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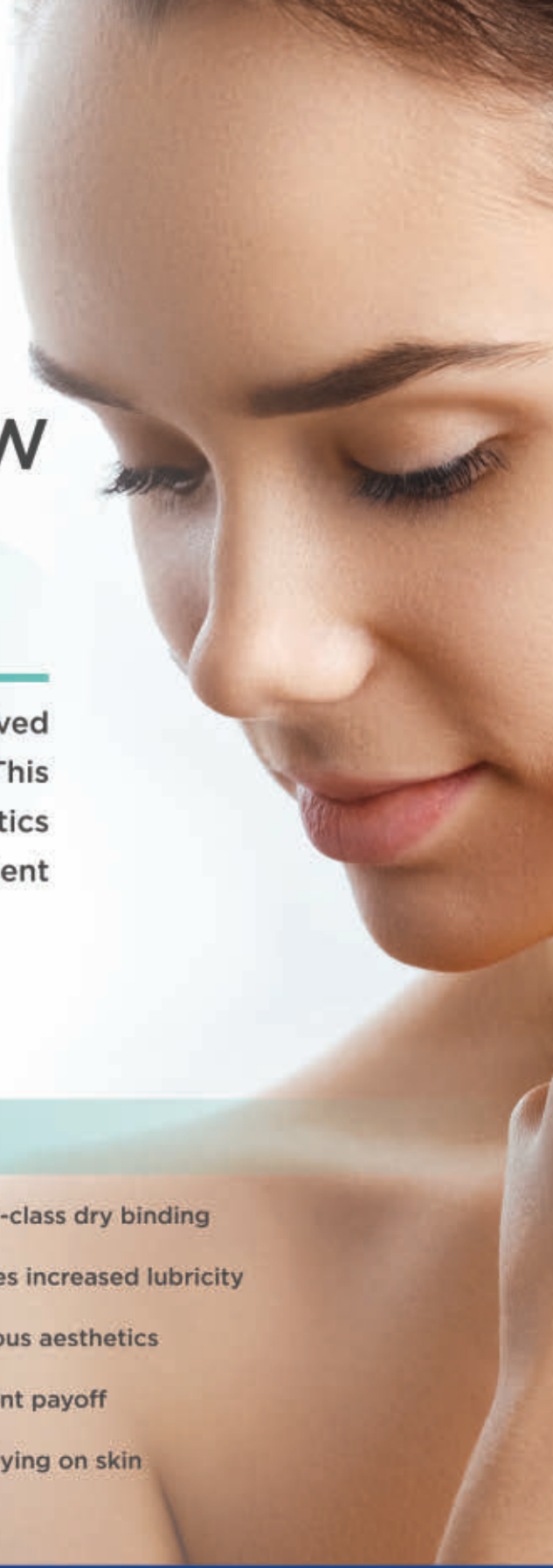

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Pixie Dust Memories

"In the consumer's world, *natural* means safer, greener, better for the environment [and people], etc. And although this belief may not entirely be grounded in reality, the fact remains that products labeled as *natural* have been the force behind a major trend in cosmetics and personal care for years."

I wrote this eight years ago. Is it still true today? To some extent, I think yes. Regarding the consumer viewpoint, a new report* from Grand View Research projects that the global market for organic and natural personal care will reach US $25.1 billion by 2025; expanding at a significant 9.5% CAGR from now until then. So, naturals are still a major growth category.

But what of the industry's perspective? In the past, formulators argued that hints of natural ingredients were sprinkled like pixie dust into products to support novel marketing stories. These were not necessarily active—let alone safer or better—and were most likely not sustainable. Based on their placement in the ingredient disclosure, many formulators were probably right.

Interestingly, though, in the current Grand View Research report*, the firm cites consumer demand for natural skin and hair care and cosmetics as a driving force due in part to *growing health awareness*. This points to a more "functional" aspiration for naturals.

Where does this lead us?

Into new territory, where naturals are designed to meet both eco-friendly and functional expectations. Innovators continue to demonstrate impressive feats in these areas; the latest of which are presented in this July/August issue. Specifically discussed are solutions with high efficacy and safety, and low environmental impact; along with guidelines to meet preservative test standards and natural packaging—pulling it all together, nicely.

In another eight years, as consumers look to minimalist formulations and maximum efficacy, "pixie dust" applications will likely fade away as mere memories.

Rachel L. Grabenhofer
C&T Managing Editor

* grandviewresearch.com/press-release/global-organic-personal-care-market

PHARCOS

Natural Cosmetic Ingredient

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Japanese Sake lees (Sake-kasu)

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IECIC 2015 Wamino-BonBon is listed in the Inventory of Existing Cosmetic Ingredient in China 2015.

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Validating the Naturals Phenomenon

Burt's Bees may be best known for its lip balm, but things could change if Celeste Lutrario has it her way. As vice president of R&D, she explained new sensitive skin research findings, presented at the AAD, and the company's larger drive to establish the credibility of naturals. Following is an excerpt adapted from our podcast; hear it all at CosmeticsandToiletries.com/multimedia.

Cosmetics & Toiletries (C&T): *Explain Burt's Bees positioning in the naturals market.*

Celeste Lutrario (CL): Burt's Bees has been a pioneer in natural skin care for more than 30 years. This goes back to the founders, who believed in the power of naturals to nourish and care for skin. A unique thing about Burt's Bees is we formulate under specific principles—rooted in the belief that healthy skin is balanced skin. We look to nature for ingredients that have the right nutritional profile, including vitamins, minerals, essential fatty acids and antioxidants, to feed skin and maintain its health.

C&T: *Describe your sensitive skin findings.*

CL: Perhaps seven years ago, we noted more than 50% of the global population felt they have sensitive skin. This was very interesting to us, and we created a sensitive skin care line with a special focus to *not* overload ingredients or over-cleanse the skin.

We then commissioned a study with our sensitive skin care line, presented at the AAD, to prove that nature-based skin care products are equal to synthetic products for addressing highly sensitive skin. Zoe Draelos, M.D., led a double-blind, randomized and controlled trial of 120 subjects comparing our products against the leading dermatologist-recommended synthetic regimen. The study found that the Burt's Bees products actually outperformed the dermatologist-recommended synthetic regimen in visual and tactile smoothness, clarity and radiance at weeks two and four. We were looking for parity but got even better results. By week four, all improvements were statistically significant.

Celeste Lutrario
Vice President of R&D
Burt's Bees

C&T: *What are the larger implications of this study for naturals in general?*

CL: I'm not saying natural is better, but we've believed for some time that natural products work *as well as* synthetic products. I believe this study shows they can.

Want More?
For more insight from Celeste Lutrario, log onto **www.CosmeticsandToiletries.com**

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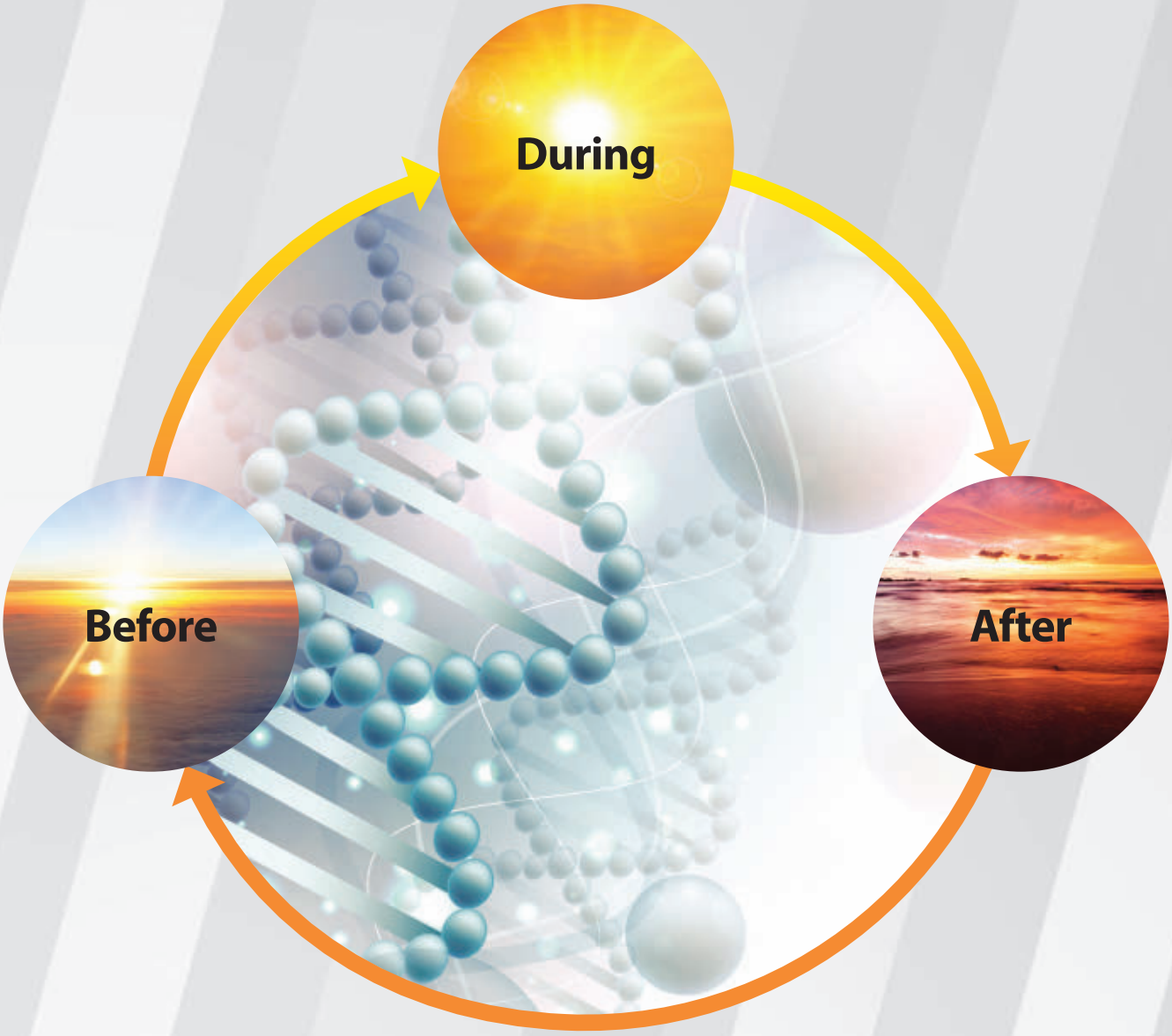

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Technology Launches

Fermented Shield

Deinove and Greentech rolled out Hebelys (INCI: Propanediol (and) *Sphingomonas* Ferment Extract), which is designed to protect skin against oxidation while stimulating collagen, elastin and fibrillin synthesis. The active is produced via the fermentation of a *Sphingomonas* bacterium, and also inhibits the expression of the p16 IINK4A protein, which contributes to premature cellular aging.
www.deinove.com

Circadian Relief

Clariant launched B-Circadin (INCI: Propanediol (and) Water (*aqua*) (and) *Lespedeza Capitata* Leaf/Stem Extract), designed to fight damage caused by disruption to the skin's circadian rhythm. The active works to improve skin's complexion while decreasing puffiness and dark circles via the synchronization of the circadian cycle. The South Korean *Lespedeza Capitata* plant contains flavonoids carlinoside and isoschaftoside, which emulate cell abilities.
www.clariant.com

Stable Emollient

AAK Personal Care introduced Lipex SMP (INCI: Hydrogenated Vegetable Oil), a solid emollient that can be used in various formulations including skin care, body care and cosmetics. The company recommends it as a base for aerated products and to texturize cosmetic pencils, sticks and crayons. The ingredient has high oxidative stability to help improve the shelf-life of finished formulations.
www. aakpersonalcare.com

Epigenetic Glow

Sederma's Crystalide (INCI: Not Available) peptide includes palmitoyl tetrapeptide-10, and delivers a soft-polished effect for a uniform glow while promoting clear and luminous skin, à la the "glass skin" concept in Korean beauty. The ingredient utilizes epigenetic regulation to allow skin to mature harmoniously and improve its surface quality.
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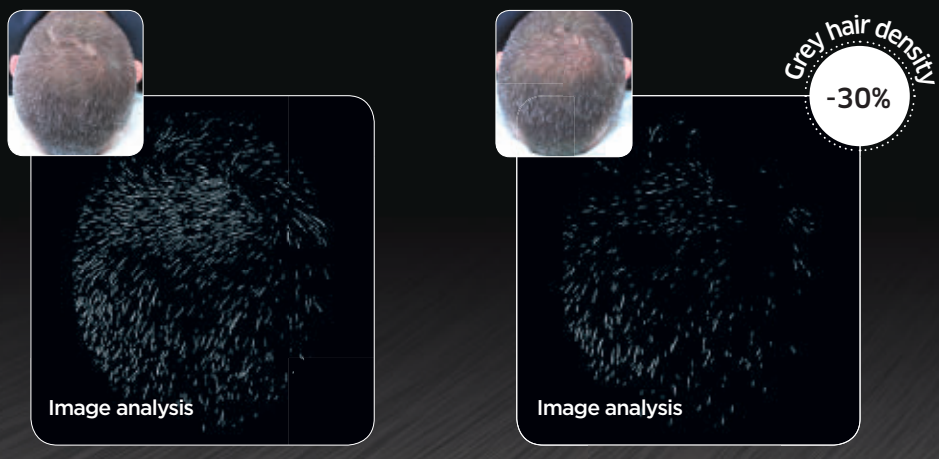

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Bio-Botanica Inc.
www.bio-botanica.com/product/puresterol-pueraria-mirifica-pe/
Puresterol (INCI: Glycerin (and) Water (*aqua*) (and) *Pueraria Mirifica* Root Extract) is an active derived from White Kwao Krua, or *Pueraria mirifica*—Thai for "miracle root," named for its anti-aging benefits.

2. **Coconut Oil**
Arista Industries, Inc.
www.aristaindustries.com
Coconut Oil (INCI: *Cocos Nucifera* (Coconut) Oil) is said to reduce inflammation, moisturize, soften skin and enhance hair health in addition to a host of health benefits. Arista Industries offers various grades of high quality natural and organic coconut oil, including organic refined, bleached and deodorized and virgin as well as conventional grades from renewable sources.

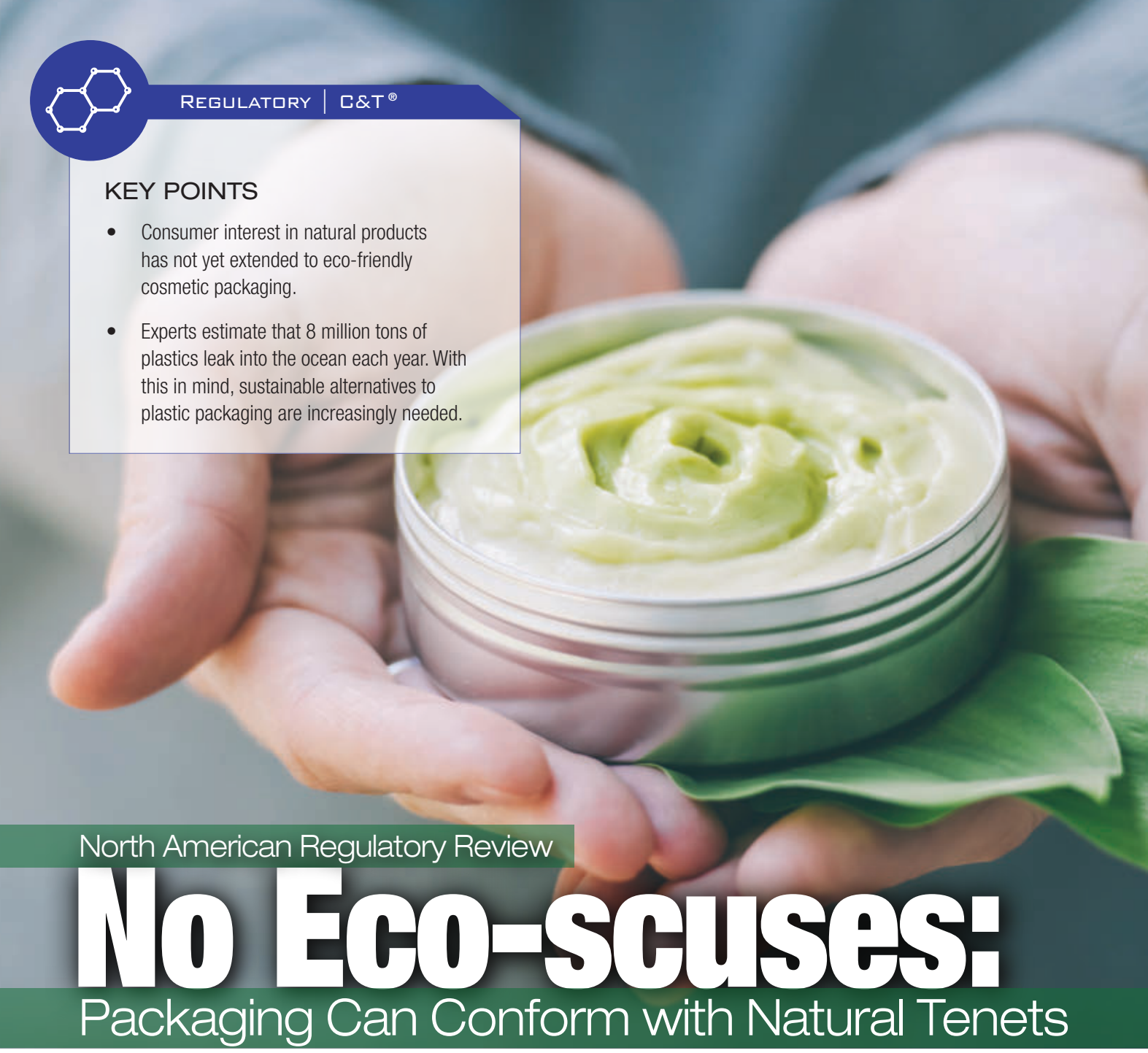
3. **Synastol TC**
Sytheon
www.sytheonltd.com
Synastol TC (INCI: *Terminalia Chebula* Fruit Extract) meets the requirements of sustainability, is Ecocert-compliant and COSMOS-certified and is globally approved for topical use. *Terminalia chebula* is one of the key ingredients in India's herbal Ayurvedic remedy. The ingredient is enriched with bio-actives (> 60%) and is suitable for anti-aging, anti-pollution, skin-brightening and toning applications.

