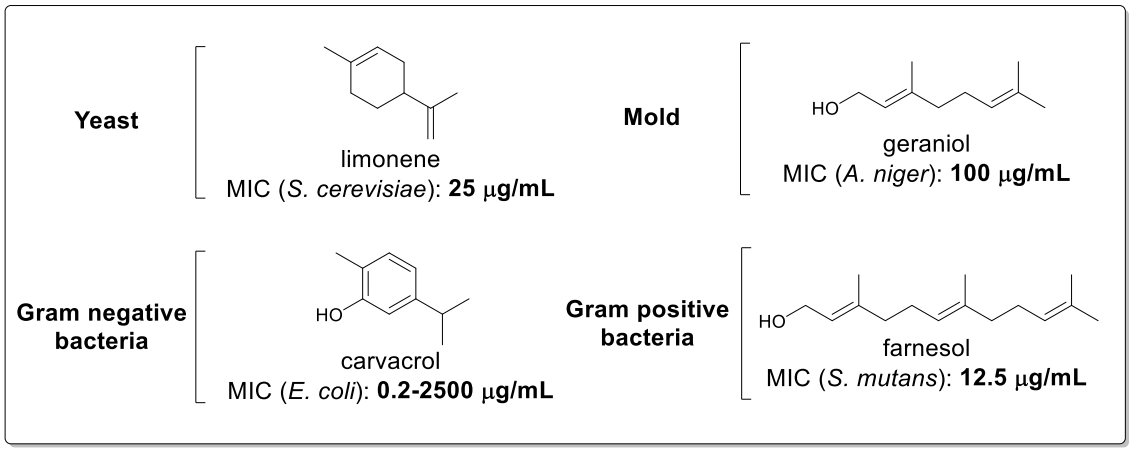
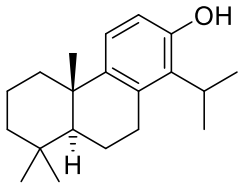


Figure 3.2. Selected antimicrobial terpenes that are particularly effective against the specified organism. Large variations in minimum inhibitory concentration bserved among better-studied compounds and organisms (see carvacrol, bottom left), which is contingent on the strain of microbe used in the study (references 9-17).

3.2. State of the Art

Due to their structural variability and versatility, terpenes are among the most widely used and effective small molecule antimicrobials produced biologically. One noteworthy compound ((+)-totarol) was identified in the wood of *P. totara*, which has long been recognized as extremely rot resistant.[18] This compound has been used for over 100 years in the traditional medicine of the Maori people, treating ailments ranging from cholera to venereal disease.[19] Although its antimicrobial activity is comparable to clinical antibiotics,[20] (+)-totarol's mechanism of action is unclear. Consequently, its use has generally been limited to personal care products, which are produced in a more liberal regulatory space than pharmaceuticals.[19]



(+)-totarol

Figure 3.3. (+)-totarol, an isolate from *P. totara*, is currently used in cosmetics and exhibits extremely high antimicrobial potency.

As pure compounds or essential oils, terpenes are widely used in cosmetics and personal care products by Beautycounter and Seventh Generation, often for their scent. Thymol, carvacrol and related essential oils are choice antimicrobials in many products including the CleanWell product line. Antimicrobial terpenes that have generally been recognized as safe will continue to provide a source of useful antimicrobials in green home and personal care products.

3.3. Hazard Information

Terpenes constitute an enormous class of chemicals, some of which are known toxins or hormones that are clearly not under consideration as potential antimicrobials. The hazard information we have provided about common plant-produced antimicrobials is therefore generalized (and oversimplified).

Many of the compounds we recommend have been in regular contact with humans for thousands of years through normal environmental exposure (pine tree sap) or use of herbs in cooking (e.g. oregano, thyme, mint). Still, "the dose makes the poison" in many cases, and the toxicological literature documents that harmful, even fatal levels of otherwise benign essential oils (or pure compounds) can be administered to various mammals, where toxicity is observed on the order of grams per kilogram through dermal exposure. Dermal sensitization, oral toxicity, asthmagen activity, and respiratory and eye irritation is sometimes observed at lower exposure levels. As natural products, terpenes are generally biodegraded rapidly; however, at high levels they may exhibit aquatic toxicity. Before any prospective terpene antimicrobial is used in a product, it should be rigorously screened for the aforementioned hazard endpoints using the toxicological data available or by independent experiment.

The hazard traits for this class of compounds have been well explored, although information on carcinogenicity and developmental toxicities/endocrine activities is less prevalent. Certain terpenes can cause skin, eye, and respiratory irritation at high exposure levels. This effect which is generally observed in less than 1% of surveyed populations (oxidized byproducts may also induce sensitization).[21–28] Oral toxicity information is readily available, and in all cases is sufficiently low to permit considering these compounds as viable preservatives. Terpenes are generally prone to facile biodegradation.

3.4. Recommendations for Seventh Generation and Beautycounter

Pure terpene compounds and essential oils are currently used in many products from these two companies, often for their scent. The concentrations of these components may need to be increased, or compositions varied, in order to achieve broad spectrum antimicrobial activity. Variation in antimicrobial and hazard properties between formula and pure additive is impossible to predict from the available literature; these concerns will need to be addressed by independent testing. Still, there exist ample examples of antimicrobial terpenes with the potential to replace preservatives currently used in these companies' products.

3.4.1. Data gaps

Quantifying the exposure levels that bring about the unfavorable hazards described above is a challenging prospect, given the inherent variability of toxicological testing. Other hazard endpoints are also rare or absent in the literature, and should be investigated or independently tested if this exposure route is likely for the intended product application (see Section 7).

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4. Alternative Recommendations: Antimicrobial Peptides

Being derived from the same building blocks and chemistries that make proteins in all forms of life, peptides are a promising class of molecules for inclusion in consumer products. Several classes have well-established antimicrobial properties, which we outline below.

4.1. Overview of Antimicrobial Peptides

4.1.1. Structure

Polypeptides are constructed from amino acid monomers, which have the general formula shown in Figure 4.1. The backbone of amino acids is generally the same, and they vary based on their side chains (shown as R). There are approximately 20 amino acids that occur commonly in nature, as well as others that are uncommon and those that have been prepared only synthetically. Consequently, a vast library of peptides can be constructed by systematically varying the amino acids.

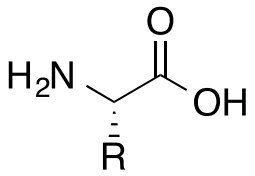

Figure 4.1. General structure of an amino acid. R represents any side chain.

Polypeptides derive their primary structure through covalent linkages between amino acids. These take place by way of a condensation reaction between the amine group of one amino acid and the carboxylic acid group of the next (Figure 4.2). In most amino acids this reaction takes place as shown between the adjacent amine and acid groups (those attached to the “alpha” carbon), however if the R-group of an amino acid also contains an amine (e.g. lysine) or carboxylic acid (e.g. aspartic acid) group, then structural variants can occur.

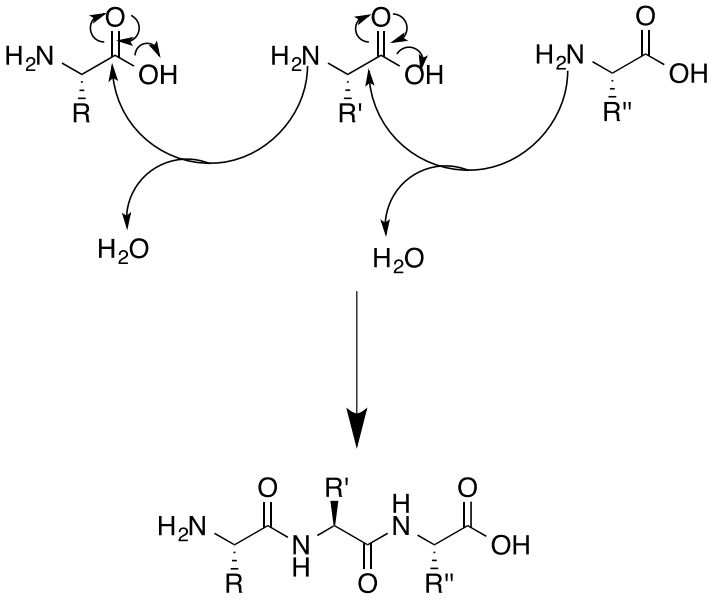

Figure 4.2. Condensation reaction of amino acids to form a polypeptide.

The properties of a polypeptide depend both upon the characteristics of its side chains and on its folding structure. For small polypeptides such as those we discuss below, the properties of the side chains predominate the functionality (charge, polarity, hydrophilicity), but in large polypeptides and proteins, biologically guided folding also contributes to the structure and function of the molecule.

An interesting subclass of polypeptides that we have encountered in this work is cyclic polypeptides. Unlike traditional polypeptides, which have a linear structure that forms as shown in Figure 4.2, cyclic peptides can form in several ways. The simplest of these are composed only of normal peptide bonds forming a closed loop; more complex structures generally result from branching that is possible due to amine and carboxylic acid groups on the amino acid side chains.

4.1.2. Synthesis

Polypeptides can be prepared either through traditional chemical synthesis or biosynthesis. They can also be extracted from natural sources and isolated for use. While the latter method is useful in early identification of active compounds, it is uncommon for a polypeptide used in a commercial product to be accessed in this way unless it is part of a complex extract.

Biosynthesis of a polypeptide typically involves splicing genetic material encoding that amino acid sequence into the genome of a nonpathogenic bacterium or yeast, and then providing growth conditions that promote the expression of that genetic material.[1,2] For example nisin is produced from the nonpathogenic bacterium *Lactococcus lactis*.

Chemical synthesis of polypeptides is generally labor intensive and expensive. Solid phase synthesis (on surface-functionalized beads) is the most common method, and it involves the stepwise addition of each amino acid, often with the use of protecting groups, followed by

intervening purification steps.[3] Other methods also require multiple steps and as such are not a good route for production of a commodity chemical.

In the case of homopeptides (repeating chains of the same amino acid, e.g. polylysine, polyaspartic acid), both biosynthesis and chemical synthesis can be accomplished more easily than for complex peptides, provided that a distribution of chain lengths is acceptable. For biosynthesis, rather than a DNA-templated reaction, an enzyme-catalyzed reaction *in vivo* can be promoted by a nutrient stock rich in the desired amino acid. Polylysine is prepared by fermentation with the nonpathogenic bacterium *Streptomyces albulus*.[4]

Chemical synthesis of homopeptides can be achieved thermally or catalytically. Polyaspartic acid, in particular, can be prepared by a variety of routes. The most common in the literature is a thermal polymerization that produces a mixture of alpha- and beta-linkages.[5] Other methods are possible to obtain enantiomerically and isomerically pure homopeptides.

4.1.3 Mechanism of Action

As will emerge as a common theme in all classes of molecules that we explored as preservative alternatives, antimicrobial peptides most often act through some form membrane disruption.[6] Amphiphilic peptides have the ability to interact both with the hydrophilic surfaces the hydrophobic interior of the cell membrane.

Membrane disruption as a primary antimicrobial mechanism is an interesting and valuable tool for differentiating activity against prokaryotic (bacterial) and eukaryotic (human) cells. Bacterial cell membranes have a significant negative charge at the outer surface, which gives them a stronger initial binding affinity for positively charged species than less strongly charged eukaryotic cell membranes (see Figure 4.3).[7–9] As a result, while the various mechanisms of membrane disruption can also disrupt eukaryotic cells, antimicrobial peptides are strongly selective for prokaryotic cells.