4. **Vegeluron Gel**
MMP Inc.
www.mmpinc.com/
Vegeluron Gel (INCI: Water (*aqua*) (and) Propanediol (and) *Tremella Fuciformis* (Mushroom) Extract) is a clear, colorless, viscous gel of high molecular weight acid polysaccharide from the mushroom *Tremella fuciformis*. The ingredient has moisturizing properties (economical alternative to hyaluronic acid); confers softness and slip (natural alternative to silicone); and provides light film-forming to protect the skin from oxidation caused by pollution stress.

5. **NEEM Leaf Liquid B**
Ichimaru Pharcos Co., Ltd.
www.ichimaru.co.jp/english/
NEEM Leaf Liquid B (INCI: *Melia Azadirachta* Leaf Extract) provides slimming, skin-lightening and anti-aging benefits. In terms of supply chain, Ichimaru Pharcos has committed to contributing to the material's country of origin by cooperating in the planting of neem trees in production areas, and also returning profits based on the spirit of "Access & Benefit Sharing."

KEY POINTS

- Consumer interest in natural products has not yet extended to eco-friendly cosmetic packaging.
- Experts estimate that 8 million tons of plastics leak into the ocean each year. With this in mind, sustainable alternatives to plastic packaging are increasingly needed.



North American Regulatory Review

No Eco-scuses:

Packaging Can Conform with Natural Tenets

Robert Fichtner and
Ann Francis
Focal Point Research, Inc.
Mississauga, Ontario

Consumers have voted with their dollars—their verdict? They want more natural products and ingredients. We have natural diapers, paper towels, cosmetics and even foods (e.g., no hormones) but frankly, consumers have been inconsistent in their interest in what is good for both themselves and the environment. They have been far less reactive to the packaging their products come in, compared to the ingredients within. Each of us brings home mountains of packaging from stores each week: paper, glass, cardboard, plastic and aluminum. Some of the waste from this packaging enters

We have plastic bottles, jars, pumps, overwraps, closures, trays and tubes—but where do these go after use?

recycling streams but sadly, a great deal is deposited into landfills and our oceans and lakes. This has been an environmental blind spot in the movement toward natural and a contributor to frightening levels of environmental abuse.

In the natural cosmetics movement, plastic microbeads became a target. Consumers and regulators adopted a sudden abhorrence to the use of these tiny plastic particles that provide abrasiveness in cosmetic products, leading many countries to ban them outright.[1] While they represented a relatively minor contribution to worldwide plastic waste, they did bring attention to the unnecessary use of plastics in some applications. In the case of cosmetic products, these microbeads could be substituted by other, more natural ingredients (see Page 56 for more on this topic).

Plastic production went from 50 million metric tons in 1964 to 311 million metric tons in 2014. This amount of plastic has been estimated to equal 900 Empire State buildings, and is forecasted to double over the next 20 years. Approximately 26% of all plastic produced is used in packaging; on a global basis, that comes to about 90 million tons per year. It has been estimated that only about 14% of plastic packaging is collected for recycling—the remainder goes to landfills or elsewhere in the environment.[2]

Most troubling is the estimate that there are more than 150 million tons of plastics in our oceans today. At least eight million tons of plastics leak into the ocean each year, which is the equivalent to dumping the contents of one garbage truck into the ocean every minute. By 2025, there will be one ton of plastic for every three tons of fish; and by 2050, there will be more plastic in the ocean than fish, by weight.[2]

It is Time to Ask: 'Where Does it Go?'

In hindsight, it is easy to see why plastics became so popular as packaging components. They are lightweight, waterproof, flexible, moldable and relatively inexpensive, when made in large quantities. Many also are recyclable, where recycling systems exist. They also make outstanding packages for personal care products and over-the-counter drugs.

Before plastics, glass was the high benchmark for packaging in these industries. Glass ensured that products were protected from water loss and oxygen ingress. But glass was breakable and heavy, so plastics were the solution. We have plastic bottles, jars, pumps, overwraps, closures, trays and tubes—but where do these go after use? Plastic bottles can be incorporated into recycling programs but many of the other components simply cannot.

Natural and organic cosmetics sales increased by 21% in Asia during 2017; however, North American and European markets experienced single-digit growth.

Source: Global Cosmetic Industry (*www.GCImagazine.com*)

There are estimates that the cosmetics and personal care industry accounts for one third of all landfill waste.[3] Yet, in spite of their enthusiasm for natural products, it seems consumers too rarely consider where the personal care product packaging goes once it is emptied. Too many purchasing decisions seem detached from environmental concerns about packaging.

Circular Economy Strategy

The European Commission recently adopted a circular economy strategy on plastics, which is meant to increase plastic packaging recycling to 55% and reduce landfill to 10% by 2030. Some European countries have deployed container deposit schemes. This approach is taken in the United States as well—currently, the country's overall recycling rate is 34% but in states where container deposit laws are enforced, there is an average rate of 70%.[2]

Another adaptor of the circular economy is the "New Plastics Economy." This initiative is led by the Ellen MacArthur Foundation, an organization established in 2010 to promote the development of a circular economy worldwide. The organization works with businesses, governments and educational institutions to encourage innovative thinking and develop circular economic initiatives. The New Plastics Economy aims to rework the way our society uses plastics by working with companies, retailers, plastic producers and packaging manufacturers, as well as local municipalities and businesses, to encourage the sustainable utilization of plastic.[2]

New Plastics Economy suggests the following measures to reduce plastic waste.

- Set up a global, industry-wide, ongoing effort to develop and facilitate the adoption of globally recognized plastic packaging design standards.
- Converge toward clearly defined global labeling and material marking standards.
- Establish a global framework for the implementation of modular and reusable business-to-business (B2B) packaging.
- Scale-up the use of industrially compostable plastics for targeted applications—this was shown to be successful in the city of Milan and during the London Olympic Games.
- Transform and strengthen markets for recycled plastics, including: supporting smaller reprocessing companies and those that source recycled content at the small- to medium-scale; allowing for more granular and standardized material specifications to better match supply and demand; and strengthening demand for recycled content through industry commitments or policy.[2]

There is reason to believe the move to reduce plastic use has begun among consumers. More are looking at the environmental impacts of product packaging and will prefer brands that shown to be environmentally friendly. Millennial women were raised to be environmentally responsible; 51% of millennials have stated they are willing to pay extra for sustain-

able products. In addition, the same percentage say they check product packaging and labeling for sustainable measures.[4]

How Companies are Reducing Packaging Waste

The Estée Lauder Corporation, which presides over 29 brands including Origins and Aveda, has set design guidelines to make sustainable packaging a priority in the company.

"We can make small redesigns in the packaging that have a huge impact and that the consumer may not even notice," John Delfausse, Estée Lauder's former chief environmental officer for corporate packaging, has previously said. For example, lightweighting, i.e., slimming the shape and weight of a bottle, tube or jar while maintaining the same volume of product—has been a common practice with Estée Lauder products. It is being adopted throughout the industry, too.[5]

Companies are keeping a close watch on what may be the next wave in packaging: bioplastics and bioresins—material made from corn and sugar substances that are renewable resources. These are already used in some cosmetic and skin care containers.[5]

In addition, many small cosmetic companies are employing reusable/refillable packaging in their product lines; such as zao Organic Makeup, whose packaging is made from bamboo. Another company that uses bamboo for packaging is Elate Cosmetics. This eco-friendly company sells products in similar reusable, refillable palettes for products including lipsticks, mascaras and eyeliner. Their products are even shipped in envelopes comprised of seed paper that can be planted after use.[6]

Ecovative, a small, U.S.-based company, has been developing an array of environmentally friendly materials that perform like plastics but are made by mushrooms. The company's technology uses the webs of thread-like mushroom roots, known as *mycelium*, which consume crop waste.[7] These materials can be grown and recycled, as opposed to being drilled, pumped, refined and discarded. Under the right conditions, the mycelium turn waste into a material with similar properties to polysytrene foam in just a few days and similarly, the mushroom packaging can be molded into any shape.[8]

Finally, paper can be used in cosmetic packaging as well. The Chicago Paper Tube and Can Company is a manufacturer that sells packaging for various uses, including for cosmetics. Specifically, these materials are 100% recycled fibers and up to 95% post-consumer content. These paper containers are also said to demonstrate high levels of biodegradability. In addition, the adhesives used by the company are water-based, and the dyes used for printing are vegetable- and soy-based inks. These paper containers have a water-resistant coating to allow for the storage of balms and lotions.[9]

The Right Direction

These steps all signal movements in the right direction. However, a major shift will be needed to stop the worrisome trends we see today. Additionally proposed steps include improving the economics and uptake of recycling, drastically reducing the leakage of plastics into the natural environment, and decoupling plastics from fossil fuel feedstocks by exploring and adopting renewable feedstocks.

The personal care industry has shown it is capable of constant change and for innovation in its products. The time to rethink packaging is now. No doubt, significant competitive advantage will be gained by any brand that successfully executes natural packaging alongside its natural product positioning.

References

All websites accessed on March 8, 2018

1. *indy100.com/article/microbeads-harmful-where-are-they-banned-countries-7549811*
2. *weforum.org/docs/WEF_The_New_Plastics_Economy.pdf*
3. *theguardian.com/sustainable-business/2014/nov/18/online-shopping-holidays-packaging-waste-recycling*
4. *beautypackaging.com/issues/2017-04-01/view_features/eco-responsible-packaging-opportunity-knocks-for-beauty-brands/*
5. *nytimes.com/2010/03/25/fashion/25skin.html*
6. *treehugger.com/organic-beauty/6-cosmetics-companies-refillable-packaging.html*
7. *ecovativedesign.com/home?ref=lp*
8. *theguardian.com/sustainable-business/mushrooms-new-plastic-ecovative*
9. *chicagopapertube.com/About/History/history.html*

C&T

KEY POINTS

- As consumers become more decidedly "anti-preservative," formulators are faced with the need for versatile preservation systems.
- Caprylyl glycol's flexible nature allows it to be incorporated into a variety of formats while serving consumer needs.

Ingredient Profile

Caprylyl Glycol

A Versatile Material to Boost Preservatives

Jon Toliver and
Saroja Narasimhan, Ph.D.
Johnson & Johnson
Skillman, NJ USA

In recent years, there has been a decline in the palette of options available for product preservation. Consequently, there is growing interest in preservative-boosting systems that use other combinations of antimicrobial choices, such as organic acids and aromatic alcohols.[1] In many formulas, the efficacy of the core preservative has been supplemented by the addition of cosmetic ingredients with one or more specified functions and an added boosting effect on the preservative system.

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PATENTS: US 7,217,546; IN229695

www.sabinsacosmetics.com

SABINSA 30 YEARS OF INNOVATION

A decline in the palette of preservatives available for formulations has inspired a growing interest in alternative preservative-boosting systems.

Caprylyl glycol, for example, is a versatile material used within personal care as a humectant and conditioning agent, as well as to modify product viscosity.[1] Other chemical names for caprylyl glycol include: 1,2-dihydroxyoctane; 1,2-octanediol; and 1,2-octylene glycol. Its product applications include eye, skin and hair care, and to a limited extent, nail care; but its predominant focus is in leave-on dermal products.[1] In addition, caprylyl glycol has the functional benefit of increasing the efficacy of preservative systems.

Process and Manufacture

Caprylyl glycol is a 1,2 glycol that may be produced as a natural derivative or via a full synthetic process. Natural derivation feedstock requires a sugar, which can come from a source such as corn. The natural sugars from corn are converted to the final glycol through a series of reactions. Catalyzed hydrogenation is employed for sugar conversion to a lactic acid intermediate. Subsequent reactions convert the lactic acid into the final caprylyl glycol.[3] This process avoids the use of petrochemical feedstocks, but it is more expensive.[3, 4]

Due to the complexity associated with natural derivation, caprylyl glycol is most often produced using catalytic oxidation of the 1-octene oxide or reduction of 2-hydroxyoctanoic acid.[2] Industrial synthesis is commonly conducted using a two-step process, consisting of catalytic oxidation of the 1-octene oxide followed by hydrolysis with distillation and recovery of caprylyl glycol.[5] The feedstock consists of alpha olefin and one or more oxidizing agents.

Caprylyl glycol is manufactured synthetically using an unsaturated linear 1-octene alpha olefin.[6, 7] General synthesis begins with the reaction of a 1-octene with hydrogen peroxide in the presence of formic acid. The hydrogen peroxide oxidizes the unsaturated α-bond on the 1-octene structure for conversion into the alkane oxide.[2, 8, 9] When in combination with formic acid, hydrogen peroxide fosters an in situ production of performic acid to aid in the epoxidation of the 1-octene precursor.[8, 9]

Formic acid has an added benefit as a solvent for the reaction and hydrolyzing agent for the epoxide ring on the alkane oxide, creating caprylyl glycol through saponification of resulting 1,2 alkane diol monoformate.[9] Additionally, the use of hydrogen peroxide and formic acid has been found to reduce the residual hydrogen peroxide at the end of the reaction, which aids in suppressing unintended byproducts.[8] Using this combination further helps to prevent the generation of odor-causing byproducts in the final caprylyl glycol, such as liberated sulfur, when using a sulfur catalyst and maintaining control of side reactions between the epoxy and diol that lower purity.[8, 9]

The reaction mixture is continuously distilled during the process to increase purity and caprylyl glycol yield.[2] Monohydric

The worldwide cosmetics ingredients market is expected to grow to a value of US $9.03 billion by 2023, with Asia-Pacific accounting for the dominant market share.

Source: Market Research Future

alcohols are utilized as well in the process, to maintain the purity of the final glycol. Methanol is one type of alcohol that can be used to remove additional byproducts of formic esters formed from the reaction through transesterification and evaporation of methyl formate from the non-azeotropic mixture.[8, 10] The final extraction of the caprylyl glycol is conducted using an organic solvent that is non-miscible with water, such as toluene, for the final recovery.[9] Depending on the synthetic pathway and conditions, there may be variations in the purity of the caprylyl glycol.

Alternative routes of synthesis are emerging to create caprylyl glycol. Dihydroxylation catalyzed by osmium is one method being explored, although this is currently not feasible for commercial use. Osmium catalysts are more expensive and face issues relating to recycling the metal and waste, depending on the amount used for the catalyst.[5]

Chemical Structure and Properties

Caprylyl glycols are low molecular weight aliphatic organic compounds with two hydroxyl groups per molecule.[11, 12] Commercially, they are supplied at approximately 98–100% active wt, with common use levels between approximately 0.5–5.0% wt.[2, 13] Under normal storage conditions the material is stable; however, oxidation may occur when exposed to air and heat. Stabilizers may be added to prevent the oxidation and degradation into carbonyl compounds and acid byproducts.[11] Depending on the storage temperature, heating may be required prior to use. Below 30°C, caprylyl glycol is present as a waxy white solid; at higher temperatures, it is a clear and colorless liquid with low odor.[13–15]

In personal care, caprylyl glycol's utility is based on its amphipathic nature. The balance of hydrophobic and hydrophilic content alters the emolliency of the material due to the contribution from hydroxyl groups and the fatty alkyl chain (see **Figure 1**).[15, 16] The hydrophilic moieties on the structure enable the absorption and retention of moisture from the atmosphere. During product use, the water absorbed by the caprylyl glycol structure aids in moisture-retention in the upper layers of skin, providing a moisturizing benefit.[11] The polyhydroxy component of the structure promotes the absorption of water from the atmosphere and permits water solubility at all use levels.