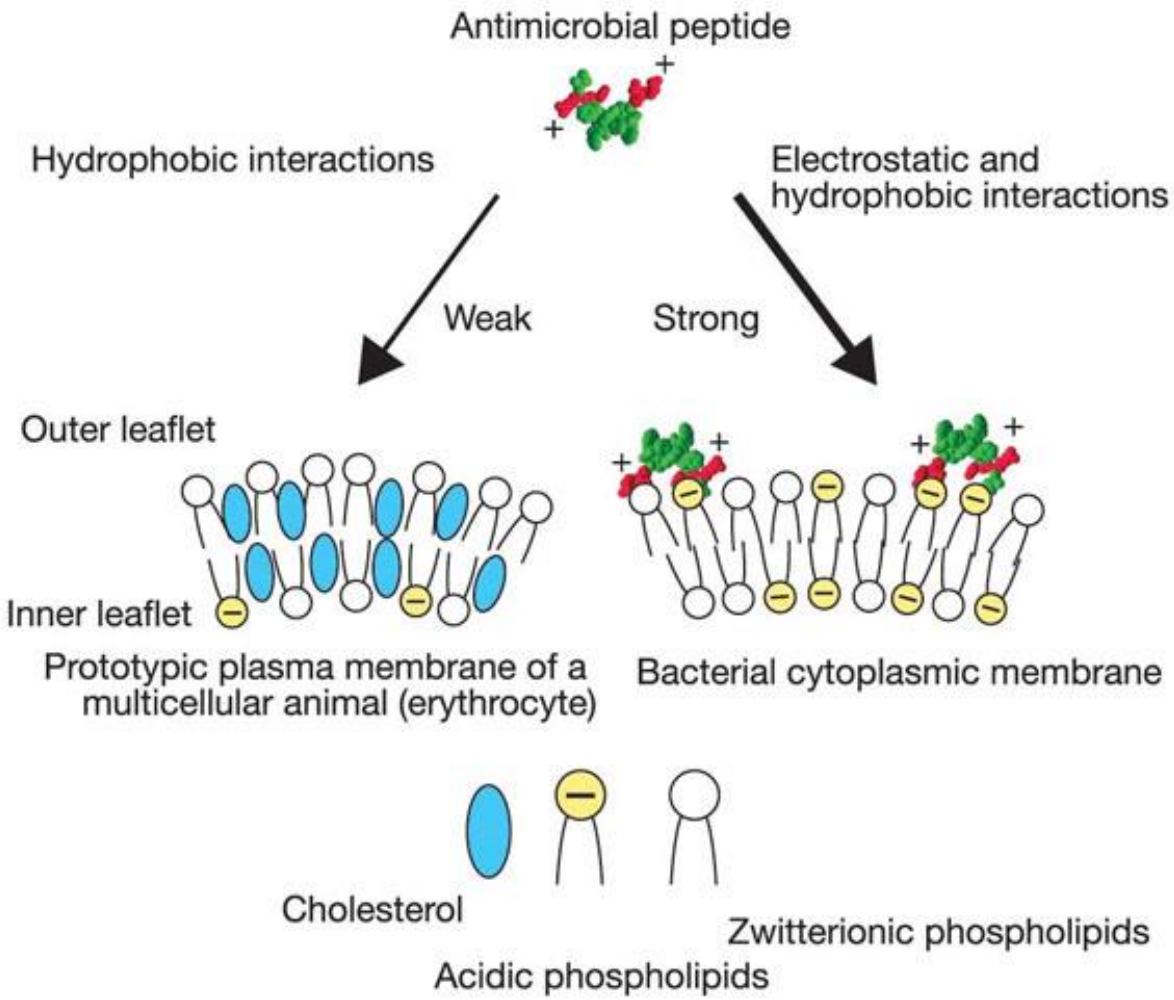

Figure 4.3. Selectivity of cationic antimicrobial peptides for bacterial cell membranes. The bacterial outer membrane is negatively charged, whereas multicellular organisms have neutrally charged outer cell membranes. Image reproduced from Zasloff.[9]

Interaction with the cell membrane can result in one of several modes of activity for a peptide.[10] The first is direct disruption of the cell membrane, resulting in pore formation that permeabilizes the membrane and disruption of concentration gradients (e.g. charge, proton, and ATP) that are vital to cell function. The second is passing through the membrane and then disrupting an intracellular process. The third mode of activity is synergistic. A polypeptide that is innocuous can either insert into the membrane or pass through it carrying another class of antimicrobial molecule that would normally be screened out by the cell's protective mechanisms.

Another mechanism of action for antimicrobial activity can be the control of nutrient availability through metal chelation. This is proposed to be one of the mechanisms of action of the highly phosphorylated protein phosvitin, which is found in egg yolk.[11,12]

4.1.4 Spectrum of Antimicrobial Activity

Antimicrobial peptides are generally most effective against Gram-positive bacteria, although in some cases activity against Gram-negative bacteria as well as yeast and mold species have been reported. Appendix E provides MIC data for several polypeptide classes. The three major peptide types we examined were homopeptides, nisin as a representative cyclic peptide, and linear tripeptide units as an emerging class of molecule.[13]

In the case of homopeptides, we found no information on MICs of polyaspartic and polyglutamic acids and some information indicating that they did not have useful activity compared to other known antimicrobials.[14] Both do have some activity in interaction with thrombin for blood clotting.[15] Polyaspartic and polyglutamic acids were of interest both because of their amphiphilicity and their ease of synthesis. The lack of antimicrobial activity from these negatively charged peptides is in fact consistent with repulsion by a negatively charged bacterial cell membrane. By contrast, we found an extensive study on polylysine demonstrating antimicrobial activity against 25 species across Gram-positive bacteria, Gram-negative bacteria, fungi, and yeast.[4]

Nisin in particular is known to be effective against Gram-positive bacteria,[12] although it should be noted from the MIC tables that effectiveness per unit weight is quite low when compared to small molecule antimicrobials[16,17] and even with polylysine. It is common throughout the peptide literature to report antimicrobial effectiveness in terms of IU (international units) per mL; this convention accounts for the common practice of keeping antimicrobial peptides in the medium where they were grown and accounts for variation in "active molecule" concentration in that medium.

4.2. State of the Art

Currently, antimicrobial peptides are not widely used in home and personal care products. However, there are several examples that are used in food preservation and in medical applications. These provide a good basis for considering antimicrobial peptides in new applications, both because they are commercially available and because more work has been done to understand their effectiveness and potentially their toxicity than for antimicrobial peptides and proteins that have been isolated or prepared only in research laboratories.

4.2.1. Food Preservation

Nisin is a naturally occurring cyclic polypeptide from a class known as bacteriocins. It has been used for several decades as a healthier alternative to salts and nitrites as food preservatives.[16] Nisin is by far the antimicrobial peptide that sees the most widespread use in food products,[18] and is sold under the trade name Nisaplin® by Danesco. In addition, roughly a dozen new bioactive peptides (not all antimicrobial) have been approved by the FDA since 2001.[19] A search for antimicrobial peptides on Sigma Aldrich (a major chemical supplier) reveals an abundance of similar compounds that are commercially available on a research scale.[20]

There are several ways in which nisin can be incorporated into packaged food. In some cases it is suspended as an ingredient in the bulk of liquid food, but it can also be sprayed as part of a mixture onto the surface of food or packaging.[21] Of great interest from the perspective of considering exposure levels (even for a low toxicity compound) is the incorporation of nisin into plastic film for food packaging. Nisin has been incorporated in traditional packaging materials,[22] in slow-slow release formulations,[23] and in polylactic acid (PLA) biodegradable packaging materials.[24]

4.2.2. Medical Use

Polylysine, specifically ε-poly-L-lysine (the predominant form produced in biosynthesis) has been studied for use as an antibiotic in vaginal creams.[25] It is of interest because unlike traditional antibiotics, the homopeptide will not promote the growth of resistant strains of *Gardnerella vaginalis* and other pathogens.[26,27] In both this application and in oral care applications,[28] polylysine has been shown to have enhanced activity when working in synergy with other antimicrobials, including nisin. Polylysine is soluble in water, and has also been studied for approval as a preservative in food.[29]

4.2.3. Biological Use

Antimicrobial peptides are ubiquitous in biology. Due to their low abundance, extraction is typically not practical if a microorganism cannot be cultured to selectively produce the antimicrobial peptide in large quantities (as with nisin and polylysine). However, examples are widespread. Frogs[30] and a diverse set of antimicrobial peptides in aquatic systems[31] provide just a few examples of inspiration for industrial applications.

4.2.4. Emerging Products

Because of issues of supply chain reliability and cost, which we discuss below, newly discovered or developed polypeptides are not likely viable candidates for use in home and personal care products in the near future. However, there are several classes of polypeptides that warrant brief mention for the sake of providing complete information.

- Amphiphilic repeating-unit peptides: the patent literature contains many examples of patents on antimicrobial peptides. Of particular note is an example that contains repeating peptides of the formula $(XZX)_n$ where Z = lysine and X = any non-polar amino acid.[13] This relatively simple formulation stands out because the patent literature contains data about antimicrobial activity. This patent specifically proposed these molecules for use in dishwashing liquids.
- Phosvitin: another proposed antimicrobial for use in the food industry. A phosphorylated protein found in egg yolk, phosvitin is recognized as an antioxidant, and has been studied for its activity against *Escherichia coli*.[11,12]
- Other antimicrobial/antibiotic cyclic peptides: straptogramin Type B derivatives appeared frequently in our searches of the patent literature for antimicrobials in detergents. These structures are typically characterized by adjustments that do not change the activity of the peptide, but modify the backbone to prevent enzymatic hydrolysis.[32]
- Peptoids: an alternate synthetic structure with similar functionality to peptides, poly-N-substituted glycines, or peptoids, have been shown to have antimicrobial activity.[33] They

are less expensive to synthesize than mixed polypeptides, and may be a promising class of compounds as antimicrobials in the future.

4.3. Hazard Information

All cells possess peptidase enzymes capable of breaking down polypeptides into their amino acid monomers. Because these monomers all have low toxicity both to humans and aquatic ecology[34] at relevant concentrations, most proteins are good choices from the hazard perspective. The only health hazards seem to occur in populations with uncommon disorders, and even then warnings are related to taking dietary supplements.[35] Specific evidence exists suggesting that polylysine exhibits low toxicity,[36] (although in the high concentrations found in snake venom it can cause necrosis)[37,38] and it is classed as "Generally Recognized as Safe" (GRAS) by the FDA. In the absence of such definite toxicology data (which is not available for newly engineered peptides such as the $(XZX)_n$ structures discussed above),[13] all of the homopeptides and linear polypeptides discussed above fall under this categorization.

Cyclic polypeptides are more robust against degradation by peptidases,[39] and so should be used with more caution in the absence of extensive toxicity data. However, because they are directly ingested by humans, preservatives in food are regulated by the FDA in a manner that is more stringent than for many other consumer products. Nisin falls into this class, and has been studied extensively enough to be classified as GRAS for this use. Additionally, all studies that we found indicated that it had low toxicity under the conditions evaluated in rat models.[40,41] Information on the biodegradability of nisin is not readily available. However, it is naturally occurring and biologically synthesized, so it is likely that enzymes exist that can facilitate nisin degradation.

For any polypeptide that is prepared through biosynthesis, it is important to be mindful of the nutrient and material source used by the nonpathogenic engineered bacteria and any potential allergies that could be linked to impurities. Nisin, for example, is produced in a milk-based environment[42] and sometimes sold mixed with milk solids, and phosvitin is extracted from egg yolk.[12] While food sensitivities exist to both of these products, there is minimal risk of triggering an allergic reaction through the normal use of home and personal care products. However, it is important for Seventh Generation and Beautycounter to be mindful both of the potential hazards of other biologically derived proteins and to be confident in defending the safety of polypeptides from any chosen feedstock.

4.4. Recommendations for Beautycounter and Seventh Generation

Our evaluation of antimicrobial peptides leads us to recommend that Seventh Generation and Beautycounter consider two antimicrobial peptides for further research. Both polylysine and nisin are good candidates for use in home and personal care products subject to the following qualifications.

- Price. Both of these compounds are available at costs as low as $1/kg.[43] Prices for a grade suitable for home and personal care products may be higher. However, these costs are typically 1000 fold lower than for other antimicrobial peptides that are not

used as broadly, and the widespread use of both products should mean that the supply chain and quality available are reliable.[44]

- Effectiveness at other pH levels. The pK_a values[45] of the two NH_3^+ groups of lysine are 8.95 and 10.53, meaning that the protonation state of polylysine should be similar in the majority Beautycounter and Seventh Generation's products, which range in pH from 4.5 to 9. However, the MICs of both compounds may be different at the pH of the product than in the experimental data we have gathered.
- No peptidases, proteases, or amide-cleaving enzymes. Some laundry detergents use peptide-cleaving enzymes as part of the cleaning process, which maydegrade polypeptide antimicrobials.
- Better understanding for nisin persistence. It is clear that polylysine will break down rapidly in the environment. Further data should be obtained to confirm that nisin will not bioaccumulate.
- Spectrum of antimicrobial activity. Generally, antimicrobial peptides are most active against Gram-positive bacteria.

As was mentioned previously, preservative incorporation in packaging rather than in the liquid formulation may be an interesting avenue for Beautycounter and Seventh Generation to pursue both for polypeptide antimicrobials and for other bioactive compounds.

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5. Alternative Recommendations: Antimicrobial Flavonoids

5.1 Overview

Flavonoids are a group of heterocyclic organic compounds. As plant metabolites, they are widely present in fruits, vegetables, seeds, and nuts.[1] They are also common in plant-based food and drinks such as honey, propolis[2], wine,[3] green tea, and traditional herbs and plant-based medines.[4–6] Flavonoids have various functions in plants, such as pigments in flowers, UV radiation protection agents in leaves, and antimicrobial agents against fungal pathogens.[7]

5.1.1. Structure

There are two major structural features that dictate the properties of flavonoids. While there is a great deal of diversity in these compounds, two important characteristics to consider is their heterocyclic nature and composition of conjugated double bonds.

- Heterocyclic. Flavonoids are a group of heterocyclic organic compounds. The basic structure of flavonoids is the flavone nucleus, which includes two aromatic rings (ring A and B, Figure 5.1) linked through a pyran ring (ring C)[7] with a total of 15 carbon atoms in the main structure. For some groups of flavonoids, such as the chalcone and dihydrochalcone groups, the pyran ring (ring C) is opened or missing, meaning ring A and ring B are linked by a carbon chain. The core structure of flavonoids is shown in Figure 5.1.

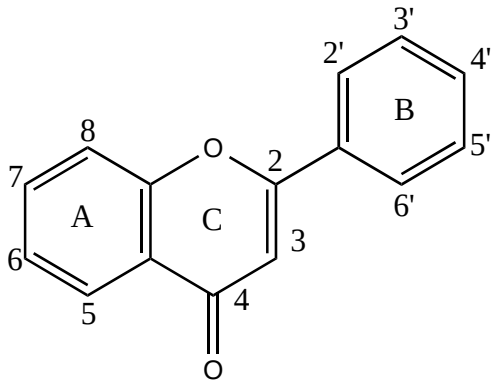

Figure 5.1. The skeleton structure of flavonoids, with standard numbering of rings and carbon atoms. Adapted from Lamb *et al.* [8]

The range of structures possible for ring C and different chemical groups attached to the three rings account for the diversity of flavonoid compounds. Currently, there are 14 subclasses of flavonoids, according to their biosynthetic origin and structural features. The 14 subclasses of flavonoids and their names are shown in Figure 5.2.

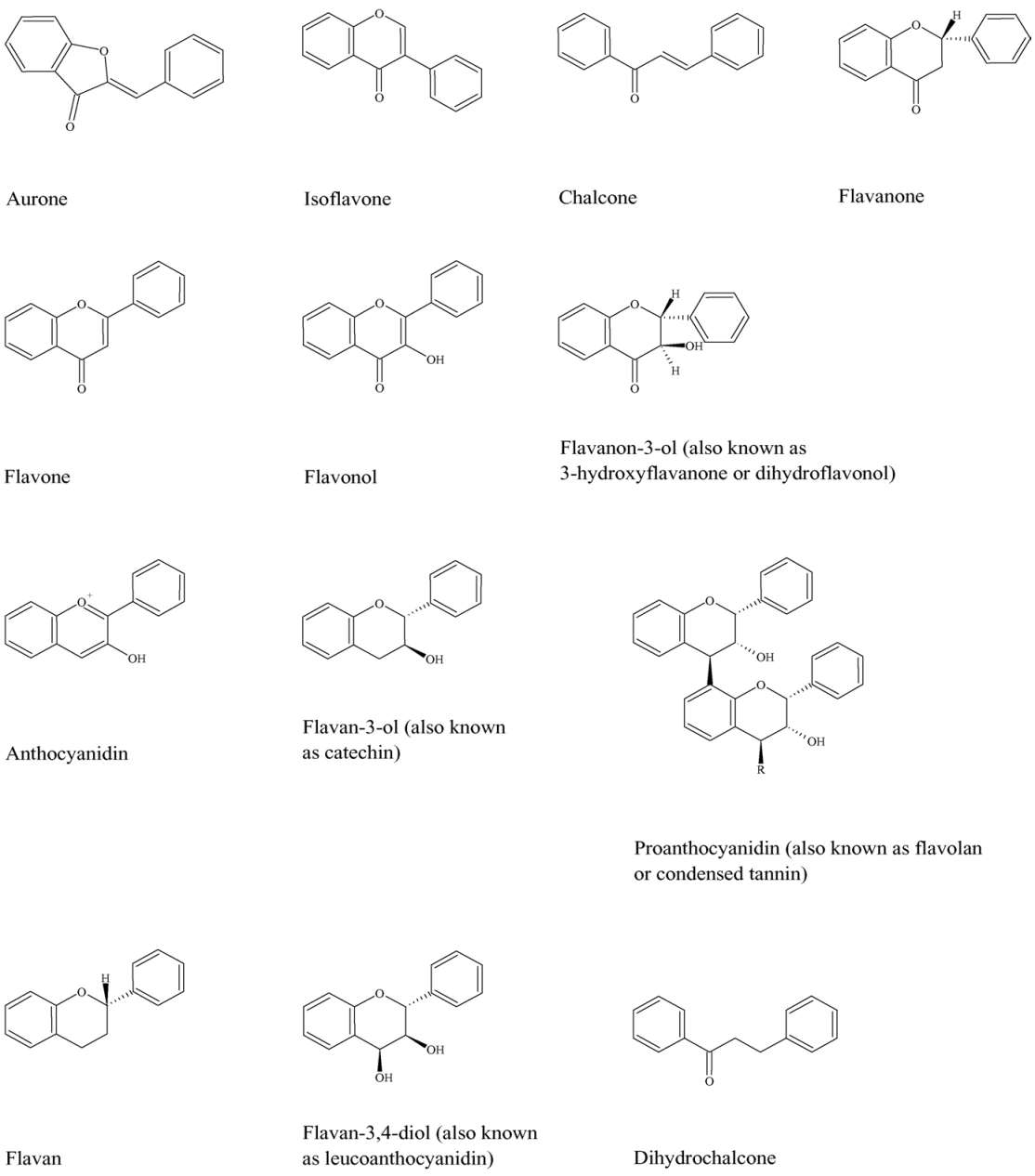

Figure 5.2. Fourteen subclasses of flavonoids. Adapted from Lamb *et al.* [8]

- Conjugated double bond systems. Conjugated double bonds systems absorb strongly in the visible spectrum of light. The presence of these structures allows many flavonoids to act as natural pigments in plants, attracting pollinators and providing protection against strong sunlight. Flower and fruit colors ranging from pale yellow, orange, and red to magenta, violet, and blue can be attributed to flavonoids such as anthocyanins.[8] Substitutions on ring B (shown in Figure 5.1) at the 3' and 5' positions change the electron distribution in flavonoid compounds, resulting in this broad diversity of color.[9] The oxidation or reduction

of a molecule can also influence the color,[10] as can pH, temperature, types of solvents and the presence of co-pigments, metals, or salts.

5.1.2. Mechanism of Action

Over the past several decades, the antibacterial activity of flavonoids and products containing high level of flavonoids have been reported in the primary literature and summarized by reviews.[7,11] In particular, green tea, honey, propolis, and various herbs have been shown to be good sources for antimicrobial flavonoids. We have chosen four flavonoids compounds from plant sources with a high level and broad spectrum antimicrobial activity,[1,4–6] and propose them as potential alternative preservatives in home and personal care products. Apigenin is a flavone compounds from Berg fruits (*Feijoa sellowiana)* in South America.[1] The other three compounds are extracted from herbs in Moraceae family in South America and Africa.[4–6] Structures of the proposed flavonoids are shown in Figure 5.3.

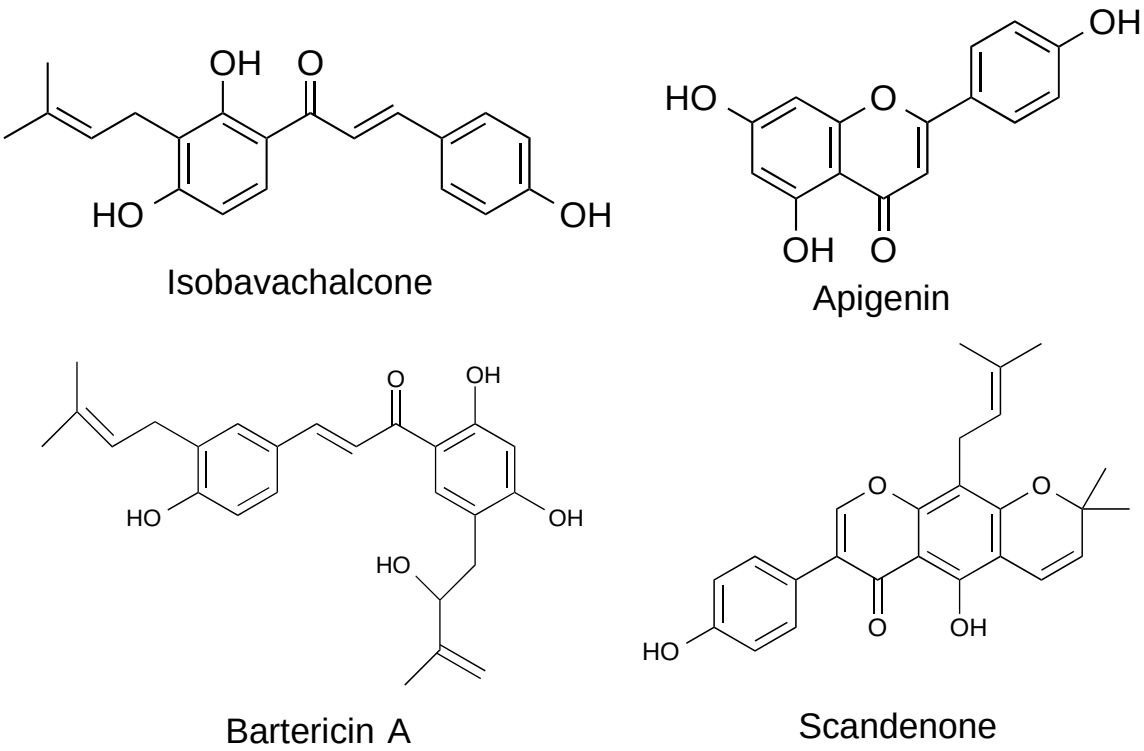

Figure 5.3. Selected flavonoids with antimicrobial activities[1,4–6]

The general mechanism of antimicrobial activity is not yet well understood. To date, several mechanisms have been observed from different flavonoids compounds, which can be grouped into three categories: inhibition of nucleic acid synthesis, inhibition of energy metabolism, and cellular membrane disruption.[11]

- Inhibition of nucleic acid synthesis. There are several studies indicating that flavonoids such as epigallocatechin in green tea exacts and quercetin in soy bean products can inhibit DNA and RNA synthesis[12,13] through non-covalent binding with nucleic acid bases or binding to the enzyme topoisomerase in the DNA replication process.[13] Some of the

inhibition mechanisms are nonspecific and target both eukaryotic and prokaryotic cells, such as nucleotide binding and mispairing activities during the replication and translation phases.[12] However, most inhibition mechanisms are specific to prokaryotes and only inhibit the topoisomerase enzymes involved in prokaryotic DNA replication.[13,14] These features of flavonoids contribute to their high antimicrobial activities towards prokaryotic microorganisms.

- Inhibition of energy metabolism. Similar to the inhibition of DNA synthesis, the inhibition of energy producing processes is mainly targeted at a prokaryote-specific respiratory electron transport chain.[15] It is reported that licochalcone, echinatin and other chalcones can inhibit oxygen consumption in susceptible bacterial cells by disturbing the electron transport chain between coenzyme Q and cytochrome C.[15]
- Membrane disruption. Another widely proposed antimicrobial mechanism of flavonoid compounds is to disrupt the cellular membrane, a mechanism that is common to many other classes of antimicrobial compounds.[11] Usually the membrane disrupting mechanism is bactericidal, meaning it will not only inhibit growth of the microorganism, but will also kill the cells.[16] Epigallocatechin in green tea extracts, for example, damages bacterial membranes by this mechanism. Identification of the mechanism of action explains why cell membrane structure influences the effectiveness of epigallocatechin, which is more effective against Gram-positive bacteria than the Gram-negative bacteria.[16] Besides damaging the structure of cell membranes, some other flavonoids such as sophoraflavanone G can exert an antimicrobial effect by reducing membrane fluidity.[17] This effect is determined by the polarization and structure of the molecule.[17]

Despite the diversity of mechanisms identified, all of the above studies are based on only a few compounds. These mechanisms cannot be generalized to all flavonoids,[7,11] and so more research on structure-activity relationships should be done to predict the antimicrobial activity of unknown flavonoid compounds.

5.1.3. Spectrum of Antimicrobial Activity

Due to the diverse mechanisms of antimicrobial activity of flavonoids and the use of some flavonoids as antimicrobial agents against both prokaryotic and eukaryotic pathogens, flavonoids are believed to have very wide spectrum of antimicrobial activity. The compound we recommend—isobavachalcone, apigenin, bartericin A and scandenone—all manifest broad antimicrobial activity against yeast, mold, and Gram-positive and Gram-negative bacteria.[1,4–6]

Current research focusing on functional compounds in traditional herbs reveals that some flavonoids are responsible for the antimicrobial activities of plants known for thousands of years to heal wounds and act as anti-inflammatory agents. Further antimicrobial testing of these flavonoid compounds has also shown them to have both high potency and a broad spectrum of activity.[1,4–6]

5.2. State of the Art

5.2.1. Historical Use

Historic evidence suggests that bee products such as honey, propolis, and pollen have been used in the treatment of human diseases for millions of years.[10] All of these products, especially propolis, contain considerable amounts of flavonoids. In ancient times, propolis and honey were used to treat inflammation of the skin and throat and for sterilization. Flavonoids and other polyphenols are now known to be what provides this functionality.[2,18]

In addition to bee products, many traditional herbs contain flavonoids as functional ingredients. Our ancestors used poultices, infusions and balms containing flavonoids as active constituents for centuries in many cultures, including Chinese,[19] South American,[4–6] and African.[4–6] Many herbs belong to the plant families Moraceae and Fabaceae.