The combined hydrophilic and hydrophobic nature of the structure creates surface active proper-

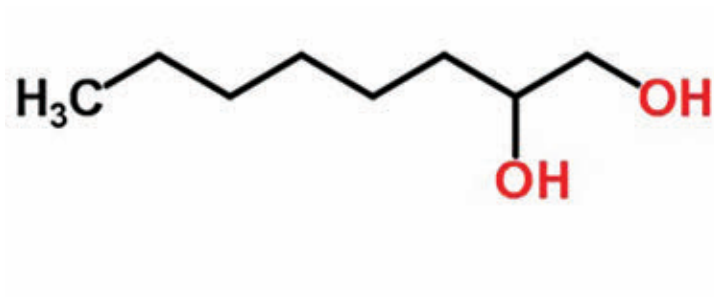

Figure 1. Caprylyl glycol structure

ties and promotes its use as a solubilizer for active ingredients; as such, in combination with other systems, within formulas the ingredient can reduce the need for other solubilizers.[12, 16] Its action as a solvent is based on the polyhydroxy moiety in combination with the carbon chain length of the structure.[12] Caprylyl glycols may also enhance visual clarity for formulations by incorporating immiscible ingredients into aqueous systems via coupling.

The structure of caprylyl glycol is also responsible for its antimicrobial benefit, which boosts total preservation efficacy in conjunction with a variety of preservatives. The alkyl chain length provides the preservation benefit, although its potency decreases rapidly beyond the C8 carbon chain length. This octyl carbon chain length has been shown to disrupt cell membranes or outer cytoplasmic membranes, accelerating anti-microbial activities.[13, 15, 18, 19] Caprylyl glycol itself may function as a preservative against bacteria within oil and water formulations; however, it has limited efficacy against fungi.[20, 21] Consequently, for broad-spectrum antimicrobial protection, caprylyl glycol is often used in conjunction with other preservatives in a system.[13, 20, 22]

The enhanced antimicrobial efficacy that caprylyl glycol lends to other preservatives is based on its ability to increase the partitioning of preservatives into the aqueous solution, wherein microbial efficacy is desired.[17, 23] Caprylyl glycol's function as a cosolvent imparts this action by increasing the aqueous wetting and solubility of some traditional preservatives, such as parabens and phenoxyethanol.[21, 24, 25] Reductions in use levels of traditional materials are thus possible when efficacy is increased in this manner.[25]

The optimum use levels of caprylyl glycol must be considered due its surface-active nature. Caprylyl glycol may be incorporated into the micelles of nonionic surfactants, thereby reducing the preservation of the system due to inavailability.[26] The interactions with emulsifiers and cleansers in the system may also promote instability of the formulation, in addition to lowering preservation efficacy, depending on the composition.[16]

Technology and Applications

The antimicrobial properties of caprylyl glycol have made it one of the predominant multifunctional ingredients to help reduce the use of traditional preservatives. It also holds promise to aid in preservation for applications in systems with extreme pH conditions and reduced free water content.[27, 28] As stated, in conjunction with other preservatives, there is also an increase in the antimicrobial efficacy due to its innate structure and function; combination blends of caprylyl glycol with chloroxylenol or chlorphenesin or both have shown broad-spectrum preservative efficacy.[29]

Finally, synergistic preservative systems including caprylyl glycol and enzymatic compositions demonstrate enhanced antifungal, antibacterial and antimicrobial efficacy.[30] This flexibility also allows for a variety of formats for use when considering non-conventional preservation systems and consumer benefits such as conditioning, emolliency and humectancy in cosmetics.[31] The robust nature of caprylyl glycol and associated multifunctional benefits provide an advantage for the development of future personal care products.

References

(All websites accessed June 12, 2018)

1. *happi.com/contents/view_features/2011-05-03/a-matter-of-self-preservation*
2. *online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/PR576.PDF*

3. A Braux, *GMO 101: A Practical Guide*, Alain Braux International Publishing, LLC (2014)
4. *cosmeticsbusiness.com/news/article_page/Naturally_well_preserved/49778*
5. U Sundermeier et al, Recent Developments in the Osmium-catalyzed Dihydroxylation of Olefins, in *Modern Oxidation Methods*, JE Bäckvall (ed), John Wiley & Sons (2000)
6. EF Lutz, Shell higher olefin process, *J Chem Educ* 63(3) 202 (1986)
7. *Chemistry and Technology of Surfactants*, R Farn (ed), Blackwell Publishing, Ames, IA USA (2006) pp 119–121
8. JP Pat 2,008,007,463 A, *Method for producing 1,2-alkanediol*, T Yamamoto et al, assigned to Osaka Organic Chem Ind Ltd (Jan 17, 2008)
9. UK Pat 2,145,076 A, *A process for producing 1,2-alkanediols*, Z Mazour and P Radimerski, assigned to Ciba-Geigy AG (March 20, 1985)
10. US Pat 7,385,092 B2, *Process for preparing alkanediols and alkenetriols having a vicinal diol group*, C Osterholt et al, assigned to Evonik Degussa GmbH (June 10, 2008)
11. *msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_091b/0901b8038091b508.pdf*
12. *Interfacial Phenomena in Apolar Media*, HF Eike and GD Parfitt (eds), Marcel Dekker, Inc., New York (1987)
13. *inolexcosmetics.ulprospector.com/en/na/PersonalCare/Detail/353/10336/Lexgard-O*
14. *minasolve.com/wp-content/uploads/2018/03/MINSO_BOOK2017_A4_REV2_SCREEN.pdf*
15. H Iwata and K Shimada, *Formulas, Ingredients and Production of Cosmetics: Technology of Skin- and Hair-Care Products in Japan*, Springer Science & Business Media (2012)
16. *eservices.personalcarecouncil.org/Science/15SS/paperlesssites/micro/schnittgerpresentation.pdf*
17. *academia.edu/23891939/Effect_of_ethanol_propylene_glycol_and_glycerol_on_the_interaction_of_methyl_and_propyl_p-hydroxybenzoate_with_Staphylococcus_aureus_and_Pseudomonas_aeruginosa*
18. *CosmeticsandToiletries.com/formulating/function/preservatives/premium-12-alkanediols-for-cosmetic-preservation-228198361.html*
19. P Ziosi et al, Caprylyl Glycol/Phenethyl Alcohol Blend for Alternative Preservation of Cosmetics, *Cosmetics & Toiletries* 128(8) 538–549 (2013)
20. *products.symrise.com/cosmetic-ingredients/actives/hydroliter-5/*
21. A Thiemann and J Jänichen, *The Formulator's Guide to Safe Cosmetic Preservation*, Personal Care Europe Nov 39–43 (2014)
22. *cen.acs.org/articles/95/i12/new-tack-discovery-funding.html*
23. A Varvaresou, Self-preserving cosmetics, *International Journal of Cosmetic Science* 31 163–173 (2009)
24. *in-cosmetics.com/__novadocuments/5121*
25. *akema.it/pdf/KEM_DIOL.pdf*
26. *cbinet.com/sites/default/files/files/English_Don_Pres%20day%202-updated.pdf*
27. *midwestscc.org/blog2/wp-content/uploads/presentations/Jan2012CurrentTrendsinCosmeticPreservation.pdf*
28. A Kerdudo et al, Optimization of cosmetic preservation: Water activity reduction, *International Journal of Cosmetic Science* 37(1) (2014)
29. US Pat 7,854,940, *Broad spectrum preservation blends*, D Ciccognani et al, assigned to Arch Chemicals Inc (Dec 21, 2010)
30. US Pat 20,080,226,568, *Synergistic preservative systems and their use in topical compositions*, E Rozsa et al, assigned to Playtex Products, Inc. (Sept 18, 2008)
31. DS Orth, Preservative-free (Self-preserving) Products, in *Insights into Cosmetic Microbiology*, Allured Publishing Corp, Carol Stream, IL USA (2010) pp 154 C&T

KEY POINTS

- Nanofibers from marine collagen were tested for their ability to deliver hyaluronic acid and grapeseed and kiwifruit bioactives into skin. Results showed they penetrated in under one minute.
- This sustainable and natural platform removes the need for emulsifiers, penetration enhancers and other transporter ingredients. It also enables the encapsulation of a wide range of water- and oil-based actives.

A New Spin on Delivery

Electrospun Collagen Drives Actives to New Depths

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Revolution Fibres Ltd., New Zealand

The pursuit of younger-looking skin drives cosmetic market growth. Youthful, glowing skin is achieved by advanced formulations and their accurate delivery to defined areas of the skin; typically via creams, gels or serums. Too often, the active formulation stays on the stratum corneum layer, where it delivers only minimal and temporary results. Thus, for acute delivery and deeper penetration of actives, penetration enhancers and delivery systems have been developed both by biomedical and cosmetic researchers.[1]

In relation, nanomaterials have been employed to both protect active ingredients and control their delivery so they can work within skin to improve its appearance. As an alternative to traditional nanoemulsions and nanoliposomes, a novel nanotechnology was developed to encapsulate and deliver bioactive agents into the skin layers quickly, for heightened benefits. Here, the authors described its manufacture and put it to the test.

The technology[a] comprises marine collagen nanofibers (MCN)[2] that can incorporate various bioactives, such as the hyaluronic acid (HA)[b] and polyphenolic compounds[c] utilized here. These actives were chosen for both their known cosmetic benefits as well as challenging characteristics for encapsulation; i.e., molecular complexity, hydrophilicity and tendency to separate out during formulation.

[a] ActiVlayr is a trademark of Revolution Fibres Ltd.
[b] FDA-grade Hyaluronic Acid (INCI: Sodium Hyaluronate), was purchased from Certified Nutraceuticals, USA.
[c] Vinanza extracts, derived from byproducts of kiwi fruit and grape skin using Aquapure technology, were purchased from NZ Extracts.

An intelligent delivery system will carry actives deeper into skin where they can live up to their full potential and work within skin for long-term effects.

Collagen is used widely in medicine and oral health supplements, usually in the forms of small peptides or gelatins, to enhance extracellular matrix construction (ECM) and the synthesis of essential nutrients for skin, hair and nails.[3] The collagen protein has a high substantivity to the skin[3, 4] but cannot permeate due to its bulk structure. Therefore, in topical formulations, its use is limited to moisturizers or natural humectants;[3, 5] or, in cosmetic surgery, it is directly injected into skin to treat minor imperfections and wrinkles.

The main objective of the present work was to construct and use collagen as a carrier and delivery vehicle for actives. The authors therefore fabricated a specialized marine collagen—i.e., disentangled α-chains of high molecular weight (HMW), denatured whole chain collagen (DWCC), with active bonding sites—in a nanofiber form using electrospinning. The combination of this unique DWCC format and electrospinning technique resulted in a highly functional material with increased surface area and superior skin penetration efficiency.

Furthermore, the fabricated MCN matrix is found to break up rapidly into small, dissolvable pieces due to its high surface area in water, deploying a strategic release of actives. The working mechanism of MCN is explained in detail and put to the test in the forthcoming sections.

The global personal care delivery systems market will be valued at US $543.37 million by the end of 2020, expanding at a CAGR of 7.8% since 2014.

Source: Persistence Market Research

Materials and Methods

Collagen sourcing: The DWC collagen was derived from the unused skins of hoki fish (*Macruronus novaezelandiae*) (see **Figure 1a**), sourced from the deepest regions of the Southern and Western oceans around New Zealand using sustainable practices[d]. The fish skins are cleaned of adhering scales, fat, pigments and muscles before being subjected to the denaturalization process. The resulting collagen was used to construct MCN using a proprietary electrospinning process[e].

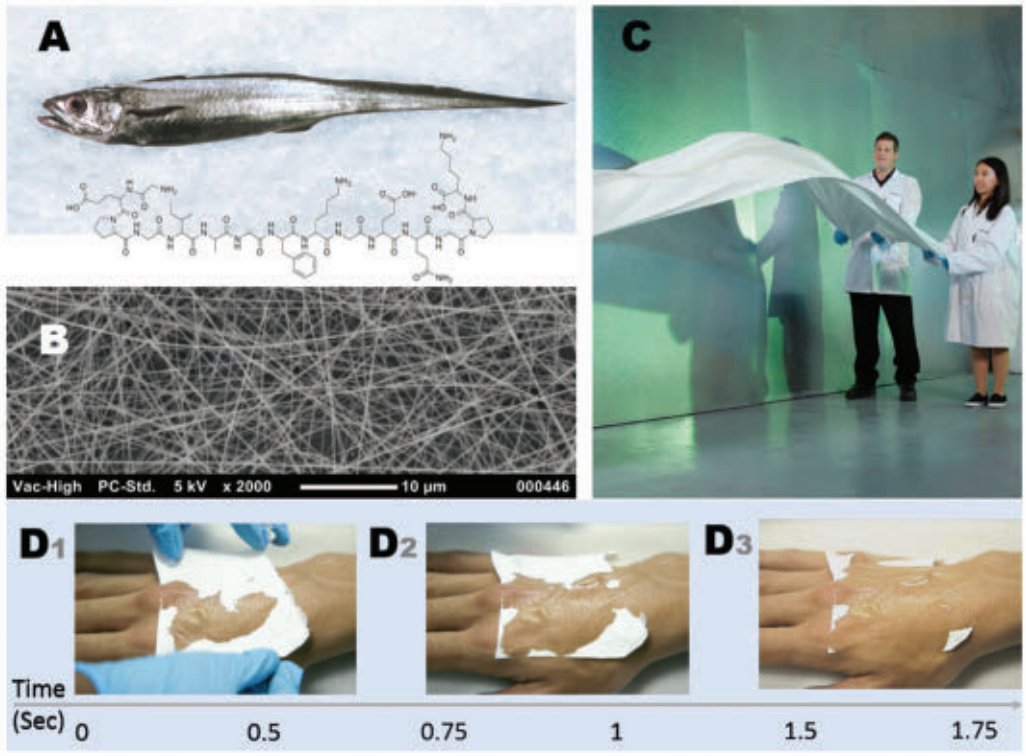
Morphology and characterization: Scanning electron microscopy (SEM)[f] (see **Figure 1b**) and ATR-Fourier-transform infrared (FTIR) spectroscopy were used[g], respectively, to examine the morphology of fibers and to characterize the MCN samples.

Permeation: Piglet skin was chosen for penetration studies due to its physiological and anatomical similarities with human skin, specifically its permeability behavior.[6] For example, the skin layers in both species have a relatively thick epidermis that is separated from all the sub-layers. The dermis of both species also contains an abundance of elastic tissue with similar collagen fibril thicknesses.[7, 8]

Stillborn piglet skin samples of different sizes were sourced from a pig farm, immediately processed, cut to the necessary size and preserved following a thorough tissue preservation protocol. The samples were dissected in dimensions to fit the size parameters of the imaging system's probes. On average, skin samples were 10 mm × 10 mm, with a thickness of up to 4 mm.

OCT: Optical Coherence Tomography (OCT) was used[h] to assess the permeation of bioactives

[d] *Sanford Ltd., New Zealand*
[e] *Revolution Fibres Ltd, New Zealand*
[f] *Jeol-5000 SEM under high vacuum with an AC voltage of 10 kV*
[g] *Nicolet 8700 ATR-FTIR spectrometer*
[h] *Standard swept source OCT, OCM1300SS, Thorlabs, Inc.; tests were performed at Otago University*



** 1) MCN touches moistened skin, 2) dissolves rapidly and 3) penetrates 100% into skin leaving dry fibers behind*

Figure 1. Hoki fish (inset: molecular structure of type I collagen) (a); SEM of MCN (b); MCN at industrial scale (c) and rapid absorption of MCN on wet hand (d)*

into skin. The system comprises a light source with a central wavelength of 1,325 nm and a bandwidth of approximately 100 nm; having a scanning rate of 16 kHz; and an output power of the probing light at 12 mW. This system is capable of acquiring 3D volumes of 1,024 pixels × 1,024 pixels × 512 pixels—i.e., up to 10 mm × 10 mm × 3 mm and containing 1,024 images—within approximately 40 sec, with axial and lateral resolutions of approx. 13 µm and 25 µm, respectively. Permeability changes can be examined up to 2.5 mm in depth.

Fundamentally, the diffusion of bioactives into a substrate changes the scattering properties, which are detectable by OCT. By utilizing specialized software, double-correlation analysis of 2D/3D OCT images are reorganized for validation of the skin penetration outcomes. OCT is a proven scientific method for non-invasive and non-destructive, functional, real-time spectral imaging. As noted, it can provide images in two and three dimensions, in vitro and in vivo.[9]

MCN Properties and Construction

Apart from cultural reasons and a low risk for transmitted disease,[10, 11] marine collagen is preferred for its proline and hydroxyproline profile, which is high enough to deliver moisturizing effects yet low enough to electrospin. For example, the reported values of the proline plus hydroxyproline content, as a percentage of the total amino acid profile in collagen, are 14.7% for hoki skin,[12] compared with 23.2% for calf skin.[13]

In general, the presence of proline and hydroxyproline molecules in collagen restricts the rotation of the backbone of the protein chain in all species (including humans), which supports the helix formation in the individual alpha chains of the collagen. The individual alpha chain then cross-links with two more alpha chains to form a super triple helix structure of the native collagen molecule. The hydroxyproline is also responsible for

Results also showed the diffusion behavior of actives does not prefer a single pathway for permeation.