Another category of flavonoid-containing products is beverages, specifically tea, coffee and wine. Tea is an important source of catechins, a group of flavonoids that have relatively high antibacterial activities.[16] Coffee and wine also contain flavonoids, such as isoflavonoid. Some of the cardiovascular health effects of tea, wine and coffee consumption are due to the flavonoids in these products.[10] Flavonoids are also found in fruits and vegetables, where they have antimicrobial activity[1] as well as many other health benefits.[20]

5.2.2. Modern Medical Use

The antimicrobial properties of flavonoids make them a good source of new antimicrobials and antibiotics to solve increasing bacterial resistance issues with current antibiotics. Current research on the traditional herbs used in Chinese, South American, and African cultures has led to the isolation of flavonoids that have high antimicrobial activities.[11] Additionally, research from the pharmaceutical industry has shown that modification of certain flavonoid compounds through processes such as alkylation can increase their efficacy. Specifically, alkylation of epigallocatechin gallate makes it more active against Gram-positive pathogens,[21] and amine or amide modification of epigallocatechin can increase its synergistic effects with antibiotics by over 500-fold.[22] The increasing attention paid to the antimicrobial features of flavonoids from medical use of flavonoids make this class of compound a potential future source of preservatives.

5.2.3. Home and Personal Care Product Use

Flavonoids are currently added to household and personal care products. A common example is the addition of green tea extracts to skin care products as antioxidants and for aromatherapy.[23,24] While the purpose of adding plant extracts that contain flavonoids to these products is not preservation, the antimicrobial activity of flavonoids means that they may also serve as preservatives in the products.

5.3. Hazard Information

Despite the apparently beneficial health effects of flavonoids, several studies demonstrate the toxicity of flavonoids through oral consumption.[25] Other studies reveal genotoxicity,[26–30] developmental toxicity,[31–33] skin and eye irritation[34] to human beings, and ecotoxicity against insects.[35] The most relevant toxicity pathways limiting the use of flavonoids as preservatives will be chronic effects such as estrogenic effects and carcinogenicity, as well as irritation and sensitization of skin and eyes.

Some research indicates that an overdose of flavonoids in the diet can lead to the formation of reactive compounds, damaging DNA[25] and binding to the estrogen receptors,[20] mimicking the effects of estrogen. However, for many adverse health effects such as mutations and cytotoxicity, the level greatly exceeds that in normal dietary sources unless an individual is using supplements containing considerable amounts of flavonoids.[25] Additionally, many studies on flavonoid consumption through whole plants and fruits have shown beneficial health outcomes and reduction in chronic diseases, cardiovascular diseases and cancer.[36,37] It is generally believed that low or moderate consumption of flavonoids from natural products promotes health and prevents cancer, but that higher doses will overwhelm the body system and thus lead to adverse health effects.

For the proposed flavonoid preservatives, there are large data gaps about health hazard information. Some studies shown that they can bind to estrogen receptor *in vitro*,[38,39] but no human health information exists for those compounds; they may be either hazardous or beneficial due to the complexity of the biological system. Several studies show that isobavachalcone can prevent cancer in mouse skin[40] and scandenone can suppress cancer cell propagation in rat brain cells,[41] but no individual human studies have been conducted. Our evaluation of the flavonoids suggests that they are generally safe to use as preservatives, but more research should be done about health effects resulting from dermal or respiratory contact with flavonoids.

5.4. Uncertainties and Data Gaps

5.4.1. Antimicrobial Effects

Uncertainties concerning the antimicrobial activities are low, but still exist. A lot of flavonoids with high antimicrobial activities have been reported and tested, and it is not hard to test the antimicrobial activity of unknown compounds. However, current research is mainly focused on the extraction and testing of antimicrobial compounds rather than on mechanism,[11] due to the diversity of the structures of flavonoids and the fact that glycoside flavonoids may have different absorption rates and mechanism compared to single flavonoid molecules.[25] As a result, there are still uncertainties about the mechanisms of the antimicrobial activity and the structure-activity relationships that dictate antimicrobial activities.

5.4.2. Health Hazards

For flavonoids used as preservatives in household products, there are two main uncertainties/data gaps regarding health hazards. The first is the uncertainties associated with health effects, as discussed in Section 5.3. Current dietary intake of flavonoids is safe, but data for health hazard

information of single molecules is lacking. Another uncertainty is about the exposure pathway. For nearly all existing research, the exposure pathway is through oral ingestion; however, people are exposed to preservatives in products mainly through dermal contact, which, while it tends to result in lower doses, is different from oral ingestion. More research should be done to fill these two data gaps and reduce uncertainty.

5.5. Recommendations for Seventh Generation and Beautycounter

Our analysis of antimicrobial flavonoids leads us several recommendations for Seventh Generation and Beautycounter.

- The four proposed antimicrobial flavonoids are plant-based compounds and have high level and broad spectrum of antimicrobial activity.
- Some flavonoids are primarily used in pharmaceuticals, and so the current price may restrict their use in products. However, if we consider using plant extracts that are commercially available as source of flavonoids, such as green tea extracts, the price will be much lower than for synthesizing pure compounds.
- Health hazards are a potential concern for flavonoids used as preservatives. However, current research suggests that normal dietary use of flavonoids is safe, even though there are data gaps to be filled about hazard information. Using plant extracts that contain flavonoids may reduce health hazards as compared to using isolated compounds.
- Chemical modification of flavonoid compounds may induce higher antimicrobial activity, but more research on structure-activity relationships is needed and data gaps on health hazards should be filled.

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6. Alternative Recommendations: Antimicrobial Lipids

Lipids play essential roles in biological systems as signaling molecules, structural components of cell membranes, and energy storage compounds.[1] Certain lipids are also important in the innate immune defense of humans and other organisms due to their natural antimicrobial activity.[2,3] Human skin is naturally abundant in antimicrobial lipids, which control skin microflora and prevent infection.[2] Their broad antimicrobial activity and ubiquity in biological systems suggest this class of compounds can be a promising strategy for preserving home and personal care products with low risk to human and environmental health.

6.1. Overview of Antimicrobial Lipids

Lipids are a broad class of compounds that includes biomolecules with a diverse range of chemical structures. Specific lipids with well documented antimicrobial activity include fatty acids and monoglycerides. For simplicity, the term "lipids" in the remainder of Section 6 will refer only to those with antimicrobial activity (i.e. fatty acids and monoglycerides).

6.1.1. Chemical Structure

Fatty acids are carboxylic acids characterized by a long, hydrophobic fatty acid chain. Monoglycerides are fatty acid monoesters with glycerol. Both are amphiphilic molecules characterized by a hydrophobic hydrocarbon tail and a hydrophilic head.

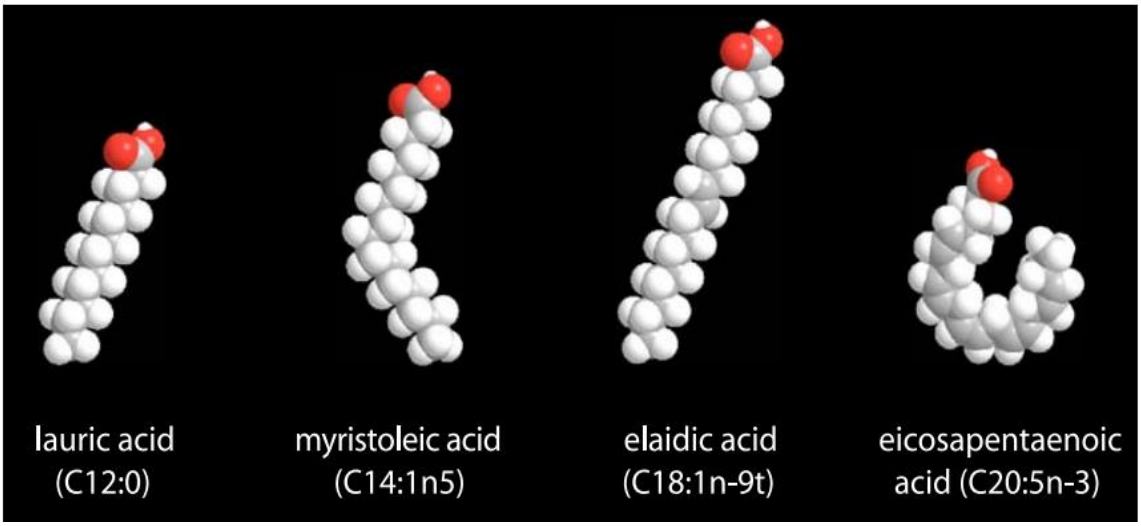

Figure 6.1. Impact of the number and orientation of double bonds on the three-dimensional structure of fatty acids. Numbers in fatty acid formula (CX:Yn-Z) denote: X = number of carbon atoms, Y = number of double bonds, Z = location of first double bond from tail end. Adapted from Desbois & Smith (2010).[1]

The structures of antimicrobial lipids vary in their carbon chain length, degree of saturation, and orientation of double bonds. Common lipids synthesized in nature have an even number of carbon atoms, with chain lengths typically ranging from 10 to 28 carbons.[1] Most unsaturated lipids (i.e. lipids with at least one carbon-carbon double bond) in biological systems have double bonds in the *cis* orientation. In contrast to the *trans* orientation, *cis* double bonds create a "kink" in the hydrocarbon chain. The non-linearity of the hydrocarbon chain increases with degree of unsaturation (Figure 6.1).

6.1.2. Mechanism of Action

Microbial cell membranes are composed of phospholipids, which have a similar structure to fatty acids and monoglycerides. The amphiphilicity of antimicrobial lipids allows them to partition into cell membranes, with the hydrophobic tail aligning with the hydrophobic interior of the membrane (Figure 6.2). Interacting with cell membranes in this way results in cell death through disruption of membrane structure and cell lysis, inhibition of membrane-bound enzymes, or disruption of energy production machinery.[1]

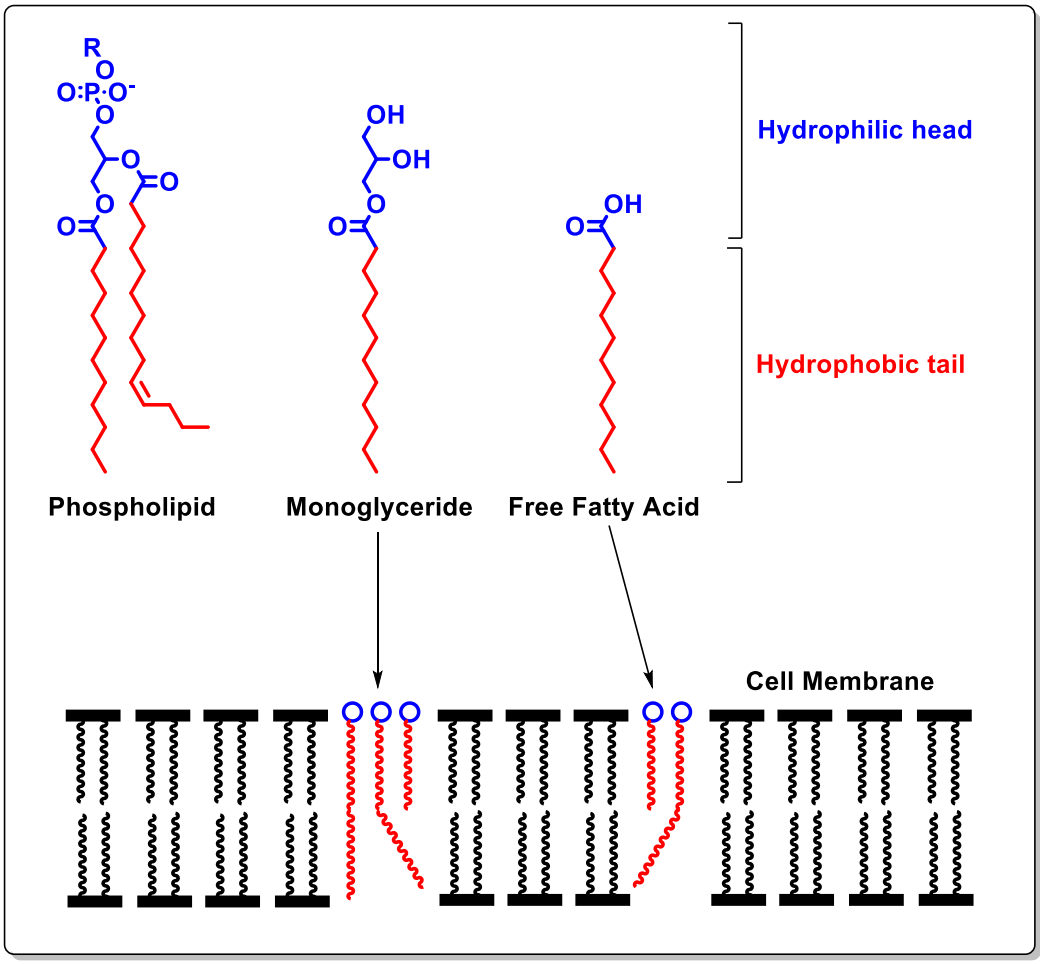

Figure 6.2. Structural comparison of phospholipids, monoglycerides, and fatty acids.

Trends between lipid structure and antimicrobial activity have been identified. In general, unsaturated lipids exhibit greater antimicrobial activity that saturated analogs of the same carbon chain length.[1] This may be due to the non-linearity of carbon chains in unsaturated lipids, which makes them more effective at disrupting membrane structure. Although polyunsaturated lipids often exhibit very high antimicrobial activity, the large number of double bonds increases their reactivity and can make them unstable.[1] Saturated lipids with the greatest antimicrobial activity are those with 10 or 12 carbon atoms.[4]

Esterification of the carboxyl group typically decreases the antimicrobial potency of fatty acids, with the notable exception of glycerol monoesters. Several monoglycerides exhibit comparable or better antimicrobial activity compared to their fatty acid analogs.[4]

6.1.3. Spectrum of Antimicrobial Activity

Antimicrobial lipids exhibit activity against a broad range of microorganisms. Researchers have reported very low minimum inhibitory concentrations (on the order of 10 μg/mL) against Gram-positive bacteria,[4] yeast,[5,6] and mold.[7] It is generally recognized that antimicrobial lipids have low activity against Gram-negative bacteria. This phenomenon is attributed to the outer membrane of Gram-negative bacteria, which is a highly effective barrier to amphiphilic molecules.[8] Several studies describe inactivation of Gram-negative bacteria, but only at concentrations much higher (on the order of 1000 μg/mL) than those required to kill other types of microorganisms.[9,10]

6.2. Precedent for Use in Home and Personal Care Products

Monoglycerides are already widely used in the personal care industry. Their amphiphilicity makes them useful as surfactants and emulsifying agents. The glycerol moiety at the hydrophilic head is highly hygroscopic, making it effective at retaining water molecules. Due to this property, monoglycerides are most commonly used as skin moisturizing ingredients (emollients).[11] Several products marketed as skin conditioning additives for cosmetics contain a monoglyceride active ingredient that also exhibits high antimicrobial activity. Commonly used monoglycerides with known antimicrobial activity include glyceryl caprylate, glyceryl caprate, glyceryl laurate, and glyceryl undecylenate.[11]

Although the presence of fatty acids as ingredients in home and personal care products is less common, it is not without precedent. The most notable example of fatty acids in the personal care industry is soap, which is composed on fatty acid salts. Oleic acid has also been used in laundry detergents as an anti-foaming agent.[12,13]

6.3. Hazard Information

Despite their integral role in biological systems and natural presence on human skin, antimicrobial lipids have some toxicity concerns that are relevant to expected routes of exposure to consumers and the environment.

6.3.1. Skin and Eye Irritation

Medium-chain saturated fatty acids have the greatest potential for skin and eye irritation, with hazard varying with chain length. Tests for primary skin irritation indicate that caprylic (C8), capric (C10), and lauric (C12) acids are irritants, but irritation potential decreases significantly for acids with 14 or more carbons. The same trend was observed in eye irritation tests.[14,15] In 4-hour skin corrosivity tests, caprylic and capric acids caused necrosis, but lauric acid and higher exhibited no toxicity.[14]

Irritation data is more limited for monoglycerides. Glyceryl laurate at 20% concentration was considered a mild skin irritant, though another study noted that it is about one-fifth less irritating that sodium lauryl sulfate. In contrast, glyceryl laurate was not considered an ocular irritant at the same concentration.[11]

6.3.2. Skin Sensitization

In clinical studies, glyceryl laurate at 50% concentration induced mild to moderate sensitization in 7 of 74 subjects. Another study evaluating a 25% glyceryl laurate emulsion concluded that it is not a skin sensitizer at that concentration. Similarly, 15% glyceryl caprate did not induce a skin sensitization reaction.[11] A recent case study has attributed adverse skin reactions in one patient to sensitization induced by glyceryl caprylate present in a cosmetic product. The study noted that the patient is considered a polysensitized individual and risk of similar reactions in the general public may be low.[16] Although there is little data available about skin sensitization reactions induced by fatty acids, one study reports that nonanoic acid may accelerate allergic reactions induced by other sensitizing agents.[17]

6.3.3. Chronic Toxicity

Toxicological data for chronic endpoints is largely unavailable for antimicrobial lipids. However, the presence of certain impurities in monoglyceride raw materials may create potential for adverse chronic health effects. Monoglyceride additives are sold as mixtures of mono-, di- and triglycerides, with the purity of the mixture varying among different products. Although most of the diglycerides present in these mixtures are expected to be 1,3-diglyceride isomers, 1,2-diglycerides may be present in low concentrations. The latter acts as a promoter of protein kinase C, which has been linked to human skin, breast, and colon cancer.[11] Because 1,2-diglycerides are expected to be present at low concentrations in raw materials—and even lower concentrations in final product formulations—the cancer risk to consumers is expected to be very low.[11] Many monoglyceride products also contain heavy metals as impurities. Because compounds such as glyceryl laurate function as penetration enhancers,[11] the identity and concentrations of impurities in such products should always be carefully evaluated to minimize the risk of chronic toxicity due to heavy metals.

6.3.4. Ecotoxicity

As with most biocidal compounds, antimicrobial lipids pose a risk of acute aquatic toxicity,[15] though it is unclear if the concentrations that might occur in wastewater effluent are sufficiently high to elicit such toxic effects. Based on EPI Suite predictions, fatty acids and monoglycerides

are expected to be rapidly biodegraded, suggesting they may be partially removed during wastewater treatment and are unlikely to become persistent in the environment.

6.4. Recommendations for Beautycounter and Seventh Generation

Of the many lipids with reported antimicrobial activity, a handful of specific compounds are the most promising for use as preservatives in home and personal care products. These compounds include:

- Medium-chain saturated fatty acids and their monoglycerides. Capric and lauric acid and their monoglycerides (glyceryl caprate and glyceryl laurate) are often cited as the most effective antimicrobial lipids.[1] Glyceryl caprylate also has good antimicrobial activity and is available as a mixture with glyceryl caprate as a skin conditioning ingredient for cosmetics. Glyceryl undecylenate is also a promising option, since it is reportedly active against Gram-negative bacteria.
- Medium-chain monounsaturated fatty acids. Myristoleic acid and palmitoleic acid have high antimicrobial activity against a range of microorganisms (refer to Appendix E for more detailed MIC data). These compounds are especially promising because they are expected to have more favorable hazard profiles than medium-chain saturated acids.

Due to Gram-negative resistance to antimicrobial lipids, adoption of this alternative will require mixed strategies to achieve complete product preservation. The easiest option would be to formulate antimicrobial lipids with other preservatives with demonstrated activity against Gram-negative bacteria. Another strategy is to include additives that increase the permeability of the outer membrane. Outer membrane permeabilizing agents include chelators, polycations such as polylysine (see Section 4),[8] lactic acid,[18] and chitosan.[19]

Adoption of monoglycerides as preservatives in products with pH greater than 7 also merits special attention due to the potential for abiotic hydrolysis at high pH. Hydrolysis of monoglycerides will eliminate their emollient properties. In the context of product preservation, however, monoglyceride hydrolysis may not be problematic as long as the corresponding fatty acid has comparable antimicrobial activity and the change in chemical structure does not significantly alter product performance.

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7. Comparison of Alternatives

To compare the hazard profiles and overall performance of the four proposed alternatives (terpenes, peptides, flavonoids, and fatty acids), we created standardized evaluation frameworks. Adoption of these frameworks permits rational comparisons among the four alternatives and conventional preservatives already being used in the home and personal care industries.

7.1. Hazard Assessment

We conducted a hazard assessment to characterize the human health and environmental safety profiles of the four proposed alternatives. Our approach was guided by the methodology described by Clean Production Action's GreenScreen For Safer Chemicals.[1] Due to time constraints and data limitations, we did not complete a full GreenScreen assessment and did not assign a benchmark to any of the four alternatives. The analysis presented here permits an evaluation of the relative hazards of each proposed alternative and can serve as a platform for future assessments as additional toxicological data becomes available.

7.1.1. Methodology

Hazards were assessed for the 18 acute and chronic (eco)toxicity endpoints defined in the GreenScreen method (Table 7.1). We selected a set of representative compounds for each compound class. Based on the hazard levels (high, medium, or low) assigned to the representative compounds, a generalization was made for the entire compound class. We included phenoxyethanol and methylisothiazolinone in our assessment to compare the hazard profiles of our proposed alternatives to those of preservatives that are already widely used in the home and personal care industries.

We consulted several data sources for assigning hazard levels. These sources are described below in the order of precedence.

- Authoritative lists: hazard classifications from recognized national and international government agencies based on a thorough evaluation of the toxicological data available for specific chemicals of concern. Classifications from authoritative lists were converted to hazard levels for our assessment according to guidelines described in the GreenScreen method.
- Scientific literature: toxicology studies reported in any peer-reviewed journal. In some instances where no studies were available for the specific compound undergoing assessment, we relied on data reported for structural analogs.
- Non-peer reviewed safety assessments: toxicological evaluations typically commissioned by regulatory agencies. Such assessments are generally conducted by experts in the relevant field, but have not undergone peer review.
- Material safety data sheets (MSDS): fact sheets published by chemical manufacturers. MSDS were consulted for relevant physical/chemical properties and physical hazard data. Toxicity data from MSDS were not considered for any hazard endpoints except reactivity and flammability.

Table 7.1. Definitions of toxicity endpoints evaluated for the hazard assessment. Adapted from Clean Production Action (2013).[1]

Hazard Endpoint	Code	Definition
Carcinogenicity	C	Capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity.
Mutagenicity & Genotoxicity	M	The term mutagenic and mutagen will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. The more general terms genotoxic and genotoxicity apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication.
Reproductive Toxicity	R	The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents.
Developmental Toxicity	D	Adverse effects in the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation.
Endocrine Activity	E	An endocrine active substance is a substance having the inherent ability to interact or interfere with one or more components of the endocrine system resulting in a biological effect, but need not necessarily cause adverse effects.
Acute Mammalian Toxicity	AT	Refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours.
Systemic Toxicity & Organ Effects (including immunotoxicity)	ST	Includes all significant non-lethal effects in a single organ that can impair function, both reversible and irreversible, immediate and/or delayed, not included in any other endpoints previously addressed; or generalized changes of a less severe nature involving several organs.
Neurotoxicity	N	An adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent.
Skin Sensitization	SnS	A skin sensitizer is a substance that will lead to an allergic response following skin contact.
Respiratory Sensitization	SnR	Hypersensitivity of the airways following inhalation of the substance.
Skin Irritation	IrS	Skin irritation is the production of reversible damage to the skin following the application of a test substance for up to 4 hours.
Eye Irritation	IrE	Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.
Acute Aquatic Toxicity	AA	The intrinsic property of a substance to be injurious to an organism in a short-term, aquatic exposure to that substance.
Chronic Aquatic Toxicity	CA	The intrinsic property of a substance to cause adverse effects to aquatic organisms during aquatic exposures which are determined in relation to the life-cycle of the organism.
Persistence	P	The length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes.
Bioaccumulation	B	A process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment, e.g., dietary and ambient environment sources.
Reactivity	Rx	Reactivity is the tendency for a substance to undergo chemical reactions.
Flammability	F	The ease with which a substance ignites and burns rapidly.

- Modeled data: *in silico* predictions of physical/chemical properties and environmental fate parameters. We used EPI Suite[2] to predict octanol-water partitioning coefficients, bioconcentration factors, and degradation half-lives in air, water, soil, and sediment.

Hazard levels were assigned for persistence and bioaccumulation based on model predictions and the guidelines described in the GreenScreen method. Modeled data were not applied to human health or aquatic toxicity endpoints due to the low level of certainty in model predictions and the lack of appropriate training data sets.

We listed endpoints as data gaps if sufficient safety information was unavailable from these five data sources. In many cases, enough data was available to make a judgment about the relative hazard of the compound, but only with a low level of certainty. The level of confidence was assigned along with each hazard level based on the quality of the data used to assign the hazard level.

- High confidence: hazard level assigned based on an authoritative list or unambiguous results reported in scientific literature or safety assessments.
- Low confidence: hazard level assigned based on modeled data or an authoritative list with classifications that do not correspond to a unique GreenScreen hazard level.
- Very low confidence: hazard level assigned based on assumptions about the relationship between physical/chemical properties and toxicity, incomplete or conflicting reports of toxicity in scientific literature and safety assessments, or toxicity data for an individual compound that may not be reliably extrapolated to other compounds in the class.

The results of the hazard assessment are summarized in Table 7.2.

Table 7.2. Summary of hazard assessment of proposed green preservatives and industry standards. H: high hazard; M: moderate hazard; L: low hazard; DG: data gap. **bold:** high confidence; *italicized:* low confidence; *italicized* and starred (*): very low confidence.