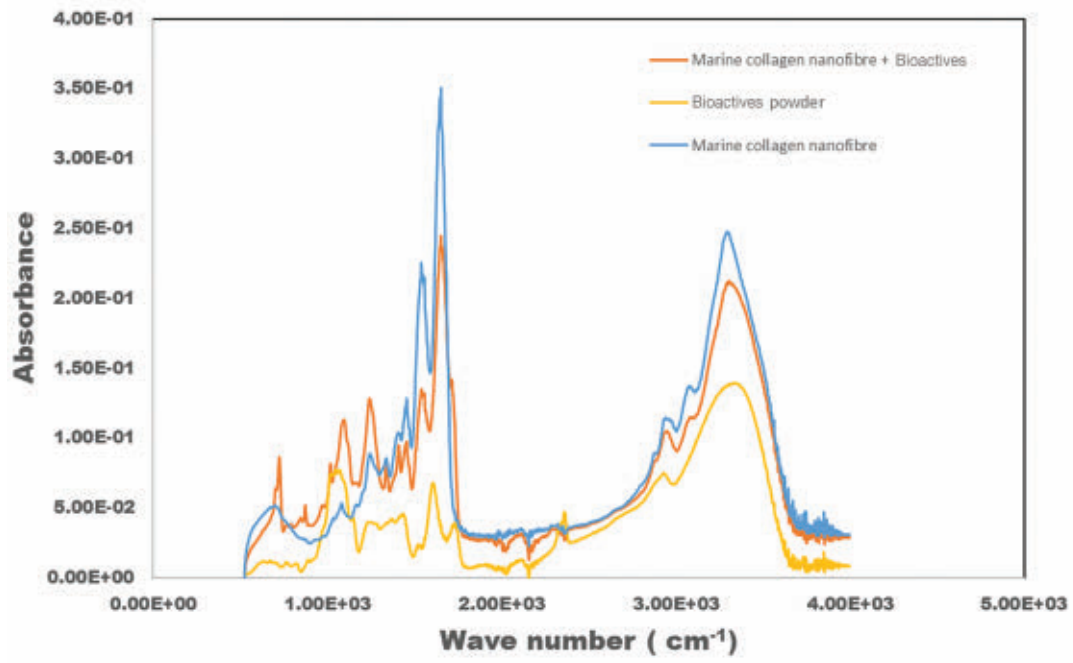
stabilizing the hydrogen bonds within the triple helix.[14] This triple helix format is required to build collagen fibrils responsible for ECM construction in the body. However, it is difficult to deliver triple helix format collagen directly through the skin without rupturing its structure.

The authors believe that electrospinning an alternate form of collagen with intact telopeptides (to represent a structure closer to that of native collagen) plus proline and hydroxyproline (essential amino acids for collagen synthesis) creates perhaps the best possible natural skin delivery platform. Therefore, a specific extraction and denaturation process was carried out to produce DWCC with intact telopeptides.

In detail, the marine collagen is extracted from an intact triple helix (tropocollagen molecules) to produce DWCC in a weak acid solution. The presence of acid-soluble covalent cross-links specific to marine species makes it possible to recover DWCC with intact telopeptides (non-helical portions of alpha chains).[12, 15] These intact telopeptides in the molecular chain make the DWCC easier to cross-link with suitable actives during electrospinning.

DWCC is denatured collagen but structurally different from gelatin. Gelatin is the complex mixture of oligomers joined by covalent bonds and partially hydrolyzed, but with alpha chains having varying molecular weights.[14, 16] In gelatin, the alpha chain loses its natural triple helix structure and becomes individual short peptide chains with no telopeptides at the end.[16]

Due to the significant structural differences between DWCC and gelatin, it was expected to electrospin in a manner different from gelatin; even with the same solvents. Interestingly, in 100% deionized water or benign acid solutions, DWCC forms a viscous liquid with solution parameters, e.g., conductivity, viscosity and surface tension, and hydrodynamic properties



The collagen-distinct peak bands at 3,330 cm^{-1} (N-H stretch), 1,655 cm^{-1} (C=O), 1,550 cm^{-1} (C-N) and (N-H) bend combination and 1,250 cm^{-1} (C-N) and (C-C) stretch present in both MCN and MCN+bioactives can be attributed to amide groups in the collagen. The signature O-H bonds, C=O bonds and C-O of the bioactive compound spectras fall in the same region as collagen; however, the distinct peaks around 1,070 cm^{-1} (C-O) stretch, and two peaks at 1,800 cm^{-1} and 1,900 cm^{-1} in both MCN and MCN+bioactives can be attributed to the C=O stretch of the bioactives.

● Figure 2. FTIR validation for the presence of bioactives in the MCN matrix

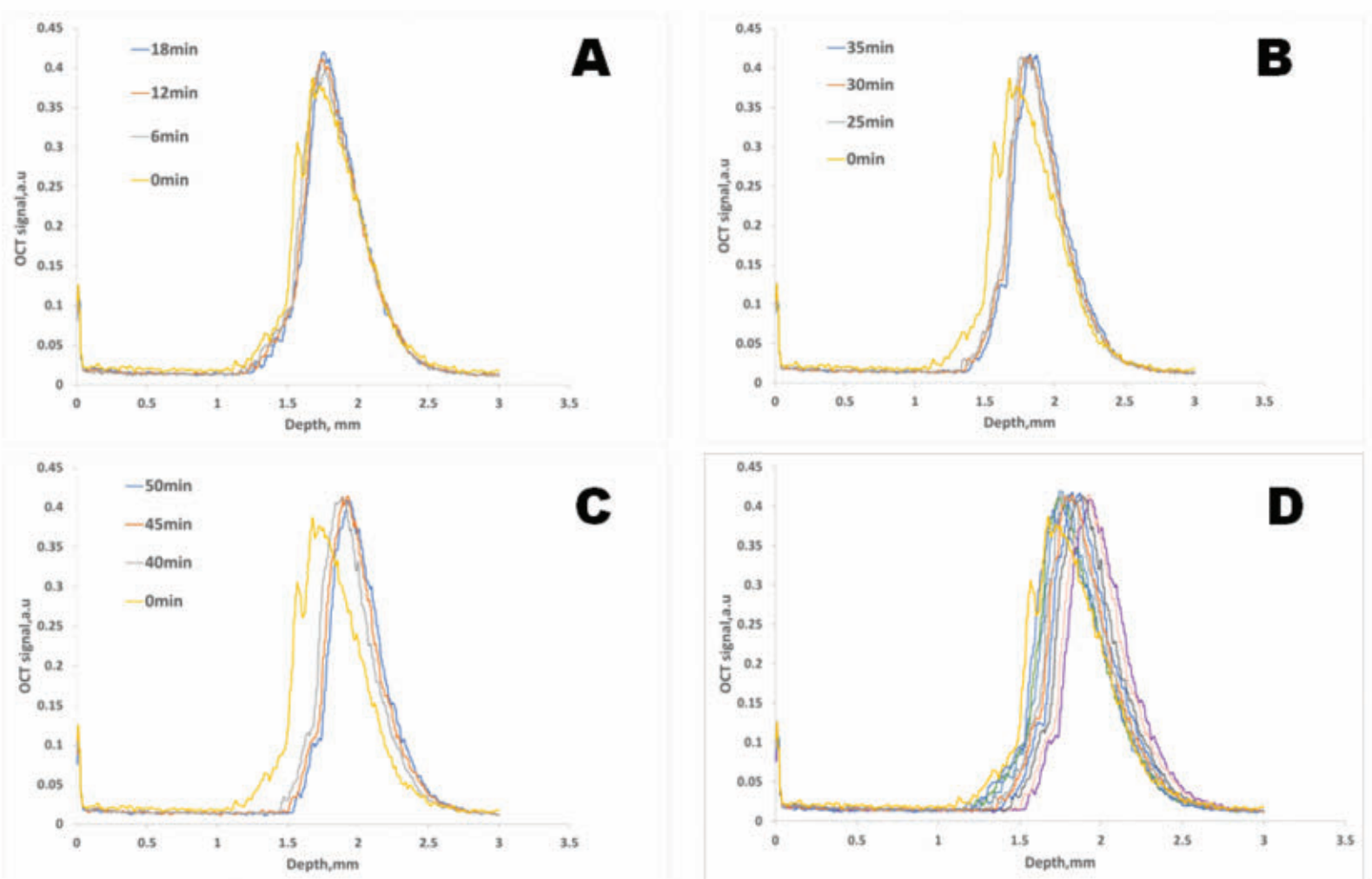
appropriate for electrospinning. In comparison, native atelocollagen or gelatin behaves like a gel at the concentration relevant to electrospinning. Therefore, stronger (often toxic) solvents are used to rupture the hydrogen bonding in gelatin to stretch it for electrospinning.

The literature suggests electrospinning native collagen extracted from mammalian species uses 1,1,1,3,3,3-hexafluoro-2-proposal (HFP) to disrupt the hydrogen bonding that stabilizes the helix.[17, 18] However, HFP is categorized as a dangerous fluorinated solvent and therefore is not desirable for electrospinning collagen nanofiber for skin care applications. In contrast, DWCC uses a benign water-acid solvent system, which makes it easier to scale-up and manufacture (see **Figure 1c**) while also remaining safe for skin.

Besides the structural benefits of DWCC, it offers a unique advantage over gelatin in that it is dissolvable even at room temperatures or lower, even in cold water, which makes it easy to apply to the skin at any temperature (see **Figure 1d**).

Sonic Electrospinning

Electrospinning in general is a nanofiber fabrication technique that utilizes an electrostatic repulsive force within an electric field to stretch a polymer droplet under high voltage.[19, 20] At this voltage, the highly conductive marine collagen solution (3.217 S/m) overcomes surface tension (220 mN/m) and the kinetic viscosity of the polymer (320 cst) to form a jet that is stretched to a collecting material. During this phase, the jet of collagen transforms to a chaotic whipping motion,[20] which is influenced by the voltage, temperature and relative humidity to form a solidified collagen fiber on the collector substrate. Sonic electrospinning, used especially to construct MCN, is a proprietary technology with a design that encourages a



At 0 min, the MCN penetrated to 1.5 mm in depth and moved to 1.75 mm after 18 min (a); at 35 min, the MCN penetrated deeper to reach 2 mm (b); at 50 min, MCN moved deeper toward 2.5 mm (c); and (d) shows the combined OCT data of a, b and c, showing the total penetration profile of OCT over a period of time

Figure 3. OCT data showing MCN penetration into the skin

Kiwi fruit and grape skin bioactives were delivered by the nanofiber to a depth of 2.5 mm or the demal layer of skin.

stable jetting process for an extended period over a large surface area.

The morphologies of electrospun jets can be categorized into five regimes.[21] At a particular regime, which starts with a jet fluctuation, the hydrodynamic interaction within the collagen solution, combined with the complex jetting mechanism in an electrically charged environment, leads the entangled DWC collagen alpha chain to stretch.

When the denatured polymer chain stretches, interactions between the molecular chains present in the solution also increase. Due to the high topological interactions in this regime, the HA present in this particular solution also stretches to form an inter-penetrated network with the collagen, resulting in increased molecular stability of HA. In the case of additional polyphenolic actives, e.g., the antioxidants from kiwi fruit and grape skins, which comprise gallic acid, proanthocyanins, catechins, and vitamin C, these are chemically bonded to the collagen backbone in the solution. Thus, during the spinning process, the collagen nanofiber carries the encapsulated/chemically bonded active compounds within the chains, protecting its potency and stability against photo-oxidation.

MCN Characterization

As noted, FTIR spectroscopy was used to analyze and assure the presence of polyphenol components in the sonically electrospun MCN (see **Figure 2**). Signature peaks specific to polyphenols confirmed they were bound to the MCN backbone. Thus, the encapsulation and protection of bioactives present in the MCN, afforded by the sonic electrospinning technique, becomes an essential component to enable the creation of a rapidly dissolving fabric for fast penetration.

Penetration Protocol

To measure the penetration of actives from MCN into pig skin, OCT was utilized. First, the test sample measurement sites and real-time measurement period, or rate, were established. The recommended timeframe of one hour (50 min) to scan and obtain all images and measurements was strictly followed.

The skin surface was wetted with room temperature water (~22°C) prior to placing the actives-embedded MCN onto it; all measurements were performed at room temperature.

The treated skin was then placed/mounted precisely under the probe, onto the OCT

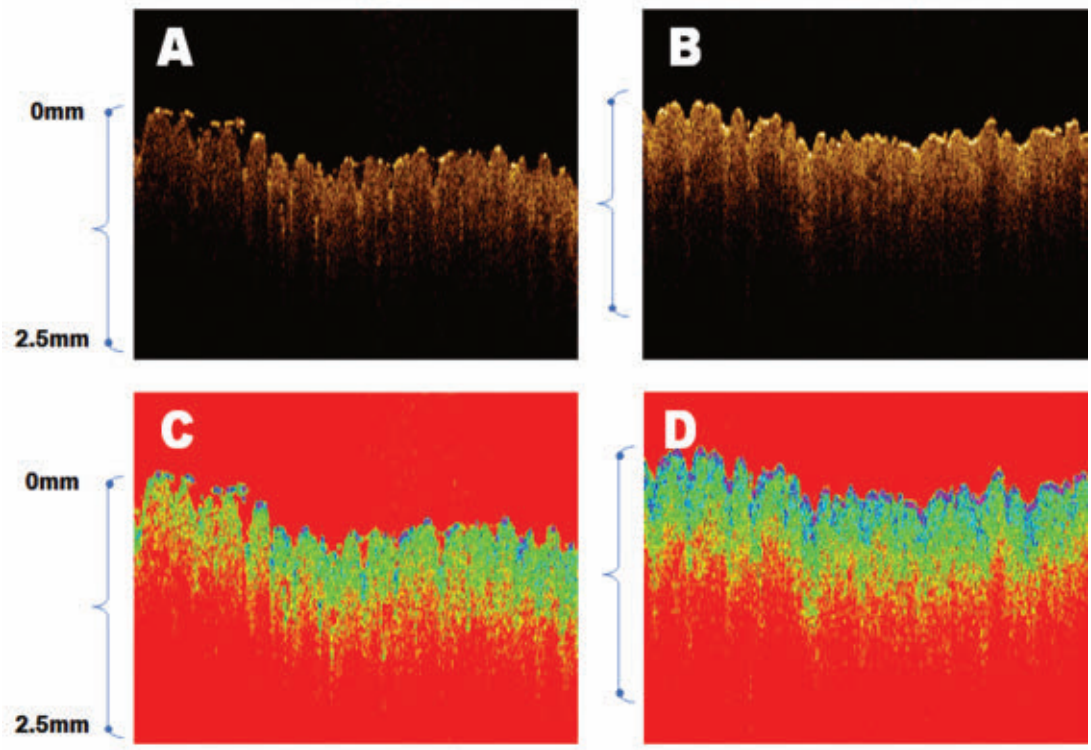


Figure 4. OCT penetration profile for MCN on a pig skin at 0 min in volume 1 (a) and at 50 min in volume 256 (b); (c) and (d) are color-coded to differentiate penetration depth

platform, where the bioactives-embedded MCN was placed, avoiding any additional compression of the sample. For each sample analysis, the OCT dynamic range was adjusted to avoid random noise and to guarantee optimal imaging with reasonable depth and good contrast. Software was used to analyze OCT data, and the images were reported as 2D or 3D image files, while the OCT signals' strength in relation to the skin depth was reported in separate graphs and elaborated using additional software.

Permeability Results

When the MCN sample was placed on the skin surface, rapid diffusion behavior was instantly observed. **Figure 3** shows the degree of penetration of the bioactives from each nanofibrous matrix. To optimize this visualization and provide a better understanding of the penetration behavior, each skin sample was arbitrarily subdivided into 256 smaller volumes during the OCT analysis, and each section was photographed and analyzed separately. As the OCT profiles reveal, the collagen nanofiber penetrated deeply, crossing the stratum corneum and reaching a depth of 1.5 mm, up to the dermal-epidermal junction (DEJ), almost immediately, i.e., at 0 min. This penetration continued further into the skin layers and reached a depth of 1.75 mm after 18 min.