	Human Health Group I					Human Health Group II							Environmental Health		Environmental Fate		Physical Hazards	
	Carcinogenicity	Mutagenicity & Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Mammalian Toxicity	Systemic Toxicity & Organ Effects	Neurotoxicity	Skin Sensitization	Respiratory Sensitization	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
	C	M	R	D	E	AT	ST	N	SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
Terpenes	DG	*M**	DG	DG	DG	**M**	DG	DG	**M**	*M*	**H**	*H*	**H**	**H**	*M*	*L*	*L*	*M*
Peptides	DG	DG	DG	DG	DG	*L**	*M**	DG	DG	*L**	DG	DG	*L**	*L**	*L**	*L*	*L*	*L*
Flavonoids	DG	*M*	DG	*M*	**H**	*M**	DG	DG	*M**	*L**	*M**	*M**	DG	DG	*M*	*L*	*L*	*L*
Lipids	*M**	DG	DG	DG	DG	*L**	DG	DG	*M**, *L**	*L**	**H**	**H**, *L**	**H**	*M*	*L*	*L*	*L*	*L*
Phenoxy-ethanol	DG	*L*	*M*	*M*	DG	**M**	DG	DG	*M**	*DG*	*M**	**H**	*L*	*M*	*L*	*L*	*L*	*M*
Methyliso-thiazolinone	*M**	*L*	*M**	*M**	DG	**M**	**M**	DG	**H**	*H*	**H**	**H**	**H**	*H*	*M*	*L*	*L*	*L*

7.1.2. Hazard Profiles for Proposed Alternatives

Toxicity information was more abundant for acute hazard endpoints than chronic endpoints. As a result, many of the data gaps for the four alternatives are associated with chronic toxicity effects. Hazard levels for endpoints associated with environmental fate and physical hazard were fairly consistent among the four alternatives and are expected to be relatively low. Greater discrepancies occurred among the alternatives for other acute hazard endpoints, with hazard levels ranging from low to high.

Of the four alternative classes of compounds, terpenes are associated with the highest level of hazard. In particular, their potential to induce irritation is well established.[3] However, terpenes are generally well described in terms of hazard. In contrast to the other proposed alternatives, the volatility of some monoterpenes have the potential for inhalation exposure and respiratory irritation.[4] Terpenes may exhibit high aquatic toxicity[3] and there is evidence that some terpenes may be mutagenic at low concentrations.[5] Still, most of the available hazard data is associated with terpene concentrations much higher than reported minimum inhibitory concentrations. While some terpenes are hazardous by certain exposure routes, there are many that are safe. It is worth noting that some oxidation products derived from certain terpenes may induce sensitization, as discussed in Section 3. We expect that at the concentrations required for product preservation, the risk to consumers will be much lower than what is suggested by the hazard assessment.

Although there is limited safety data available for antimicrobial peptides, they are expected to have a low hazard profile. Nisin and polylysine are considered GRAS by the FDA[6] and are expected to have low acute toxicity. Due their very low volatility, peptides are not expected to cause respiratory sensitization. One study indicated that polylysine may cause damage to the liver and kidneys, but only at very high exposure levels.[7] Additional research is needed to understand the chronic toxicity profile of peptides and their potential for irritation and sensitization upon dermal exposure.

Flavonoids have been the subject of extensive pharmacological research due to their observed anticancer activity.[8,9] However, one study also suggests that certain flavonoids may increase the risk of skin and lung cancers.[10] Due to these conflicting reports in the literature, carcinogenicity is still considered a data gap for flavonoids. Some flavonoids have shown potential for irritation and sensitization at high concentrations.[11] One of the greatest safety concerns with the use of flavonoids is their potential estrogen activity. Many flavonoids are phytoestrogens and can behave as estrogen mimics.[12,13] It remains unclear whether the observed endocrine activity of phytoestrogens will result in adverse health outcomes in humans, and additional data is needed to understand the risk that flavonoids might pose to consumers.

Although antimicrobial lipids generally have a moderate hazard profile, there is some discrepancy between the hazards for fatty acids and those for monoglycerides. While both can cause skin irritation, only fatty acids are considered ocular irritants.[3,14] There is evidence that some fatty acids may accelerate skin allergic reactions,[15] but other studies have concluded that some monoglycerides are non-sensitizers.[14] Carbon chain length also appears to have a significant impact on irritation potential. Fatty acids with 8, 10, and 12 carbon atoms (caprylic, capric, and lauric acids) may cause skin and eye irritation, but acids with shorter or longer chain lengths are considered non-irritating.[16] There is some carcinogenicity concern due to the presence of 1,2-

diglycerides as impurities in monoglyceride raw materials. As protein kinase C promoters, 1,2-diglycerides may be linked to human skin, breast, and colon cancers.[14] However, these impurities are expected to be present in raw materials at low concentrations and experts expect the cancer risk to consumers will be very low at the concentrations present in final product formulations.[14]

Relative to methylisothiazolinone, each of the proposed alternatives have comparable or lower hazard levels for the majority of the hazard endpoints. Antimicrobial peptides and flavonoids also have favorable hazard profiles compared to phenoxyethanol. However, phenoxyethanol was assigned lower hazard levels than terpenes and antimicrobial lipids for hazard endpoints associated with aquatic toxicity, irritation, and sensitization. We suspect that, despite the high hazard levels for some endpoints, terpenes and antimicrobial lipids may still be safer alternatives because they can achieve effective product preservation at much lower concentrations than phenoxyethanol.

7.1.3. Limitations of Safety Data

Although this hazard assessment is useful as a preliminary indication of the relative hazards of the alternatives we proposed, it should not be considered a complete representation of the risk associated with adopting any of the proposed alternatives for product preservation. Potential hazards exist for each class of compounds, but the human and environmental health outcomes for each will depend greatly on routes of exposure, exposure concentrations, and the toxicity of individual compounds within each class. Important data limitations and constraints imposed by the scope and timeframe of this project should be taken into account when interpreting the results of the hazard assessment. The most important data limitations include:

- Availability of appropriate toxicological data. In many cases, there is no data available to assess the hazard level of the proposed alternatives. In particular, toxicity information for chronic hazard endpoints is often completely unavailable, can be difficult to corroborate with multiple studies, or is ambiguous and contradictory across different studies.
- Relevance of toxicological studies. When appropriate toxicity information is available, it can be difficult to extrapolate conclusions drawn under experimental conditions to those expected in home and personal care products. In most cases, hazard data is only available for pure compounds or for solutions with very high active ingredient concentrations. We expect the high level of antimicrobial activity of the proposed alternatives will permit effective product preservation at very low preservative concentrations. However, until reliable dose-response relationships are established for each hazard endpoint, it is impossible to predict *a priori* whether preservatives will elicit adverse health effects at the concentrations required for preservation. Exposure pathways (dermal, inhalation, oral) and testing methods (*in vitro* assays, animal models, clinical studies) also impact the relevance of available toxicity studies to this project.
- Generalized hazard profiles for compound classes. Even small changes in molecular structure can impact chemical toxicity. The hazard profiles established from this safety assessment describe general toxicity trends for each compound class, but specific compounds used in products should be assessed individually for an accurate hazard characterization. Additionally, the representative compounds we selected from each compound class were those with the greatest potential as antimicrobial agents. In the absence of toxicology data for those compounds, we relied on data for structurally similar molecules that may or may not exhibit antimicrobial activity. As a result, some of the

hazard levels assigned during the hazard assessment may not apply directly to the compounds that are recommended for use as preservatives.

Despite these limitations, conclusions from the hazard assessment highlight the most important safety data gaps and can guide future areas of investigation if proposed alternatives are incorporated into existing products. Our hazard assessment can also serve as a starting point for a more rigorous GreenScreen assessment if future resources permit that level of analysis.

7.2. Multi-Criteria Evaluation Framework

Although preservative safety is the most important performance criterion for Beautycounter and Seventh Generation, the feasibility of each alternative is ultimately a function of its ability to effectively preserve products within the technical and non-technical constraints of the home and personal care industries. We developed an alternatives analysis framework to standardize evaluation criteria and highlight the advantages and challenges associated with each alternative when accounting for all relevant performance criteria. Our analysis provides a qualitative comparison of alternatives, but the framework can easily be adapted for a quantitative comparison when evaluating future alternatives.

7.2.1. Criteria Definitions and Scoring Guidelines

We identified 8 performance criteria that control the feasibility of adopting new preservatives for home and personal care products. We assigned each alternative a score of 1, 2, or 3 for each criterion according to the guidelines described in Table 7.3. The multi-criteria evaluation framework was completed separately for Beautycounter (Table 7.5) and Seventh Generation (Table 7.6) due to differences in the products and regulatory spaces of the two companies.

Table 7.3. Scoring guidelines for multi-criteria evaluation framework.

Performance Criteria	**Score**		
	3	2	1
Hazard	Low hazard level for most non-DG endpoints AND no endpoints with high hazard level	Medium hazard level for most non-DG endpoints OR roughly equal numbers of high and low hazard levels	High hazard level for most non-DG endpoints AND more medium than low hazard levels for remaining endpoints
Antimicrobial Efficacy	Average of efficacy scores = 3	Average of efficacy scores = 2-3	Average of efficacy scores = 1-2
Level of Uncertainty	Number of safety data gaps = 0-3	Number of safety data gaps = 4-8	Number of safety data gaps = 9+
Biodegradability	Low hazard level for persistence	Medium hazard level for persistence	High hazard level for persistence
Origin of Raw Materials	Natural source is available and comparably priced or cheaper than synthetic alternative	Natural source is available but much more expensive than synthetic alternative	Only synthetic options are available
Product Compatibility	Already used in product as a preservative OR expected to perform optimally in existing products with minimal (or no) changes to formulation	Expected to perform well in existing products with small changes in formulation	May be unsuitable for some products OR may require major changes to formulation to perform well
Regulatory Concerns	Already approved for use as a preservative by FIFRA or has exempt status	Has precedent for approval for other uses OR is a good candidate for FIFRA exempt status OR has additional requirements but not restrictions	Ingredient is banned or has limits on maximum allowable concentration
Cost	$0-20/kg (irrespective of source, i.e. natural or synthetic)	$20-50/kg (irrespective of source, i.e. natural or synthetic)	$50+/kg (irrespective of source, i.e. natural or synthetic)

7.2.2. Performance Comparison of Alternatives

Scores for hazard were based on the results of the hazard assessment for each alternative, as discussed in Section 7.1.2. The level of certainty inherent in the hazards profiles was similar for terpenes, flavonoids, and antimicrobial lipids. Antimicrobial peptides have a higher degree of uncertainty regarding their hazard profile, with over half of the hazard endpoints designated as data gaps.

Table 7.4. Comparison of antimicrobial efficacy based on MICs against four classes of microorganisms. Scoring ranges: 3 = MIC ~ 10 µg/mL; 2 = MIC ~ 100 µg/mL; 1 = MIC ~ 1000+ µg/mL

	Yeast	Mold	Gram-positive Bacteria	Gram-negative Bacteria
Terpenes	2	2	3	3
Peptides	2	2	1	3
Flavonoids	3	2	2	3
Lipids	3	2	3	1
Phenoxyethanol	1	1	1	1
Methyliso-thiazolinone	2	1	2	3

We assigned scores for antimicrobial efficacy based on minimum inhibitory concentrations published in the scientific literature. Detailed MIC data for each class of compounds is provided in Appendix E. Each compound class has high antimicrobial activity against some types of microorganisms (Gram-positive and Gram-negative bacteria, yeast, and mold) and more limited activity against others (Table 7.4). On average, all four proposed alternatives had similar levels of antimicrobial activity compared to methylisothiazolinone and are more effective than phenoxyethanol.

Biodegradability was predicted by EPI Suite estimates of degradation half-lives in air, water, soil, and sediment. Scores were assigned for the multi-criteria evaluation framework based on the hazard level in the safety assessment. Antimicrobial peptides and lipids are expected to biodegrade rapidly, while terpenes and flavonoids have the potential to be more persistent.

All four proposed alternatives received a score of 3 for origin of raw materials because they are all naturally derived. Synthetic terpene products are available but are not necessarily less expensive than natural options based on readily available cost data. Cost comparisons presented here represent very rough price estimates. Antimicrobial peptides and flavonoids are expected to be the most expensive alternatives. Notable exceptions for peptides include nisin and polylysine, which are available at reasonable costs due to their growing use as food preservatives.

Table 7.5. Multi-criteria evaluation framework for Beautycounter.

	Hazard	Antimicrobial Efficacy	Level of Uncertainty	Biodegradability	Origin of Raw Materials	Product Compatibility	Regulatory Concerns	Cost
Terpenes	1	2	2	2	3	2	3 1	2
Peptides	3	2	1	3	3	3	3	1
Flavonoids	2	2	2	2	3	2	3	1
Lipids	2	2	2	3	3	3	3	3
Phenoxyethanol	2	1	2	3	2	3	3 1	3

For Beautycounter, we expect that antimicrobial peptides and lipids could replace existing preservative systems with little or no disruption is product performance. Monoglycerides are already widely used in personal care products as emollients, emulsifiers, and surfactants.[14] Antimicrobial peptides can act as effective preservatives at low enough concentrations that their addition is unlikely to impact the aesthetics or performance of a product. Terpenes present in essential oils are already used in Beautycounter products as fragrance additives. However, increasing the concentration of these ingredients to achieve preservation may result in more elevated fragrance than desired. Flavonoids may also be suitable for Beautycounter products as long as they do not impart undesired color to the final product.

Under the confines of the FDCA, regulatory concerns for Beautycounter are inconsequential when selling products only within the United States. If selling products in the European Union, concentration limits have been set for fragrance additives in cosmetics with constituents known to induce allergic reactions.[17] If terpenes are adopted as a preservation system, such regulations must be considered if product sales are expanded beyond United States markets. Continued use of phenoxyethanol may also prove challenging in markets outside the United States. Japan has established a maximum allowable concentration for personal care products and it is possible that other markets may eventually follow suit.