It is documented that the size of a molecule and its lipophilicity are the major determinants of penetration processes through the stratum corneum. This means the permeability of a molecule is directly related to its hydrophilicity or lipophilicity, and inversely proportional to its molecular size. Many cosmetic or drug formulations use skin penetration enhancers to help promote the transport of actives into skin,[22] with water being the safest and most effective skin penetration enhancer; it has a high permeability constant of 5.5×10^{-6} cm/hr.[23] Therefore, simply hydrating the stratum corneum can modify its surface efficiently to promote actives penetration.

The DWCC nanofiber matrix, being a water-soluble material, naturally diffuses through

the stratum corneum pores on a wet surface due to its hydrophilicity, which in turn promotes the transport of actives attached to it, as is evident from **Figure 3a**.

Continuing its penetration, over the next 35 min, MCN crossed 2 mm of skin, which is where the dermal-epidermal junction (DEJ) reaches the dermal layer. This zone between the dermis and epidermis is the cutaneous basement membrane—a dynamic interface where the two layers adhere to one another. The DEJ typically restricts the passage of most molecules, based on charge and size, between the epidermis and dermis[1] but due to its structural advantages, MCN permeated the DEJ layer (see **Figure 3b**) and spread across the network.

The actives also continued to penetrate further, reaching 2.5 mm—a dermal layer of the skin—after 50 min (see **Figure 3c**). This layer consists of fibroblast connective tissues that produce natural collagen and other fibers. Therefore, MCN, with the appropriate actives, could potentially be used to reconstruct the ECM of skin. **Figure 4** shows the penetration level of the initial volume 1 and the final volume 256 at 0 min and 50 min. Note that all images showed a comprehensive bioactive profile across the whole sample and the clear absence of particle agglomeration. These results also show the diffusion behavior of actives does not prefer a single pathway for permeation.

Discussion

As previously described, the instant diffusion behavior was achieved by the breakdown of the soluble amino acid chain of the MCN, along with the HA molecule and bioactives, into small, nano-sized fragments in an aqueous phase that crosses the skin structures. Such an instant breakdown appears to be possible only through this high surface area-nanofiber matrix.

In comparison, triple helix collagen could not break down so rapidly, especially with water at room temperature, due to its high molecular weight. And HA, being a bulk polysaccharide, and hence classified as an injectable product, could not otherwise penetrate the skin. The observed success of the deeply penetrating actives is greatly associated with the electrospinning technique and the nature of soluble DWCC structure.

It is important to consider the diffusion characteristics of the ingredients are governed by both the skin type, i.e., its absorbing efficiency, as well as the working mechanism of the collagen nanofiber matrix. However, to some degree, the chemical nature of the actives also controls the diffusion behavior.

Conclusions

In both cosmetics and medicine, it is preferred that topically administrated actives act either dermally or transdermally. Unfortunately, most topically administrated drugs or actives cannot penetrate or permeate the skin layers without skin barrier manipulations. The key to driving the actives deeper into the skin is to design an intelligent delivery system that can carry those actives to where they can live up to their full potential and work within the skin for long-term effects.

The described marine collagen in a nanofiber matrix form, along with the inclusion of HA and natural bioactives, is shown here to provide one such intelligent skin delivery vehicle, which delivered actives in less than one minute. This exceeds all the literature values published to date for non-invasive delivery, especially as a natural product. The penetration also continued for an additional 50 min to reach 2.5 mm of a deep dermal layer of the skin, making it perhaps one of the fastest non-invasive active delivery systems in the world.

Further, the interfacial surface chemistry of the nanofiber matrix could be varied to encapsulate multiple actives, custom-designed for specific target applications, as well increase their dispersion and efficacy while decreasing formulation complexity. The technique could also potentially be used to send both water- or oil-soluble cosmetic ingredients or drugs into the skin without the use the emulsifiers, stabilizers and skin penetration enhancers.

Finally, the strong bonding of actives within the nanofiber matrix and rapid entry of those actives into skin illustrate its potential use in beauty facial masks and wound care. This process could potentially revolutionize the skin care industry and, importantly, has been shown to be 100% sustainable, reproducible, cost-effective and scalable to meet industry demand. In the future, clinical studies will assess the feasibility and efficacy of this system for dermatological applications.

References

1. MMA Elsayed et al, Lipid vesicles for skin delivery of drugs: Reviewing three decades of research, *Intl J Pharmaceutics* 332(1) 1–16 (2007)
2. US Pat 9775917B2, *Nanofiber and bioactive compositions and related methods*, IC Hosie, GF Vile et al, Active Fibres Ltd, (2017)
3. N Boyera, I Galey and BA Bernard, Effect of vitamin C and its derivatives on collagen synthesis and cross-linking by normal human fibroblasts, *Intl J Cos Sci* 20(3) 151–158 (2001)
4. Y Peng et al, Evaluation for collagen products for cosmetic application, *Intl J Cos Sci* 26(6) 313–313 (2004)
5. A Alves et al, Cosmetic potential of marine fish skin collagen, *Cosmetics* 4(4) 39 (2017)
6. N Zaman, Influence of the (ultra) structure of skin specimens used for pharmacological studies on the tissue permeability, *Department of Pharmaceutics, Laboratory for Medicinal Chemistry, Universiteit Gent, Belgium* (2014)
7. IP Dick and RC Scott, Pig ear skin as an in vitro model for human skin permeability, *J Pharma and Pharmacol* 44(8) 640–645 (1992)
8. E Abd, et al, Skin models for the testing of transdermal drugs, *Clinical Pharmacology: Advances and Applications* 8 163–176 (2016)
9. J Welzel, Optical coherence tomography in dermatology: A review, *Skin Res and Tech* 7(1) 1–9 (2008)
10. TH Silva et al, Marine origin collagens and its potential applications, *Marine Drugs* 12(12) 5881–5901 (2014)
11. S Arvanitoyannis Ioannis and A Kassaveti, Fish industry waste: Treatments, environmental impacts, current and potential uses, *Intl J Food Sci & Tech* 43(4) 726–745 (2007)
12. K Hofman et al, Effects of the molecular format of collagen on characteristics of electrospun fibers, *J Materials Sci* 47(3) 1148–1155 (2012)
13. JE Eastoe, The amino acid composition of mammalian collagen and gelatin, *Biochem J* 61(4) 589–600 (1955)
14. D Le Corre-Bordes, K Hofman and B Hall, Guide to electrospinning denatured whole chain collagen from hoki fish using benign solvents, *Intl J Biological Macromolecules* 112 1289–1299 (2018)
15. K Hofman et al, High-throughput quantification of hydroxyproline for determination of collagen, *Analytical Biochemistry* 417(2) 289–291 (2011)
16. HG Reinhard Schrieber, From collagen to gelatine, in *Gelatine Handbook: Theory and Industrial Practice*, Wiley-VCH Verlag GmbH & Co (2007) pp 45–117
17. DI Zeugolis et al, Electro-spinning of pure collagen nanofibers—Just an expensive way to make gelatin? *Biomaterials* 29(15) 2293–2305 (2008)
18. O Hartman et al, Biofunctionalization of electrospun PCL-based scaffolds with perlecan domain IV peptide to create a 3-D pharmacokinetic cancer model, *Biomaterials* 31(21) 5700–5718 (2010)
19. B Kannan, H Cha and IC Hosie, Electrospinning—Commercial applications, challenges and opportunities, in *Nano-size Polymers: Preparation, Properties, Applications*, S Fakirov, ed, Springer International Publishing, Cham (2016) pp 309–342
20. YM Shin et al, Electrospinning: A whipping fluid jet generates submicron polymer fibers, *Applied Physics Letters* 78(8) 1149–1151 (2001)
21. Y Cai and M Gevelber, The effect of relative humidity and evaporation rate on electrospinning: Fiber diameter and measurement for control implications, *J Mat Sci* 48(22) 7812–7826 (2013)
22. J Kalbitz, R Neubert and W Wohlrab, Modulation of drug penetration in the skin, *Die Pharmazie* 51(9) 619–637 (1996)
23. RL Bronaugh, The effect of cosmetic vehicles on the penetration of N-nitrosodiethanolamine through excised human skin, *J Inves Derm* 76(2) (1981)

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KEY POINTS

- Anthocyanins are flavonoid compounds found naturally in plants with promising potential for skin health and protection benefits.
- Sustaining their natural supply for industry has become increasingly important; as such, microbial routes for their production have been explored, such as those described here.

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Editor's note: The cosmetic industry's knowledge of microbes and their potential applications has grown tremendously. One area of recent interest is the skin microbiome, which can be manipulated for desired effects. Another is the utilization of microbes for the sustainable and economical production of natural materials; such as the upstream production of cosmetic pigments described here. The processes presented here are at the forefront of innovation and their use holds potential for future marketing claims to appeal to environmentally conscious consumers.

Biocatalyst for Change

Part I: The Potential for *E. coli* to Color Cosmetics

Anthocyanins are ubiquitous flavonoid compounds found naturally in plants. With promising health-promoting and skin-protection functions, they hold great potential for cosmetics. The sustainable supply of anthocyanins for industrial purposes has become increasingly important in recent years. As such, their microbial production provides a more sustainable and controllable approach over traditional plant extraction. This has successfully been achieved with the employment of novel metabolic engineering strategies.

This review first focuses on the potential of microbial-produced anthocyanins in color cosmetics and their supply. Part two, on Page DE4 of our digital edition, then looks in detail at strategies to use *Escherichia coli*. Anthocyanin production by plants is compared with engineering by *E. coli*, with a major emphasis on metabolic approaches and tactics in biocatalysis and coculturing. Specifically described are approaches to strain development such as enzyme selection, enzyme engineering and

Anthocyanins function as pigments, antioxidants and antimicrobials during a plant's growth.

the regulation of cofactor supply and anthocyanin efflux. Finally, a new biocatalytic process to enable de novo anthocyanin production from glucose via a polyculture is discussed.

Cosmetic Potential of Anthocyanins

Over the past decade, the global demand for cosmetics has continuously increased, which is expected to continue. This will require the development of more diverse and effective products.[1] Plant extracts attract consumer interest due, in part, to an innate fondness for that which is "natural." However, natural ingredients have long been incorporated in cosmetics due to the specific activities of components in them; such as scavenging free radicals, treating dry skin, improving signs of aging, etc.[2, 3]

Among these components are flavonoids; specifically anthocyanins, which are based on a wide group of flavylium compounds in plants. They function as pigments, antioxidants and antimicrobials during a plant's growth (see **Figure 1**). They also have been widely used as colorants in food and beverage manufacturing.[4]

As effective antioxidants against reactive oxygen species, anthocyanins can strongly absorb visible and UV light owing to their tricyclic aromatic structure.[5] They also can protect skin from aging and UV-induced damage,[6] such as inflammation and oxidative damage in the epidermis, dermis and adnexal organs.[4] Their fundamental mechanisms have been investigated in several in vitro cellular and animal models, although detailed in vivo studies have yet to be performed.[7, 8]

In general, anthocyanins reduce UV-induced elevations of cyclooxygenase-2 and prostaglandin E2 through NFκβ-dependent pathways. Moreover, anthocyanins decrease apoptotic cell death by inhibiting caspase-3 activation and reducing the proapoptotic Bax protein levels.[7, 9]

To date, cosmetic products incorporating pure anthocyanins have not yet been approved or marketed. However, trials have been conducted on the development of anthocyanin-colored lipsticks.[10] Also, Nutrasorb, LLC, a spin-off of Rutgers, has engineered a red lettuce for use as both a food supplement and a cosmetic additive.[11]

The inclusion of anthocyanins in cosmetics and skin care products may facilitate the alleviation of sensitivities caused either by direct contact with other chemicals in the formula, or help to rejuvenate skin by reducing wrinkles, dark spots, redness and other skin problems resulting from aging and skin damage.[12, 13] It is important to note, though, that anthocyanins currently approved for use are derived from plants. Those produced via microbes would require regulatory approval since they derive from a recombinant microorganism. However, with further investigations into the skin-protective functions of anthocyanins, along with decreasing production costs and regulatory approval, cosmetics containing anthocyanins may find their way into the market in the near future.[14]

Anthocyanin Supply

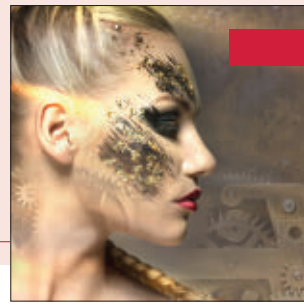
Currently, the supply of anthocyanins mostly relies on traditional extraction from plant tissues. However, concerns over seasonal supply and quality control, inherent in agricultural products, necessitate a sustainable and control-

The global color cosmetics market was valued at $5.88 billion in 2016 and is projected to reach $9.56 billion by 2023--a CAGR of 7.4%.

Allied Market Research

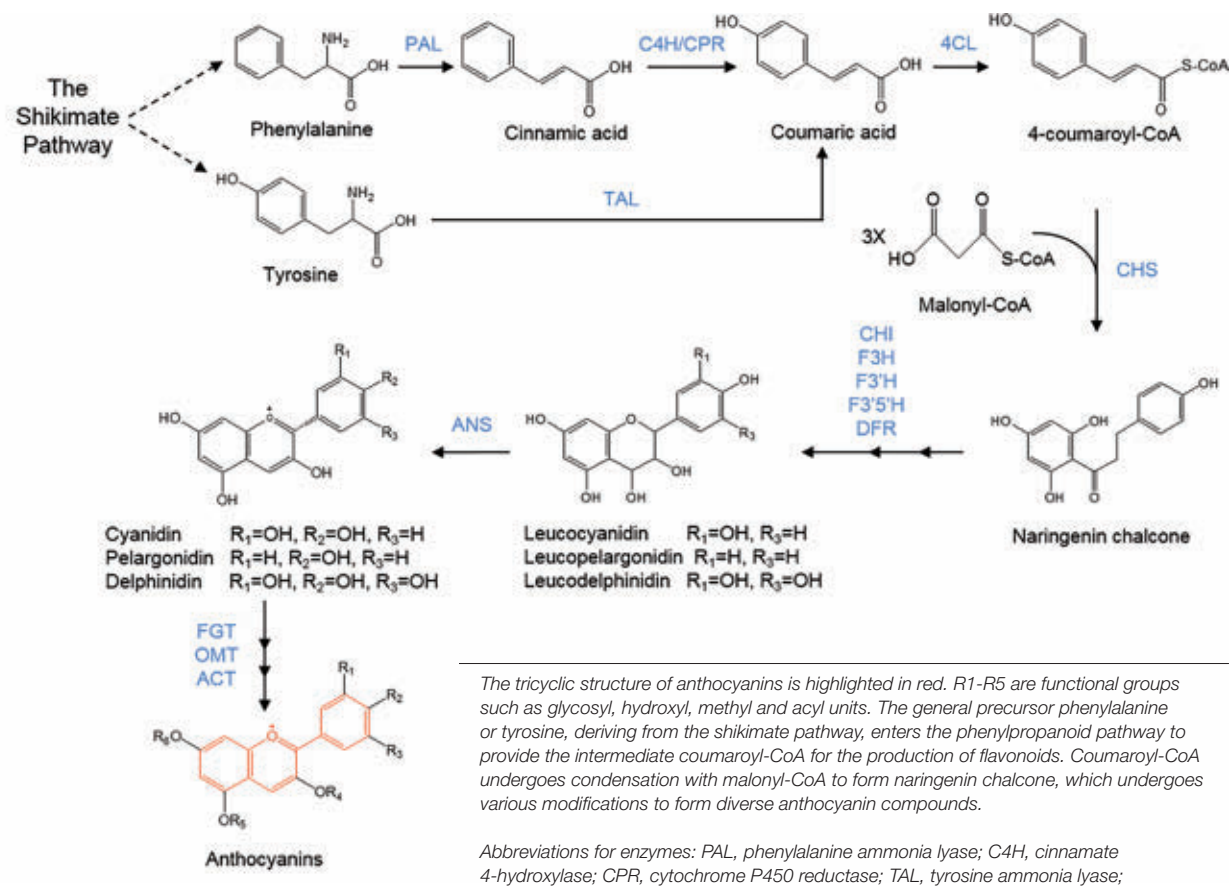
lable method to produce anthocyanins.[15, 16] Chemical synthesis has been investigated to provide regular or ^{13}C labeled anthocyanins, such as peonidin 3-*O*-glucoside and ^{13}C labelled cyanidin 3-*O*-glucoside, for clinical trials.[17, 18] This method depends upon the Robinson's acidic aldol condensation between a phenolic aldehyde and an aryl ketone. By tailoring different phenolic aldehydes and aryl ketones, non-natural anthocyanins can be created to provide a valuable reservoir for clinical use. However, chemical synthesis requires environmentally unfriendly and toxic reagents, and long and complicated reactions, leading to poor yields. To date, few studies have explored their chemical synthesis and no apparent anthocyanins created by chemical synthesis are commercially available.[18]

As such, the microbial production of anthocyanins has been explored using metabolically engineered microorganisms to produce specified anthocyanins in a controlled setting, which fits the requirement for industrial manufacturing. Part II explores these processes (see **Page DE4** in the digital edition).



Want Part 2?