Table 7.6. Multi-criteria evaluation framework for Seventh Generation.

	Hazard	Antimicrobial Efficacy	Level of Uncertainty	Biodegradability	Origin of Raw Materials	Product Compatibility	Regulatory Concerns	Cost
Terpenes	1	2	2	2	3	3 1	3	2
Peptides	3	2	1	3	3	1	2	1
Flavonoids	2	2	2	2	3	1	2	1
Lipids	2	2	2	3	3	2	2	3
Methyliso-thiazolinone	1	2	3	2	1	3	3	3

For Seventh Generation, both peptides and flavonoids may be incompatible with some of the products that we considered for this project. Antimicrobial peptides will be unable to preserve auto dish gel, laundry detergent, and stain remover because proteases present as cleaning agents with degrade the peptides in solution. Flavonoids may be unstable as well because they are prone to oxidation at the pH range of these products. Antimicrobial fatty acids are promising options for home care products because they are effective antimicrobial agents in both the associated and disassociated forms. Monoglycerides may also be feasible alternatives, but their susceptibility to hydrolysis at pH greater than 7 should be taken into account. Terpenes are an excellent option for many of Seventh Generation's products because they are already present as fragrance additives. However, relying on terpenes for preservation in scent-free products may require more challenging approaches to control the scent associated with many of these compounds.

In addition to technical feasibility, terpenes are an excellent alternative for Seventh Generation because many antimicrobial terpenes are already recognized as exempt from preservative registration requirements under FIFRA.[18] Flavonoids and antimicrobial lipids are also good candidates for FIFRA exemption status because they are multifunctional ingredients that can be included in home care products for uses other than preservation. Although antimicrobial peptides may not qualify as minimum risk pesticides under FIFRA guidelines, their use as food preservatives may help overcome some of the regulatory hurdles associated with their use as preservatives in home care products.

Based on the results of the multi-criteria evaluation framework, it is clear that the four proposed alternatives each have advantages for Beautycounter and Seventh Generation. Some challenges

must also be overcome if they are adopted as preservatives in home and personal care products, but they are promising alternatives to conventional preservatives in the near-term. As additional green preservative strategies are identified, the multi-criteria evaluation framework developed here can be adapted to include additional performance criteria and refined scoring guidelines. It is also possible to adapt this framework for qualitative alternatives analysis that accounts for the relative importance of the performance criteria.

7.3. References

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8. Formulation Strategies

Moving beyond drop-in replacement of existing preservatives, we explored strategies to enhance the activity of these molecules in the context of formulated products. Specifically, we considered the use of multifunctional ingredients, synergistic effects, functionally similar isomers, derivatization, and encapsulation. Each of these strategies is discussed below.

8.1. Multifunctional Antimicrobials

The use of a single molecular component to provide a diverse set of functions has precedence in biology. For instance, the túngara frog produces a mixture of proteins that act as surfactants and antimicrobials (see Appendix B). Although the specific use of these proteins is impractical given the current state of technology, the use of multifunctional ingredients to satisfy several desired properties of the formula under development is a fruitful strategy when considering the formulation of antimicrobial components.

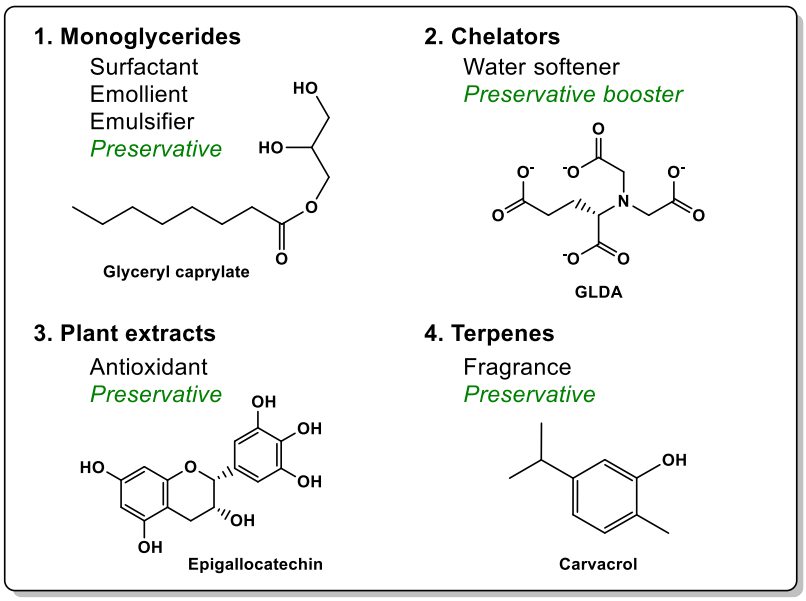

Figure 8.1. Selected multifunctional antimicrobial classes that may a secondary benefit (e.g. antioxidant, emulsifier, etc.) to a formula.

There are many advantages to multifunctional ingredients (Figure 8.1). Contingent on the molecular component under consideration, there could be cost savings by getting the function of two components for the price of one. Multifunctional ingredients also simplify formulation and minimize the likelihood of antagonism (i.e. that two components will deactivate each other). Depending on the regulatory and consumer space that the product inhabits, multifunctional components may also simplify the process of regulatory compliance. For example, use of a benign multifunctional ingredient would be preferred to using two components, which may carry with them increased uncertainty in hazard. In a parallel sense, a multifunctional ingredient whose

consumer acceptance has been well established would be a valuable component from a formulation perspective.

There are many examples of compounds that can operate in a multifunctional manner in existing home and personal care products. In addition to acting as potent antimicrobials, many monoglycerides and fatty acids are effective surfactants, emollients and emulsifiers (by virtue of their amphiphilic nature). Functioning as ion and metal-binding components, chelators can operate as preservative "boosters" by increasing a microbe's susceptibility to a biocide. Boosting may occur by the chelator sequestering ions that are structural or functional components to the microbe. Alternatively, chelators can repress microbial growth by coordinating strongly to an essential nutrient (e.g. iron, which is often a limiting nutrient for bacteria). Many chelators also act as water softeners by coordinating aqueous Ca^{2+} or Mg^{2+}, thereby preventing the formation of hydroxide or carbonate precipitates.

Polyphenol components of plant extracts are notoriously effective antioxidants and antimicrobials. Antioxidants have many uses in products; for example, antioxidant components can slow discoloration of a formula by ambient oxidation. In cases where other formula components could potentially oxidize to compounds of unknown hazard, antioxidants can minimize this hazard by slowing or preventing this oxidation. Finally, certain antimicrobials (e.g., terpene derivatives) offer aesthetic benefits, such as a pleasant aroma. The latter property can also be disadvantageous, however, if the scent of an antimicrobial component does not suit the formula under consideration. Collectively, the classes described in this report provide ample opportunities to reap the benefits of multifunctional chemical ingredients.

8.2. Antimicrobial Synergism, Antagonism and Additivity

When two or more antimicrobial chemicals are combined, the resulting formula may exhibit a degree of antimicrobial efficacy that is additive (effectively the sum of their parts), antagonistic (less than the sum of their parts), or synergistic (greater than the sum of their parts). The latter property is well represented in biology, where synergistic mixtures of peptides and small molecules are observed to produce antimicrobial efficiencies that may be orders of magnitude greater than what one would expect from the sum of individual components. The magnitude of this effect can be indexed using the fractional inhibitory concentration (FIC, eq. 8.1), where *A* is the concentration of compound A in the minimum inhibiting association, *B* is the concentration of compound B in the minimum inhibiting association, and MIC A and MIC B are MIC values acquired for the aqueous single components (Figure 8.2).[1]

$$\mathrm{FIC} = \frac{\mathrm{A}}{\mathrm{MIC\ A}} + \frac{\mathrm{B}}{\mathrm{MIC\ B}} \qquad (8.1)$$

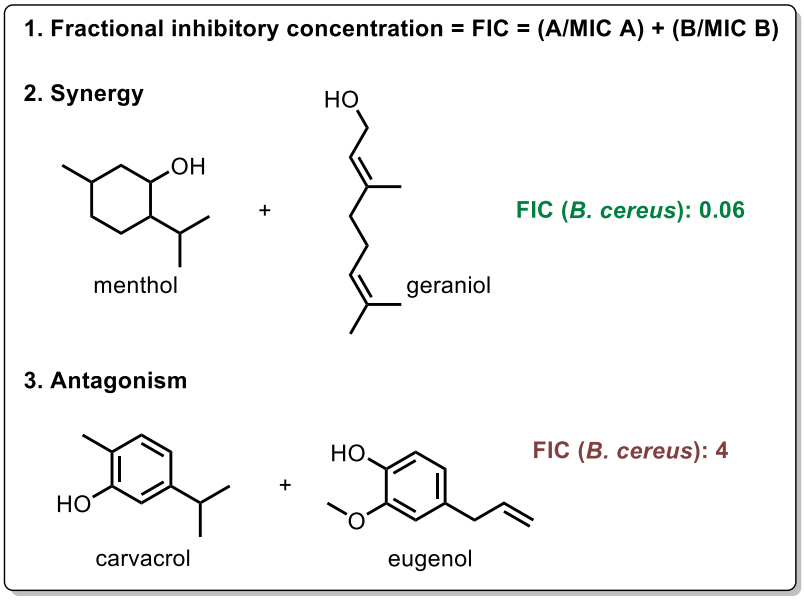

Figure 8.2. Naturally derived antimicrobials that operate synergistically or antagonistically in formulae.

Effective synergism is indicated by FIC values significantly less than 1 (generally < 0.5); antagonism is represented with values greater than 1. Based on characterized examples in the literature, additivity is more common than synergism and antagonism. Furthermore, characterized synergistic and antagonistic effects in two component (A + B) mixtures are generally less than an order of magnitude (i.e. less than a factor of 10 increase or decrease in antimicrobial efficacy is usually measured), although rare examples approach nearly two orders of magnitude.[2–7] Presumably, multicomponent mixtures (> 2 antimicrobial components) have the potential to bring about a greater degree of synergism and antagonism. The occurrence of synergism and antagonism, while widely documented in the scientific literature, is poorly understood mechanistically. How other formula components (e.g. chelators or emulsifiers) influence the antimicrobial efficacy of a commercial formula is also absent from the literature. A better understanding of synergistic and antagonistic processes in relation to different classes and species of microbes would enable more efficient formulation. More broadly, such investigations could address fundamental concerns—for instance, what is the optimal combination of biochemical processes (e.g. proton gradients, membrane structure) that should be targeted for maximum antimicrobial efficacy?

8.3. Functionally Similar Antimicrobial Isomers to Avoid Undesired Qualities

Certain classes of antimicrobials, such as those derived from terpenes, commonly exhibit potent scents. While often pleasant, these odors limit the use of these compounds as general preservatives. Put simply, it is undesirable for many types of home and personal care products to smell like pine oil. However, the use of functionally similar isomers of well-established scented antimicrobials may enable broad-spectrum antimicrobial efficacy for a variety of applications.

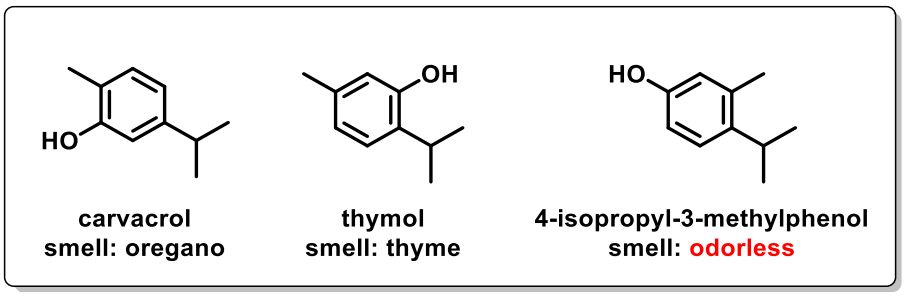

Figure 8.3. Monoterpene isomers with comparable antimicrobial activities but different scents.

Carvocrol and thymol are isomeric components of oregano and thyme essential oil, of which they constitute 90% and 20-50%, respectively.[8–10] These compounds are potent antimicrobials effective against many bacteria, but carry a strong odor of oregano and thyme, which limits their general use as antimicrobials. Odor recognition, which is strongly related to the shape and bonding arrangement of a compound in relation to a scent receptor, is molecule-specific. Despite the scent-related limitations of carvacrol and thymol, a third, odorless isomer (4-isopropyl-3-methylphenol) exists that exhibits comparable antimicrobial efficacies (Figure 8.3). This chemical is commercially available, affordable and has the potential to be used as a general antimicrobial in unscented formulae.

8.4. Antimicrobial Derivatization to Enable Diverse Antimicrobial Libraries

By covalently coupling two sets of complementary antimicrobials (e.g. by esterification), one can construct a library of chimeric antimicrobials (which are constructed from fragments of smaller antimicrobials; Figure 8.4). Doing so has the potential to address several issues:

1. Volatility may be suppressed due to an increased molecular weight, which would minimize the likelihood of respiratory irritation or sensitization.
2. Undesired odors may also be suppressed by modifying the shape of the antimicrobial and reducing its volatility.
3. Water solubility could be enhanced.
4. Antimicrobial efficacy may be enhanced by derivatization, for example by increasing microbial cell permeability.