Check out page DE4 of the July/August digital edition.



The tricyclic structure of anthocyanins is highlighted in red. R1-R5 are functional groups such as glycosyl, hydroxyl, methyl and acyl units. The general precursor phenylalanine or tyrosine, deriving from the shikimate pathway, enters the phenylpropanoid pathway to provide the intermediate coumaroyl-CoA for the production of flavonoids. Coumaroyl-CoA undergoes condensation with malonyl-CoA to form naringenin chalcone, which undergoes various modifications to form diverse anthocyanin compounds.

Abbreviations for enzymes: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; CPR, cytochrome P450 reductase; TAL, tyrosine ammonia lyase; 4CL, 4-coumaroyl-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5 'H, flavonoid 3', 5'-hydroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; FGT, flavonoid glucosyltransferase; OMT, O-methyltransferase; ACT, anthocyanin acyltransferase

Figure 1. Biosynthesis of anthocyanins in plants

KEY POINTS

- Though the number of incidents has declined, cosmetic products still hold the potential to trigger adverse reactions in users.
- This first of two articles reviews the regulatory status and adverse reporting of contact dermatitis due to cosmetics. Part II takes a closer look at ingredients of concern.

A Dermatological View

Skin in the Game

Contact Dermatitis and Cosmetics, Part I

Modified with permission from: Nanocosmetics: From Ideas to Products

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Women and men worldwide use an abundance of skin care and cosmetic products, in pursuit of cleanliness with soaps and shampoos, or everlasting youth with creams and serums. No matter what age, location or socioeconomic background, we are all exposed to the widespread cosmetics industry.

Growing public concern over the safety of cosmetics has prompted regulatory measures in the past.

The global cosmetic market was worth $460 billion in 2014 and is estimated to reach $675 billion by 2020.[1] While marketing and advertising efforts have forged a path for the industry's continued growth, this momentum has not translated to equally refreshed regulatory practices in some parts of the world.

This first in a two-part series reviews the regulatory status of cosmetics, adverse event reporting and studies attempting to link cosmetics with contact dermatitis. Part II will look at specific ingredients of concern. Together, the two identify gaps for the industry and regulators to close to improve user experiences.

Brief History of Cosmetics Safety

In the past, growing public concern regarding the safety of cosmetics in the 20th century prompted the U.S. Congress to enact the Food, Drug, and Cosmetic Act of 1938 (FDCA). The FDCA brought the cosmetics industry under the general oversight of the U.S. Food and Drug Administration; under this framework, the FDA relies on the public and physicians to alert the agency about problem products.

While select state legislatures have opted for tighter controls over cosmetics, the federal government has not enacted significant new regulatory cosmetic legislation or substantially amended the FDCA.[2] Essentially, since its passing, the FDCA has only defined the term *cosmetic* and prohibited the adulteration or misbranding of cosmetics. In addition, the Fair Packaging and Labeling Act of 1973 established further labeling provisions, while the establishment of the Voluntary Cosmetic Regulation Program (VCRP) helped to direct the Cosmetic Ingredient Review (CIR) program in prioritizing specific ingredient testing.[2]

As of March 2017, the CIR completed safety assessments for 4,740 individual cosmetic ingredients.[3] Of these ingredients, 4,611 were determined to be safe; 12 were deemed unsafe; and insufficient information was available for the remaining 117.[3] However, in cases where an ingredient is considered unsafe, neither the CIR nor the FDA possesses the power to remove cosmetics containing it from the market.[2]

The Research Institute for Fragrance Materials (RIFM) also was established to help evaluate the safety of ingredients in fragrances. Like the CIR, RIFM publishes its ingredient evaluation but has no authority to remove products containing ingredients of concern from the marketplace. There is also no outside review of the primary documents upon which their reports are based. Essentially, as the industry knows, current legislation places the responsibility of determining cosmetic ingredient safety on the manufacturer of the product.

Within this setting, Diane Feinstein, a California senator, recently introduced the Personal Care Products Safety Act (PCPSA), which seeks to give the FDA authority to recall unsafe cosmetics, impose mandatory adverse event reporting for manufacturers, and propose an annual safety review of a minimal five ingredients;[4] (see **Table 1** for regulatory timeline).

Surveillance Data

Adverse reactions to cosmetics can occur, although their reports have decreased over time.[5] Still, some reactions are not being reported,[2] so in order to encourage additional adverse event reporting, the FDA opened its reporting system—the Center for Food Safety

Anti-inflammatory ingredients—such as green tea, cannabis, turmeric and fermented options—are currently en vogue in consumer products.

Source: Global Cosmetic Industry (*www.GCImagazine.com*)

Table 1. Timeline of Laws Enacted and Review Boards Established

Year	Legislation or review board establishment	Description
1938	Food, Drug and Cosmetic Act (FDCA)	Brought the cosmetic industry under the general oversight of the FDA
1966	Research Institute Fragrance Material	Helped evaluate the safety of ingredients in fragrances
1973	Fair Packaging and Labeling Act	Provided labeling provisions, including the cosmetic name; quantity; name and place of business of the manufacturer; ingredients listed in order of predominance; and a warning label for untested ingredients
1976	Cosmetic Ingredient Review	Self-regulation panel of the cosmetic industry.
2017 (proposed)	Personal Care Products Safety Act (PCPSA)	Would give the FDA authority to recall unsafe cosmetics, impose mandatory adverse event reporting for manufacturers, and propose an annual safety review of at least five ingredients.

Table 2. Common Sites of ACD Due to Cosmetics and Their Most Common Allergens

Body Region	Allergen
Face	Tosylamide formaldehyde resin in nail lacquer[17]
Eyelid	Nail lacquer[18]
	p-Phenylenediamine (PPD), ammonium persulfate[19]
	Mascara, eyeliner, eye shadow, false eyelashes and eyelash curler metal[20, 21]
	Shellac[22, 23]
	Gold[24]
Neck	Tosylamide formaldehyde resin in nail lacquer[17]
	Fragrance[25]
Scalp	PPD in hair dyes[19]
Anogenital	Fragrances, preservatives and corticosteroids[26]
	Balsam of Peru, nickel[26–28]
	Spices and flavorings (i.e. nutmeg, peppermint oil, coriander, curry mix, peppermint oil and onion powder)[29–30]

Cosmetics and skin care products may trigger an adverse reaction of the skin, commonly inflammation.

and Applied Nutrition's (CFSAN's) Adverse Event Reporting System—to the public.[4] Since its creation, a spike in adverse events filed with CFSAN has been observed; 436 events were reported in 2014; 706 in 2015; and 1,591 in 2016.[4] The most commonly implicated cosmetic categories include hair and skin care, followed by tattoos.[4] Linda Katz, M.D., director for the Office of Cosmetics and Colors at CFSAN, attributes these increases to a few high-profile cases, as well as increased FDA outreach to consumers and health care professionals to report such adverse events.

The authors' personal experience, at the University of California at San Francisco, is that most adverse reactions—mainly irritant and allergic contact dermatitis—are not reported.

Cosmetics and Contact Dermatitis

As previously mentioned, cosmetic and skin care products may sometimes trigger an adverse reaction, commonly inflammation of the skin. Dermatitis is the general term used for inflammation that causes pruritis and erythema. When dermatitis is caused by exogenous materials coming into direct contact with skin, it is termed *contact dermatitis*.

Contact dermatitis consists of both irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD), with ICD being more common and accounting for a majority of cases.[6–10] While ACD is a type IV hypersensitivity reaction and requires prior sensitization, ICD does not involve the immune system and is not an

It is difficult to estimate the prevalence of allergic contact dermatitis caused by cosmetics but studies have attempted it.

allergy.[11] Considerable overlap exists between the two in clinical, histological and molecular presentation.[11] However, distinction between the two conditions is critical, as allergens should be generally avoided and irritants can often be tolerated in small amounts.

While it is difficult to estimate the prevalence of ACD due to cosmetics in the general population—given the prevalence is most likely underestimated since most individuals discontinue using the product and seek medical advice—studies have attempted to do so. Menkart suggested an adverse reaction to cosmetics occurs approximately once every 13.3 years per person.[12] Another review found the pooled prevalence rate of ACD to cosmetics in seven different studies to be 9.8%.[13]

For a dramatic example, the North American Contact Dermatitis Group (NACDG) patch-tested some 10,061 patients with suspected ACD over seven years—23.8% of these female patients and 17.8% of the male patients reported having at least one allergic patch-test reaction associated with a cosmetic source.[14]

Park and Zippin outlined the frequent sites for cosmetically induced ACD and the most common allergens (see **Table 2**)[15–30] since, for patients with ACD, the cornerstone of management is avoiding the triggering allergen. This has been made more feasible with the Fair Packaging and Labeling Act of 1973, requiring cosmetic ingredients to be listed on packaging. However, there remain challenges in fragrances—a leading cause of contact allergy to cosmetics—as fragrance ingredients are not required to be individually listed.

Formaldehyde avoidance also appears to be difficult. In 2000, Rastogi found that formaldehyde content was incorrectly labeled in 23–33% of products tested.[16]

Finally, in invaluable tool for physicians and patients to manage cosmetically induced ACD is the Contact Allergen Management Program, developed and managed by the American Contact Dermatitis Society. This computerized database contains thousands of cosmetics and personal care products, so after a patient has been patch-tested and the allergen pinpointed, the offending agent may be entered into the database. From this, a list is generated of all the products that are safe for the patient to use, making it easier for patients to avoid trigger agents.

In Part II, scheduled for our November issue, ongoing contact dermatitis research and specific ingredients of concern will be explored.

References

1. *researchandmarkets.com/research/f2lvdg/global_cosmetics*
2. RB Termini and LB Tressler, American Beauty: An Analytical View of the Past and Current Effectiveness of Cosmetic Safety Regulations and Future Direction, *Food and Drug Law Journal* 63(1) 257–274 (2008)
3. IJ Boyer et al, The Cosmetic Ingredient Review Program-Expert Safety Assessments of Cosmetic Ingredients in an Open Forum, *Int J Toxicol* 36(5_suppl2) 5S–13S (2017)
4. M Kwa, LJ Welty and S Xu, Adverse Events Reported to the US Food and Drug Administration for Cosmetics and Personal Care Products, *JAMA Intern Med* 177(8) 1202–1204 (2017)
5. JB Mowry et al, 2015 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 33rd Annual Report, *Clin Toxicol (Phila)* 54(10) 924–1109 (2016)
6. RM Adams and HI Maibach, A five-year study of cosmetic reactions, *J Am Acad Dermatol* 13 (6) 1062–1069 (1985)
7. AC de Groot, Contact allergy to cosmetics: causative ingredients, *Contact Dermatitis* 17(1) 26–34 (1987)
8. HJ Eiermann et al, Prospective study of cosmetic reactions: 1977–1980, North American Contact Dermatitis Group, *J Am Acad Dermatol* 6(5) 909–917 (1982)
9. NH Nielsen and T Menne, Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark, *Acta Derm Venereol* 72(6) 456–460 (1992)
10. C Romaguera et al, Patch tests with allergens related to cosmetics, *Contact Dermatitis* 9(2) 167–168 (1983)
11. J-M Lachapelle, HI Maibach (eds), *Patch Testing and Prick Testing: A Practical Guide Official Publication of the ICDRG*, Springer Science & Business Media, Berlin (2012)
12. J Menkart, An analysis of adverse reactions to cosmetics, *Cutis* 24(6) 599–662 (1979)

13. KA Biebl and EM Warshaw, Allergic contact dermatitis to cosmetics, *Dermatol Clin* 24(2) 215–232, vii (2006)
14. EM Warshaw et al, Allergic patch test reactions associated with cosmetics: retrospective analysis of cross-sectional data from the North American Contact Dermatitis Group, 2001–2004, *J Am Acad Dermatol* 60(1) 23–38 (2009)
15. ME Park and JH Zippin JH. Allergic contact dermatitis to cosmetics, *Dermatol Clin* 32(1) 1–11 (2014)
16. SC Rastogi, Analytical control of preservative labelling on skin creams, *Contact Dermatitis* 43(6) 339–343 (2000)
17. R Lazzarini et al, Frequency and main sites of allergic contact dermatitis caused by nail varnish, *Dermatitis* 19 (6) 319–322 (2008)
18. RL Rietschel et al, Common contact allergens associated with eyelid dermatitis: data from the North American Contact Dermatitis Group 2003–2004 study period, *Dermatitis* 18(2) 78–81 (2007)
19. RL Rietschel and JF Fowler (eds) *Fisher's Contact Dermatitis*, Sixth Edition, Hamilton, BC Decker (2008)
20. F Brandrup, Nickel eyelid dermatitis from an eyelash curler, *Contact Dermatitis* 25(1) 77 (1991)
21. JD Guin, Eyelid dermatitis: experience in 203 cases, *J Am Acad Dermatol* 47(5) 755–765 (2002)
22. CJ Le Coz et al, Allergic contact dermatitis from shellac in mascara, *Contact Dermatitis* 46(3) 149–152 (2002)
23. T Shaw et al, A rare eyelid dermatitis allergen: shellac in a popular mascara, *Dermatitis* 20(6) 341–345 (2009)
24. S Nedorost and A Wagman, Positive patch-test reactions to gold: patients' perception of relevance and the role of titanium dioxide in cosmetics, *Dermatitis* 16(2) 67–70; quiz 55–66 (2005)
25. SE Jacob and MP Castanedo-Tardan, A diagnostic pearl in allergic contact dermatitis to fragrances: the atomizer sign, *Cutis* 82(5) 317–318 (2008)
26. EM Warshaw et al, Anogenital dermatitis in patients referred for patch testing: retrospective analysis of cross-sectional data from the North American Contact Dermatitis Group, 1994–2004, *Arch Dermatol* 144(6) 749–755 (2008)
27. K Bhate et al, Genital contact dermatitis: a retrospective analysis, *Dermatitis* 21(6) 317–320 (2010)
28. K Kugler et al, [Anogenital dermatoses—allergic and irritative causative factors. Analysis of IVDK data and review of the literature], *J Dtsch Dermatol Ges* 3(12) 979–986 (2005)
29. H Vermaat et al, Anogenital allergic contact dermatitis, the role of spices and flavour allergy, *Contact Dermatitis* 59(4) 233–237 (2008)
30. H Vermaat et al, Vulval allergic contact dermatitis due to peppermint oil in herbal tea, *Contact Dermatitis* 58(6) 364–365 (2008)

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KINETIN 激动素

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激动素提取自青玉米和青椰子，能有效平复皮肤老化引起的皱纹。现在激动素作为神奇的抗皱治疗成分被广泛地使用在化妆品中。

DMAE 二甲氨基乙醇

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二甲氨基乙醇是抗氧化剂膜稳定剂，可增强其它抗氧化剂的效果。随着年龄增长，人体内制造的乙酰胆碱数量减少，导致皮肤下垂，起皱。将二甲氨基乙醇用于皮肤护理，几分钟就能见效，皮肤紧绷能持续一段时间，还可以减少色素沉着和皱纹。

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Lonicera Caprifolium (Honeysuckle) Flower Extract
Lonicera Japonica (Honeysuckle) Flower Extract
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Dark Ultra Brilliant Yellow 亮黄 Yellow Light 浅黄
Dark Black 黑

LIPO & OIL SOLUBLE LIQUIDS 脂/油溶性液体
Brown 棕 Red 红 Natural Yellow 纯黄 Green 绿
Red Light / Scarlet Red 鲜红 Ultra Sky Blue 天蓝
Dark Black 黑

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KEY POINTS

- This two-part article provides an overview of areas relevant to the U.S. Food and Drug Administration's (FDA's) inspection of cosmetic microbiology laboratories.
- Part 1 covered personnel, facilities and equipment, test materials and procedures, and documentation. Part 2 addresses microbial cultures and growth media, biochemical reagents, test procedures and more.

Didn't Catch Part I?

Part I of this series appeared in the May 2018 issue of Cosmetics & Toiletries. *Find it in your digital edition archive (click on "Back Issues") or online at:*

www.cosmeticsandtoiletries.com/magazine/pastissues/2018/

UPON Inspection

Part II

Is Your Microbiology Lab FDA-compliant?

Cultures, Growth Media, Reagents and Testing

Donald J. English, *Avon Products, Inc.*
Mark Entrup, *Kao USA, Inc.*
Joanne Nikitakis, *Personal Care Products Council (PCPC)*
John F. Krowka, *formerly PCPC (retired) and Kensho Farms, Boonsboro, MD USA*

This second in a two-part series provides an overview of some important areas relevant to preparing for the U.S. Food and Drug Administration's (FDA's) inspection of cosmetic microbiology laboratories. It includes discussions on microbial culture maintenance, growth media, biochemical reagents, test procedures, documentation and investigations. Part 1 addressed personnel, facilities and equipment, test materials and procedures and documentation.[1]

The microbiology quality control laboratory performs a critical function for cosmetic product manufacturing in safeguarding the quality, integrity and safety of finished products. The importance of preparation for an inspection cannot be underestimated.