Simple acetylated* derivatives of antimicrobial phenols and peptides have been shown to bring about greater antimicrobial efficacy (up to a factor of 10) compared to the parent compound.[11] Derivatization could also generate a high degree of chemical diversity for evaluation of antimicrobial efficacy by high throughput methods. Furthermore, antimicrobial derivatives may be constructed in a manner that generates predicable, safe antimicrobials upon gradual decomposition, suggesting a low likelihood for hazard.

*Acetylated compounds have an extra acetyl (-C(=O)CH_3) group added to the parent compound. See http://en.wikipedia.org/wiki/Acetylation.

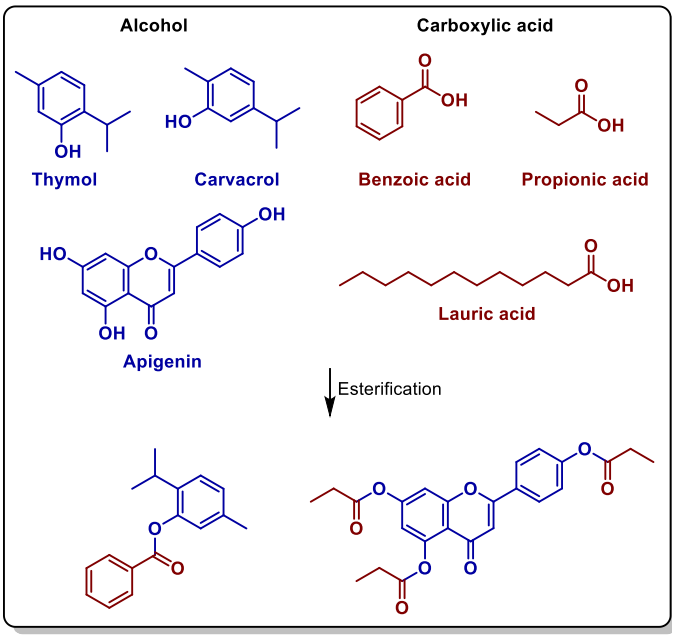

Figure 8.4. Antimicrobial derivatization using selected alcohol or phenol antimicrobials with a carboxylic acid antimicrobial affords combinatorial ester products for possible use as preservatives.

8.5. Microbial Activation of a Chimeric Antimicrobial

The approach of generating chimeric antimicrobials can be taken one step further by specifically derivatizing molecules so as to facilitate their activation by microbial enzymes *in vivo*. One approach to this strategy involves linking alcohol or phenol-functionalized antimicrobials with a carboxylic acid to generate an ester. Conjugation of this type may impart some of the practical benefits described in the previous section, such as the suppression of volatility and odor. We envision that modification of this kind could enable microbial cellular uptake by increasing the lipophilicity of the chimeric compound compared to its starting components. Upon exposure to a suitable esterase or lipase enzyme in the microbe's cytosol (both of which are produced by microbes), the ester would conceivably be cleaved and multiple equivalents of preservative generated, which may be trapped in the aqueous cell interior. Furthermore, this method could improve the antimicrobial efficacy of fatty acids by facilitating their delivery to microbial cell interiors. The general strategy of *in vivo* ester cleavage by enzymes is a well-established method to deliver and trap hydrophilic precursors to cell interiors, and has been successfully employed in the delivery of nanoparticles, drugs, polymers and luminescent probes to the cytosol (Figure 8.5).[12–16]

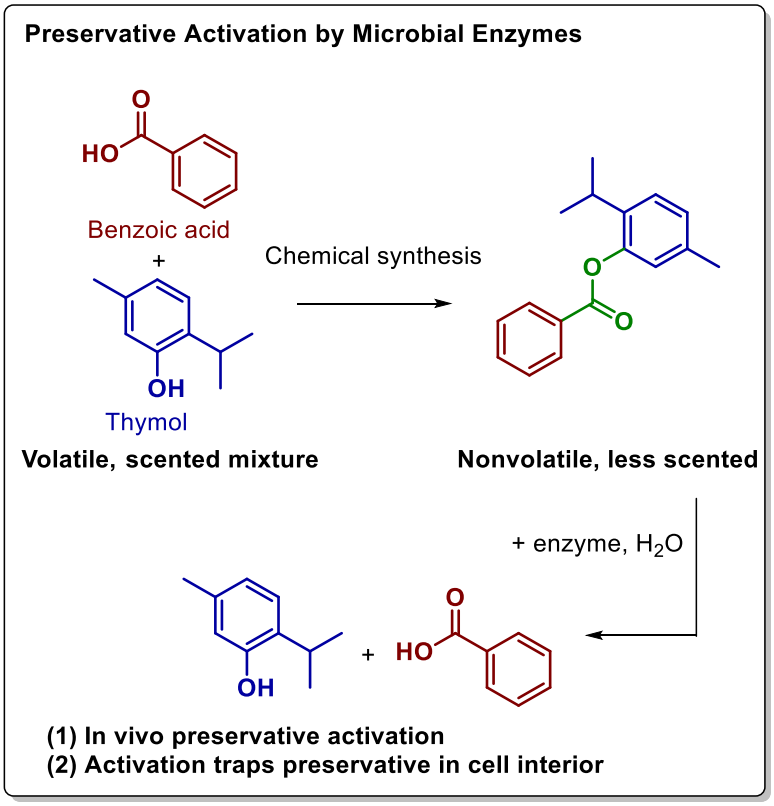

Figure 8.5. Chemical synthesis enables the preparation of a chimeric preservative that obviate possibly unfavorable characteristics such as volatility and scent. In formula, the chimeric preservative may be hydrolyzed in vivo by action of an antimicrobial enzyme, potentially generating potent antimicrobials in the microbial cell interior.

8.6. Molecular Encapsulation to Alter the Physical Properties of Antimicrobials

Dozens of commercially available, affordable, variably-sized host molecules (cyclodextrins, calixarenes, carcerands, dendrimers and other water soluble polymers) have been shown to encapsulate a wide variety of small molecule organic guests. Once encapsulated, the physical properties of the guest are sometimes dramatically altered: volatility is often suppressed—minimizing undesired scents and lowering the possible risk of respiratory irritation, water solubility may be dramatically increased, and guests may be stabilized from decomposition or oxidation (Figure 8.6).[17] These factors could enable the widespread application of preservative components whose physical characteristics would otherwise limit their general use.[18,19]

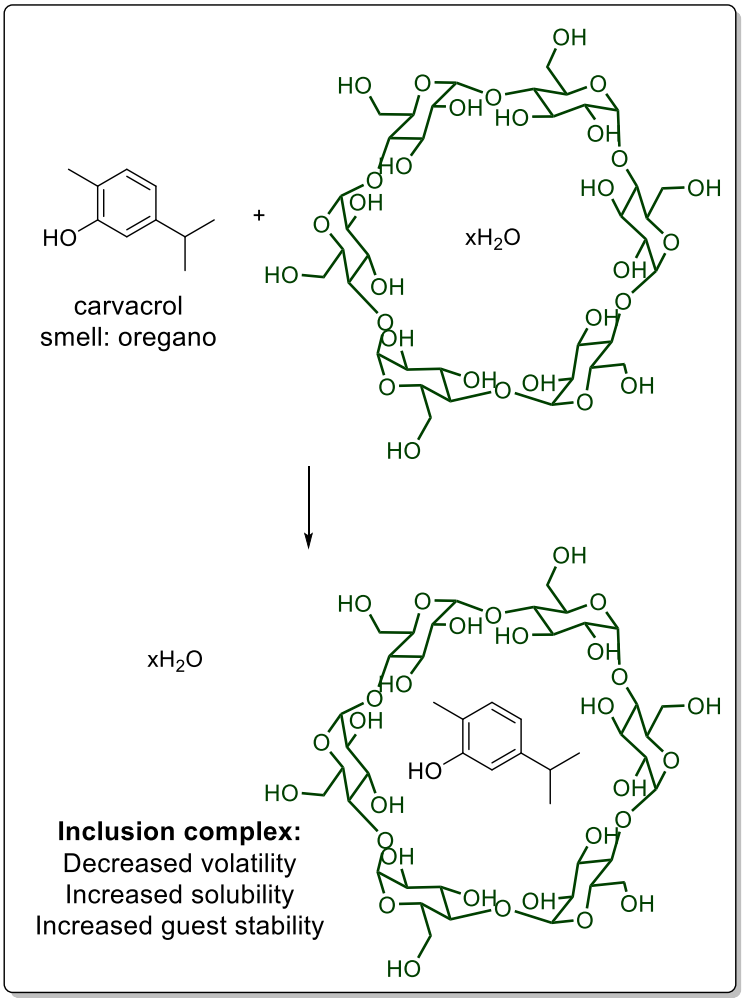

Figure 8.6. The formation of inclusion complexes to obviate unfavorable preservative characteristics.

By using some of the formulation strategies outlined in this section in conjunction with an increasing understanding of the antimicrobial activity of safer molecular alternatives, it may be possible to increase efficacy of antimicrobial components in home and personal care products, reducing the required concentrations and therefore the associated hazards.

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9. Conclusions

The ubiquity of home and personal care products in our society creates an opportunity for safety improvements that impact people of all cultures and socioeconomic groups. Through our partnership with Seventh Generation and Beautycounter, we have developed biologically inspired alternatives for preservatives currently used in their formulations and throughout industry.

We recommend four classes of compounds to be used as drop-in replacements in these products: terpenes, polypeptides, flavonoids, and lipids. Subject to limitations due to data gaps and regulation, all of these have potential to be safer, effective, and biodegradable preservatives.

Moving beyond immediate product substitutions, we believe it is important to take a longer view of product preservation including both chemical and non-chemical alternatives. In this report we discuss opportunities in synergy, encapsulation, microbial hydrolysis, and chimeric complexes. Although not discussed in this work, creative packaging strategies may provide a pathway to safer product preservation.

Finally, it is important to realize that this work offers many possibilities, but it is up to those creating products to move forward and implement the best options. Ongoing collaboration between industry and academia provides space for deeper mechanistic understanding of antimicrobial activity, which in turn will allow the design of better preservatives. Collaboration amongst industry players is also essential: while in many ways companies are competitors, this competition can drive change. In the preservative space, these players have a unique opportunity to work together to protect people, products, and our planet.

Appendices

Appendix A. About the Authors

Heather Buckley (hbuckley@berkeley.edu) is a postdoctoral fellow with the Berkeley Center for Green Chemistry. Her background is in inorganic chemistry as well as technical editing. Heather's current research involves the development of waterproofing and antifungal additives for sustainable roofing materials.

Adam Byrne (abyrne@berkeley.edu) is a PhD student in the environmental engineering program at UC Berkeley. His primary experience is in developing and optimizing sustainable technologies for detoxifying anthropogenic contaminants in soil and groundwater systems. Currently, his research is focused on understanding the impact of indigenous soil microorganisms on the fate of fluorinated surfactants in the environment.

Jiawen Liao (fredliao@berkeley.edu) is a first-year M.S. student in the Environmental Health Sciences with a special concentration Global Health and Environment (GHE), in the School of Public Health at UC Berkeley. His primary research lies in quantitative analysis of the health effects due to indoor air pollution, especially in developing countries. He will help to analyze health risk and conduct toxicological analysis of the chemicals in the Greener Solutions courses.

William Hart-Cooper (hartcoop@berkeley.edu) is a PhD student in the chemistry department at UC Berkeley. His current research involves preparing synthetic nanoscale receptors and evaluating them as host compounds and enzyme mimics using physical organic methods.

Full Appendices are listed below and are available at bcgc.berkeley.edu/greenersolutions

Appendix B. Original Biomimicry Strategies

A spreadsheet containing an extensive list of antimicrobial strategies used in biological systems. This work was compiled primarly by Mark Dorfman of Biomimicry 3.8.

See supplementary spreadsheet SI5_Antimicrobials_research_matrix.xlsx.

Appendix C. Industry Maps

A listing of each of the ingredients present in several products marketed by Beautycounter and Seventh Generation, along with a notation about the molecular class and function of each product.

See supplementary spreadsheet SI1_7Gen_Industry_Map.xlsx for Seventh Generation and SI2_BC_Industry_Map.xlsx for Beautycounter.

Appendix D. Alternative Preservatives List

Includes many potential candidate antimicrobials. Some of these chemicals did not conform to the desired product restrictions (ex. low water solubility or stability, high volatility) or did not exhibit sufficient antimicrobial activity for further consideration.

See supplementary spreadsheet SI6_Alternative_Preservative_List.xlsx for more information.

Appendix E. MIC Tables for Recommended Antimicrobials

Compiled data on Minimum Inhibitory Concentrations (MICs) for recommended chemicals in each of the four classes.

See supplementary spreadsheet SI3_AMP_MIC_Summary.xlsx for antimicrobial peptides, SI4_FA_MIC_Summary.xlsx for peptides, SI7_Terpenes_MIC_Summary.xlsx for terpenes, and SI7_Terpenes_MIC_Summary.xlsx for flavonoids.