Each lot of prepared growth media from vendors should be re-checked for pH, sterility and growth-promotion to ensure no changes occurred during shipment.

Understanding the FDA's expectations and preparing your facility accordingly will demonstrate good management practices. In a cosmetic manufacturing facility, the inspector is entitled to inspect all pertinent manufacturing and filling equipment, finished and unfinished materials, storage containers, labeling materials and personnel qualification and training records associated with the manufacture of the product.[2]

Culture Maintenance

Microbial cultures are used within microbiology laboratories to perform certain important tasks, including: promoting microbial media growth; acting as positive and negative controls for biochemical diagnostic tests; testing biochemical identification kits and cards for quality control; and validating microbial test methods. In general, it is common practice to limit the use of microbial cultures to no more than five passages or transfers from an original American Tissue Culture Collection (ATCC) vial. The purpose for this limitation is to prevent phenotypic drift in the appearance of microbial growth for a culture. In addition, proper documentation should be kept to confirm the identity of the used stock cultures.

Across categories, the global microbiology test market is forecast to reach US $5.77 billion by 2021, with an 11.5% CAGR from 2016. Instruments account for the largest share, although reagents are expected to grow faster.

Source: MarketsandMarkets

Microbial Growth Media

In-house, laboratory-prepared media: For each received lot of dehydrated microbial growth media, e.g., agar and enrichment broth, the "Date Received" and "Date Opened" should be marked on all containers. Furthermore, it is recommended that microbial growth media be rotated so the oldest container is used first—i.e., first in, first out or FIFO. This practice helps to reduce the expiration of microbial growth media, which cannot be used. In addition, microbial growth media, supplements and additives should be stored according to the manufacturer's directions.

During media preparation, supplements and additives should be added at the correct temperature to avoid their chemical degradation or destruction. Each batch of microbial growth media prepared in-house should be rehydrated with the proper type of water, e.g., distilled, deionized or the equivalent. In addition, each batch of microbial growth media must have an individual batch record including: the name of the media, date of preparation, manufacturer's lot number, quantity of each ingredient weighed, quantity prepared, signature of the preparer, volume dispensed and the number of units dispensed. This information should be reviewed for accuracy and signed by the laboratory supervisor.

After sterilization, each prepared batch of microbial growth media should be checked for pH, sterility and microbial growth promotion before being used in microbial testing. Laboratory procedures must list the acceptable pH ranges and methods for testing both pH and sterility for each in-house prepared microbial growth medium.

Finally, each prepared batch of microbial growth media should be tested for its ability to recover the presence of microorganisms in a test sample. This is achieved by inoculating the

medium with appropriate types of microorganisms at a level less than 100 colony-forming units (CFU). If a selective/differential microbial growth media is used within a laboratory, the microorganisms to be inhibited during actual testing should be included in the growth-promotion testing in order to provide accurate and characteristic biochemical reactions.

Vendor-prepared media: Each lot of prepared microbial growth media received from an outside vendor should have a Certificate of Analysis, including the following information: manufacturer's name and address of preparation facility, pH check, sterility check and microbial growth-promotion test data. However, it is recommended that each lot of prepared microbial growth media received from the outside vendor be re-checked for pH, sterility and microbial growth-promotion to ensure the media was not adversely affected during shipment.

Storage and expiration dating: Prepared microbial growth media must be stored under the appropriate conditions. This includes protection from light exposure—if it is sensitive to light—and storage at the appropriate temperature, per the manufacturer's direction. Expiration dates for laboratory-prepared media also should be established by performing growth-promotion testing on aged lots.

Microbial count diluents: Microbial count diluents are used to prepare serial dilutions of a test sample to measure a microbial count per gram or milliliter. Microbial count diluents often contain ingredients to neutralize the antimicrobial activity of preservatives present in the test samples. The neutralizing activity of the microbial count diluents should be demonstrated and documented prior to conducting the microbial testing of samples.

As with microbial growth media, microbial count diluents are prepared in-house and each must have an individual batch record indicating: their name, date of preparation, manufacturer's lot number of ingredients, quantity of each ingredient weighed, quantity

prepared, signature of the preparer, volume dispensed and number of units dispensed. Each record should be reviewed for accuracy and signed by a second laboratory individual. Furthermore, each batch should be checked for microbial sterility after sterilization and before being used in microbial testing.

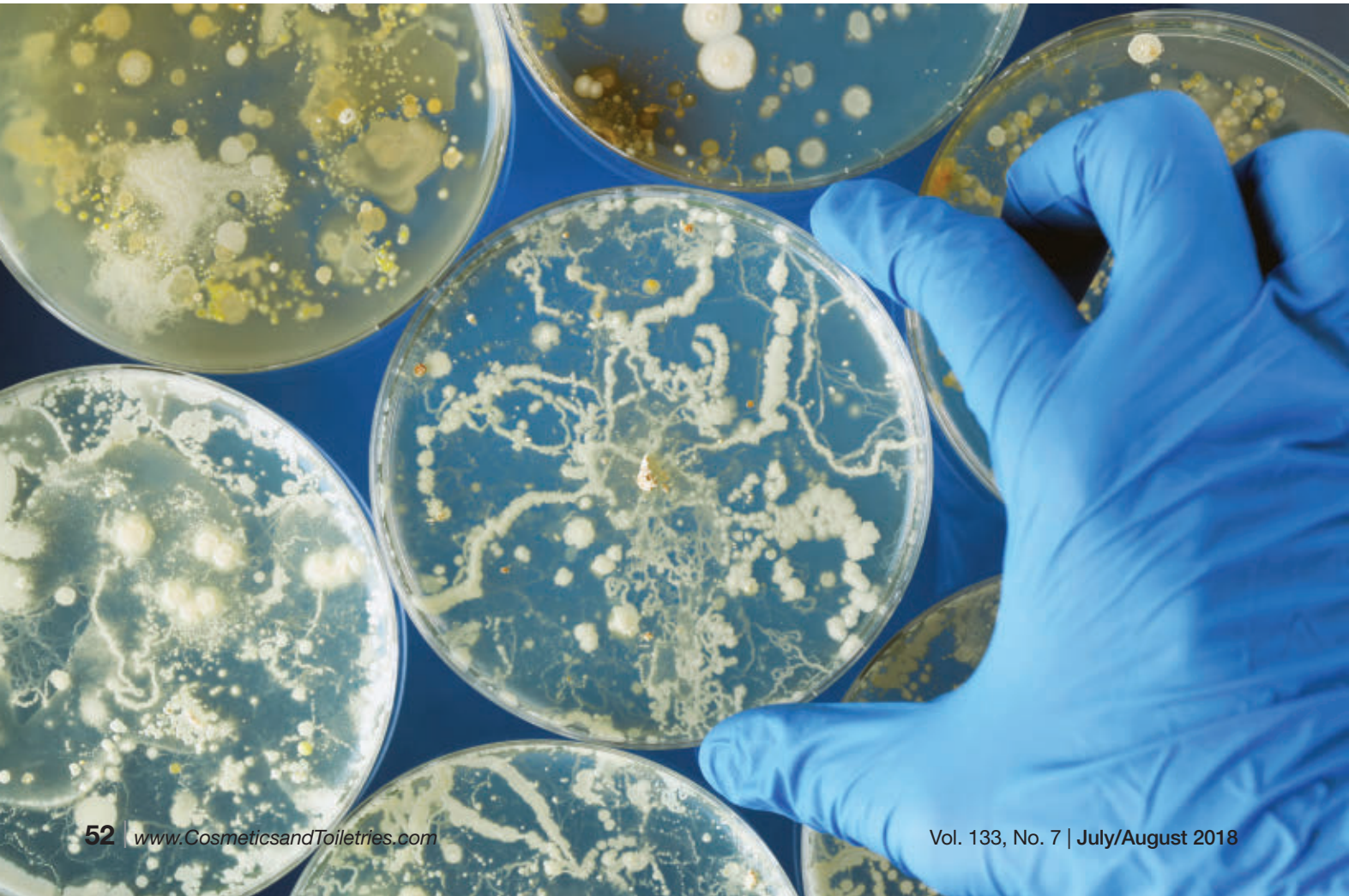
Reagents, Chemicals and Identification Kits/Cards

Chemicals and biochemical reagents such as Gram stain, oxidase, coagulase, catalase, etc., should be checked for expiration prior to their use and, if expired, be discarded. The specificity of each new lot of biochemical reagent also should be checked prior to or at the time of use with an unknown microbial isolate by using positive and negative microbial test controls.

Furthermore, each lot of biochemical identification cards or kits used to identify microbial isolates must be quality control-tested per the manufacturer's recommended quality control test microorganisms before use in order to verify the kits or cards were not adversely affected during shipping. Also, if 16S and 23S rRNA sequencing is performed on microbial isolates for identification purposes, it is recommended that each lot used for extraction and sequencing rRNA be tested on bacterial, yeast and mold reference strains to verify the reagents were not compromised during shipping. If biochemical reagents or identification cards/kits are unable to provide the necessary biochemical reactions for each isolate, they should not be used for routine microbial testing.

Microbiological Test Methods

To test the microbial bioburden of a raw ingredient or finished product, most cosmetic companies use in-house methods based upon those outlined either in the Microbial Limits Test Section 61 in the *U.S. Pharmacopeia*,[3] PCPC M-1 and M-2 guideline methods,[4] or from ISO. However, each of the microbial content



test methods used should be validated and documented to ensure accuracy of the results. In general, microbial content test methods are validated by spiking test samples with several types of microbial species at levels less than 100 CFU/gram to demonstrate the method can recover a low level of microorganisms.

For enrichment testing of cosmetic products, samples are inoculated with less than 100 CFU of the following microorganisms in an enrichment broth containing preservative neutralizers: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Burkholderia cepacia* ATCC 25416. After inoculation with a test microorganism, the enrichment broth containers with product are incubated. After incubation, the

The microbiology quality control laboratory performs a critical function for cosmetic product manufacturing.

recovery of each spiked test microorganism in the incubated enrichment broth is verified by streaking an aliquot of the broth onto appropriate selective/differential microbial growth agars and/or a general purpose microbial growth agar such as Soybean-Casein Digest Agar Medium. It is recommended the recovered microbial isolates be Gram-stained and biochemically identified to confirm the actual isolation of each spiked test microorganism.

For validating the microbial enumeration method for a test sample, the above-named microbial species can be used, as well as *Aspergillus brasiliensis* ATCC 16404. Here, each test microorganism is separately inoculated in a test tube or container of a microbial count diluent with preservative neutralizer and either a 1.0- or 10-g test sample; and a test tube or container with only the microbial count diluent with a preservative neutralizer in which the final concentration of test microorganisms is less than 100 CFU/milliliter.

Next, one milliliter aliquots from these dilutions are aseptically removed, plated and incubated. After incubation, microbial counts of the diluents with and without the sample are compared. The enumeration test method is considered to be validated if there is no more than a $\pm 0.5_{log}$ difference between the two microbial counts or if the difference in counts is not less a 70% for each test microorganism.

Documentation

The purpose of documentation in a microbiology laboratory is to provide a written record of procedures—as well as the activities—that

have occurred. For example, the operational history of a laboratory is usually maintained through the retention of documents such as logbooks, worksheets, calibration records, etc. However, any laboratory function performed must be described in the laboratory procedures, which should be reviewed and updated when appropriate.

Forms used to document test results should be periodically reviewed and updated to reflect current practices, as should written standard operating procedures and test methods. Procedures for electronic data recording and data integrity assessments should be developed, implemented and periodically checked.

Investigations and Discrepancies

Each laboratory should keep a formalized investigation procedure in place in order to manage the occurrence of finished products that register microbial test results that are out-of-specification (OOS). The purpose of this is to establish the root causes for test failures and implement corrective actions to prevent them in the future.

The procedure should include complete details related to the process, findings and results, and should be supported by adequate scientific rationale. Any discrepancies in the equipment or microbial test results for raw ingredients, finished products and environmental test samples should be investigated, documented and evaluated by the appropriate personnel.

Conclusions

In addition to the practices described in Part 1 of this series,[1] procedures for the maintenance of microbial cultures, and testing and storage of microbial growth media and diluents should be developed, implemented and documented. Finally, formalized procedures for investigations of discrepancies and OOS test results must be documented. These preparations for FDA inspections are necessary to maintain the quality of products and ensure that costly violations do not occur.

References

1. DJ English, M Entrup, JM Nikitakis and JF Krowka, Is your microbiology lab FDA-compliant? Upon inspection, part 1: Personnel, facilities and equipment, *Cosm & Toil* 133(50) 24 (May 2018)
2. JM Nikitakis, ed, FDA inspections, *Personal Care Products Council Quality Assurance Guidelines*, The Personal Care Products Council, Washington, DC (2014)
3. 341-National Formulary-364, Microbiological best laboratory practices <1117>, *U.S. Pharmacopeia*, United States Pharmacopeia Convention, Inc., Rockville, MD (2017)
4. *http://uscode.house.gov/view.xhtml?req=(title:21%20section:361%20edition:prelim)* (accessed May 24, 2018)

Additional Reading

JF Krowka and BA Jonas, eds, Microbial validation and documentation, *Personal Care Products Council Microbiology Guidelines,* The Personal Care Products Council, Washington, DC (2016)

JF Krowka, and BA Jonas, eds, Microbiology laboratory audit, *Personal Care Products Council Microbiology Guidelines,* The Personal Care Products Council, Washington, DC (2016)

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KEY POINTS

- While cosmetics represent a small source of primary and secondary microplastics in the environment, it is a source that could easily be avoided by using natural biodegradable alternatives.
- As such, described herein is a research project to identify a natural, biodegradable alternative for peel applications. Plant cellulose was of particular interest.

Replacing Microplastics

Natural Cellulose Offers Gentle and Biodegradable Exfoliation

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We live in a time when our oceans are littered with plastics. According to the literature, as much as three times more plastic than fish may be in our oceans by 2050.[1–4] Microfine plastic particles find their way back via wastewater to the sea and into our food chain. This occurs because, with a size of less than 5 mm, the particles are too small for effective removal by wastewater treatment plants. They are almost non-biodegradable and can be harmful to marine life.

The hazard potential of microplastics is mainly characterized by the following concerns.

First, microplastics can attract environmental toxins due to their surface characteristics. Throughout their lifetime, plastic particles' surfaces become rougher, potentially acting as magnets for other harmful substances present in the environment.[5] If these toxins and microplastic particles are ingested by sea organisms and released into their gastro-intestinal systems, the organisms may experience negative effects such as tissue changes, inflammatory reactions, toxicological impacts, internal injuries and even death.[6] Secondly, the morphological characteristics of the particles themselves may pose threats.[7]

Based on these risks and consumer concern, the use of microplastic particles, i.e., microbeads, in personal care products has

There are still significant scientific challenges to finding replacements for microplastic particles.

been prohibited in the United States as of January 2018. The British government also banned personal care products containing microplastics as of January 2018, and several additional countries in the EU are discussing similar bans.

While efforts are being made to phase them out, microplastics are still used in some cosmetic products; most commonly exfoliants. As soft abrasives, these formulas help to remove dead skin cells and stimulate blood circulation. In addition, microplastics are used in some countries as abrasive particles for dental care.[8]

It is true that cosmetics represent a small source of primary and secondary microplastics in the environment, compared with other sources. One report showed 4,300 tons of microplastic particles were used in EU personal care products in 2012.[9] However, it is a source that could easily be avoided using natural or biodegradable alternatives.

Yet, there are still significant scientific challenges to finding replacements for microplastic particles. Alternatives must satisfy requirements for cleansing effects but also be biodegradable and available at moderate costs. Several attempts have been made to replace plastic microbeads using natural particles such as hydrogenated waxes, silica, sugar, salt and crushed plant stones or nut shells.[10] However, their scientific features and effects have room for improvement.

As such, described herein is a research project to identify a natural, biodegradable alternative with good efficacy. The first objective was to define the criteria for the desired characteristics, to produce optimal replacements for specific microplastic particles. This included finding the ideal size, shape, hardness and surface morphologies, to obtain the desired characteristics without causing skin irritation.

From this information, biodegradable cellulose particles were derived from various sources and modified using a milling and grinding technology. Their microstructures were analyzed, and their abrasion and cleaning effects were tested for applicability in facial exfoliants and toothpastes. This article presents the results of these tests in relation to facial exfoliants.

The global exfoliators and scrubs market is projected to grow at a CAGR of 4.68% between 2016 and 2020.

Source: ResearchandMarkets

Material and Methods

Natural cellulose and reference particles: Generally, the cellulose particles used in this study[a-c] were made from organic and renewable raw materials. Natural cellulose particles vary in terms of their particle shape and size, which enables different functionalities in line with the specific targets of cosmetic applications. Cellulose from different natural sources was tested, including beech[a], oat[b] and wheat[c]. Reference particles including silica and polyethylene were used for comparison.

Exfoliant formulas: In vitro measurements for abrasion and cleansing effects were performed with a water-based exfoliant formula (see **Formula 1**). As noted, cellulose particles were compared with polyethylene and standard silica abrasives in identical formulations, each including a different particle at 3%. Two market references containing polyethylene and silica also were used as benchmarks.

Morphological characterization of cellulose particles: Characterization of the particles was carried out by scanning electron microscopy (SEM)[d]. To avoid charging effects, the particles were coated with an ultra-thin platinum film by magnetron sputtering.

[a] *Sensocel 100G and 200G globular beech cellulose particles,*
[b] *Sensocel oc 30G globular oat cellulose particles and*
[c] *Sensocel wc 200G globular wheat cellulose particles are products of CFF GmbH & Co. KG*

Validation of biodegradability: Biodegradability was verified according to DIN EN 14851 under fresh water conditions. This is accomplished by determining the ultimate aerobic biodegradability in an aqueous medium by measuring the oxygen demand in a closed respirometer. Cellulose particles were tested in comparison with polyethylene beads and standard silica of similar particle sizes and shapes. Silica was included as a reference as it became established as one of the most-used natural alternatives to polyethylene in exfoliants.

Abrasion and cleansing efficacy tests: Tests to determine abrasion and cleansing efficacy were performed in a brushing simulator[e] at a pressure of 150 g with a 1 cm hub. Mechanical abrasion was evaluated on a glass substrate (25 × 25 mm^2) coated with a 1-µm layer of chrome. This coating was deemed to be the most reproducible variant in pre-tests. The substrates were fixed on a sample holder (75 × 25 mm^2) by double-sided adhesive tape.

Before brushing, the coating was briefly wetted with water under standardized conditions.One gram of the exfoliant formulation was applied to the sample holder. The slurry was renewed after 30 min. The brushing unit[e] was equipped with a silicone layer onto which a synthetic leather[f] surface (25 × 25 mm^2) was fixed to simulate skin. Abrasiveness was determined by colorimetric measurements[g] (ΔE) on the glass substrate as well as by visual comparison prior to and after brushing for 10,000 strokes.

Analysis of the cleansing effects also was made on synthetic skin leather[f] (75 × 25 mm) as the substrate, fixed on a sample holder by double-sided adhesive tape, to which 0.1 g of makeup[h] was applied and distributed homogenously with a squeegee (15 µm gap).

Measurements were performed 24 hr after application of the makeup. One gram of exfoliant formulation and 1 mL of water were applied onto the synthetic skin leather in the sample holder. The brushing unit was equipped with a makeup sponge fixed on a silicone layer. The number of strokes was adapted to 10. Evaluations determining cleansing performance were made in accordance with those determining abrasion.

Colorimetric data for the different treatments were compared using one-way analysis of variance (ANOVA) and the Tukey HSD-test.

Results: SEM Characterization

Depending on the choice of materials as well as milling and sieving process during manufacturing, cellulose particles of different sizes (20–800 µm) and shapes (globular and fibrous) can be generated. **Figure 1** shows select SEM images of representative cellulose particles and the reference particles (silica and polyethylene). The polyethylene particles investigated had a size of 50-100 µm, whereas the silica varied between 50 µm and 80 µm.

Results: Biodegradability

The results of biodegradability tests are depicted in **Figure 2**, which show the distribu-

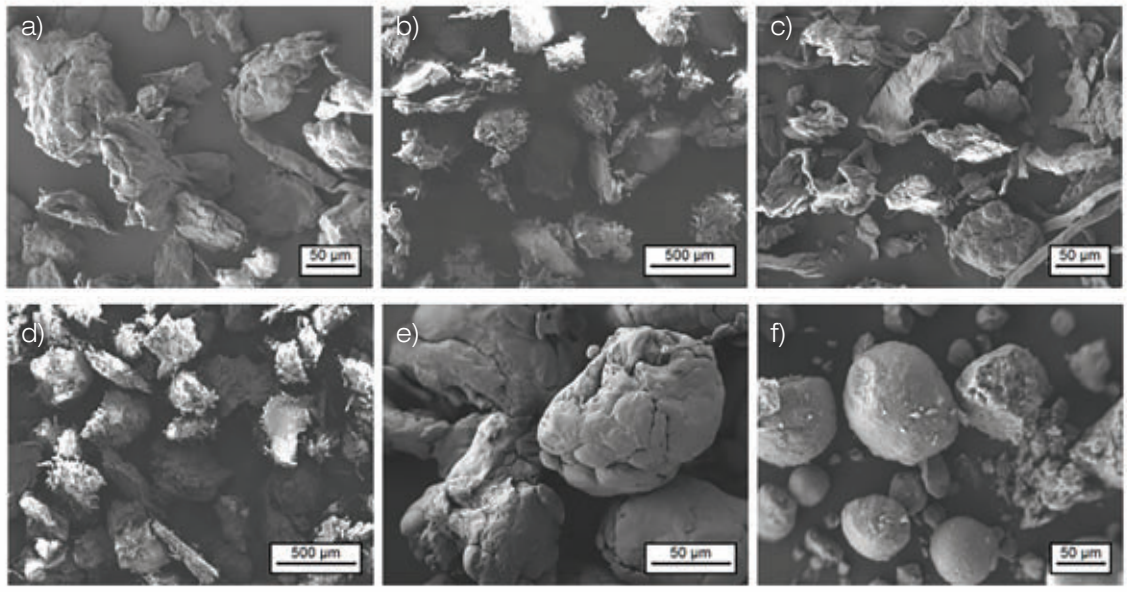
[h] *Perfect Stay Foundation 24 hr + Perfect Skin Primer, Astor*

Formula 1. Exfoliant Cleanser Formula

A. Water (*aqua*)	64.30% w/w
Xanthan Gum	1.10
Glycerine	4.00
B. Decyl Glucoside	13.00
Coco Glucoside	3.00
Cocoamidopropyl Betaine	7.00
Methyl Gluceth-10	3.00
Sodium Benzoate	1.50
Cleansing particles	3.00
C. Fragrance (*parfum*)	0.10
Citric Acid, 50%	qs
	100.00

Procedure: Suspend xanthan gum in glycerine. Add water at 50°C and stir with blade agitator until homogenous. Wait 30-60 min until swelling is complete. Homogenize if necessary. Add B to A in order, stirring until homogenous. Adjust pH value with C to 5.8-6.1 (~ 545 µL ad. 100 g).

[d] *Quanta 3D FEG, FEI*
[e] *ZM-3.8, SD-Mechatronic*
[f] *TE610, Hornschuch*
[g] *Spectrophotometer CM-3600A, Konica Minolta*



a) Globular beech cellulose particles (100 µm); b) globular beech cellulose particles (300 µm); c) globular oat cellulose particles; d) globular wheat cellulose particles; e) polyethylene (PE); and f) silica

Figure 1. SEM image of cellulose particles, polyethylene and silica from beech wood

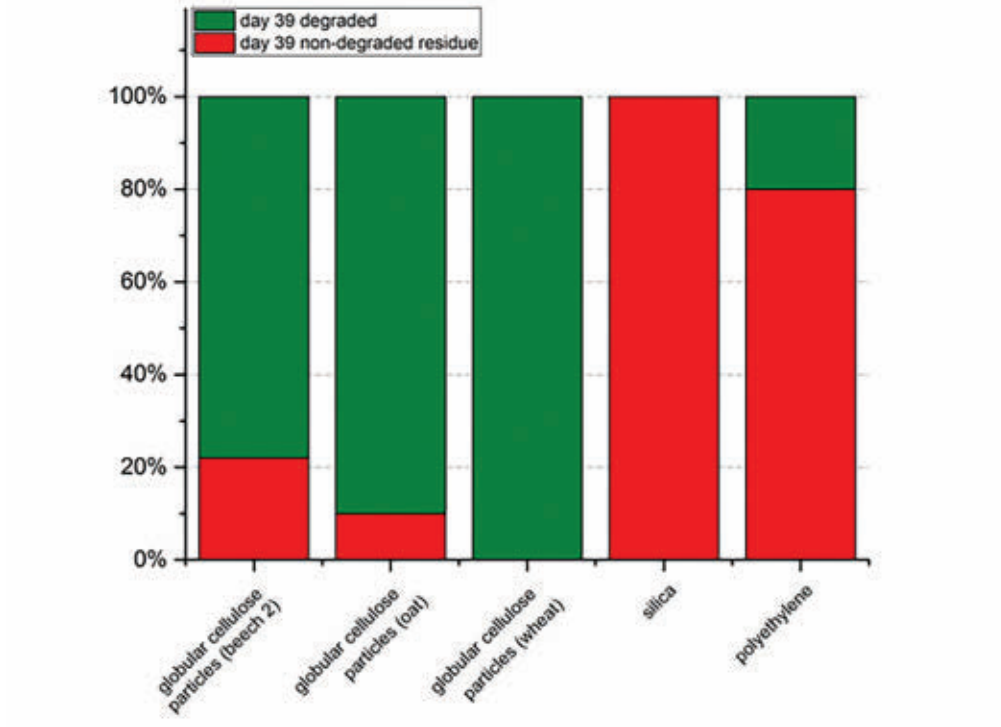


Figure 2. Biodegradability according to DIN EN 14851 of cellulose particles with varying shape from different origin, in comparison with silica and PE

Attempts have been made to replace plastic microbeads using hydrogenated waxes, silica, sugar, salt and crushed plant stones or nut shells.

tion of the non-degraded residue and degraded parts of the particles after a time of 39 days. Depending on the origin and shape of the cellulose particle, its biodegradability ranged between 78–100% after 1–2 months. While cellulose will always degrade at 100%,[11] PE particles are non-biodegradable[12] and silica does not degrade due to its mineral origin.

Results: Abrasion Efficacy

The results for abrasion are shown in **Figure 3**. All tested cellulose particles showed significantly lower abrasion in comparison with silica ($p < 0.001$). The abrasion behavior of the cellulose particles was comparable to polyethylene particles and to commercial product 1, which also contained polyethylene ($p > 0.001$). The most abrasive sample was commercial product 2, containing silica.

The measurements also showed that different cellulose particle sizes produced different exfoliation levels. The combination of two particles with different sizes, notably globular beech cellulose at 100 µm and 300 µm, resulted in higher abrasion, in comparison with the individual abrasiveness of each of these particles.

Results: Cleansing Efficacy

The results for cleansing efficacy are represented in **Figure 4**, which are confirmed by

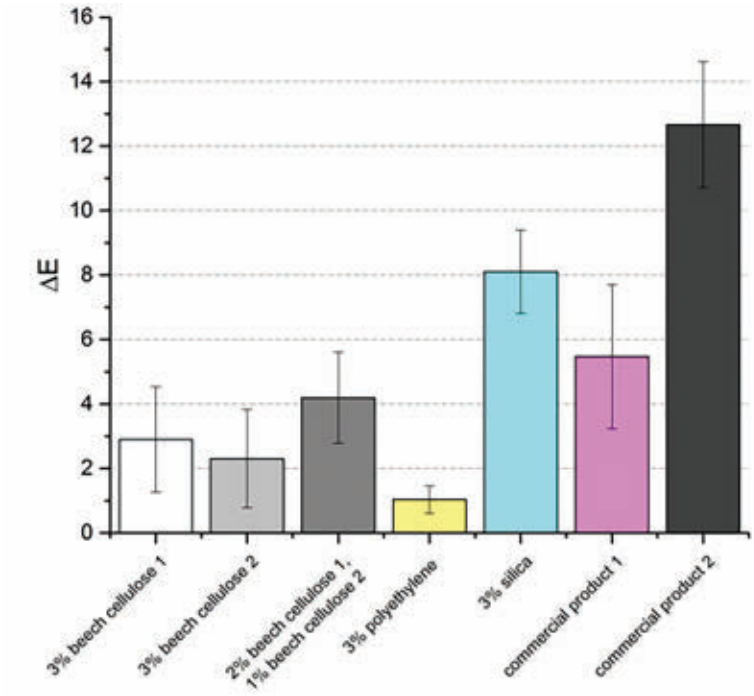


Figure 3. Abrasion of different cleansing bodies measured by change in color (ΔE)

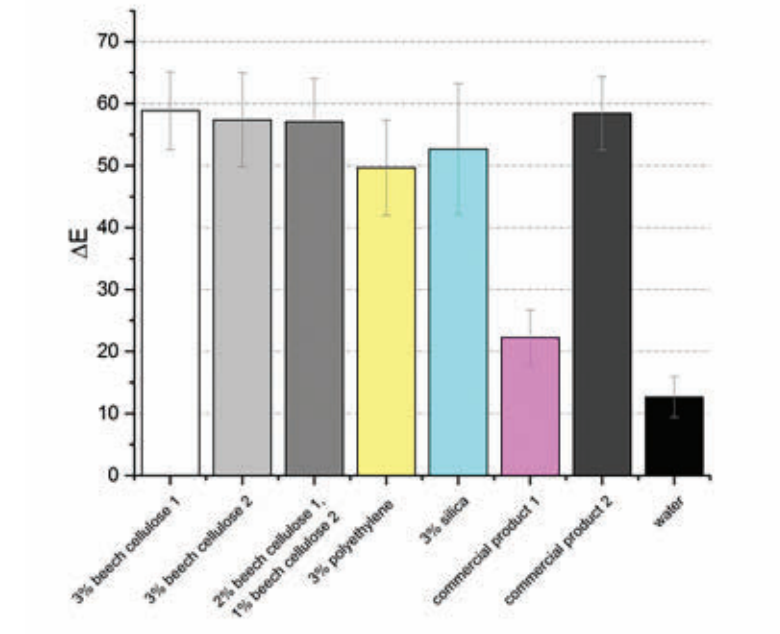


Figure 4. Cleaning efficacy (%) of different cleansing bodies

the visual comparison in **Figure 5**. Here, cellulose particles had a cleansing effect in cosmetic products that was comparable to that of polyethylene or silica. Significantly lower cleansing efficacy was found for commercial product 1 and water.

Discussion

The results for abrasion and cleaning efficacy indicate that biodegradable cellulose represents an ingredient with optimal characteristics for dermal cleansing particles. Cellulose showed very low abrasion on the skin and a mild, gentle cleansing effect. In addition, cleansing effects matched those of low abrasive polyethylene.

While standard silica shows good cleansing effects, its high abrasion must be considered. This effect is of particular concern as the effects of pollution and its impact on skin are being elucidated.

Summary

This study aimed to identify, optimize and analyze natural cellulose particles for their use as environmentally friendly, biodegradable alternatives to microplastics in cosmetics and personal care products. The results indicate cellulose scrubs offer optimal characteristics including low abrasion with high cleansing efficacy. Thus, cellulose represents a mild, patch-test

approved and non-irritating alternative to other natural scrubs and shows 100% biodegradability.

Acknowledgements: Funding by Project Management Jülich (PTJ) and German Federal Ministry of Education and Research (BMBF), project#: 031B0041A, 031B0041B, 031B0041C is greatly acknowledged. We would also like to thank Skinomics GmbH from Halle (Saale), who was mainly responsible for developing the exfoliant formulations as well for the galenic and dermatological testing of formulations.

References

1. M Eriksen et al, Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea, *PLoS One* 9(12) (2014) doi: 10.1371/journal.pone.0111913
2. KL Law et al, Distribution of surface plastic debris in the eastern Pacific Ocean from an 11-year data set, *Environ Sci Technol* 48(9) 4732–4738 (2014) doi: 10.1021/es4053076
3. AL Andrady et al, Microplastics in the marine environment, *Mar Pollut Bull* 62(8) (2011) pp 1596-605 doi: 10.1016/j.marpolbul.2011.05.030
4. L Lechner et al, The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river, *Facets* 188 (2014) pp 177-181
5. M Llorca et al, Adsorption of perfluoroalkyl substances on microplastics under environmental conditions, *Envt Pollution* 235 (2018) 680–691
6. MA Browne et al, Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity, *Current Biol* 23 (2013) 2388-2392
7. SL Wright et al, The physical impacts of microplastics on marine organisms: A review, *Envt Pollution* 178 (2013) 483-492
8. T Gouin et al, Use of micro-plastic beads in cosmetic products in Europe and their estimated emissions to the North Sea environment, *SÖFW* 3 (2015)
9. *http://mcc.jrc.ec.europa.eu/document.py?code=201606243248&title=Study%20to%20support%20the%20development%20of%20measures%20to%20combat%20a%20range%20of%20marine%20litter%20sources* (Accessed Jun 7, 2018)
10. R Mantilla and S Venkataraman, Feeling the power of nature, *COSSMA* 7–8 (2017) pp 13-14
11. J Pérez et al, Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview, *Int Microbiol* 5 53–63 (2002) doi 10.1007/s10123-002-0062-3
12. Y Tokiwa et al, Biodegradability of plastics, *Int J Mol Sci* 10(9) 3722–3742 (2009)

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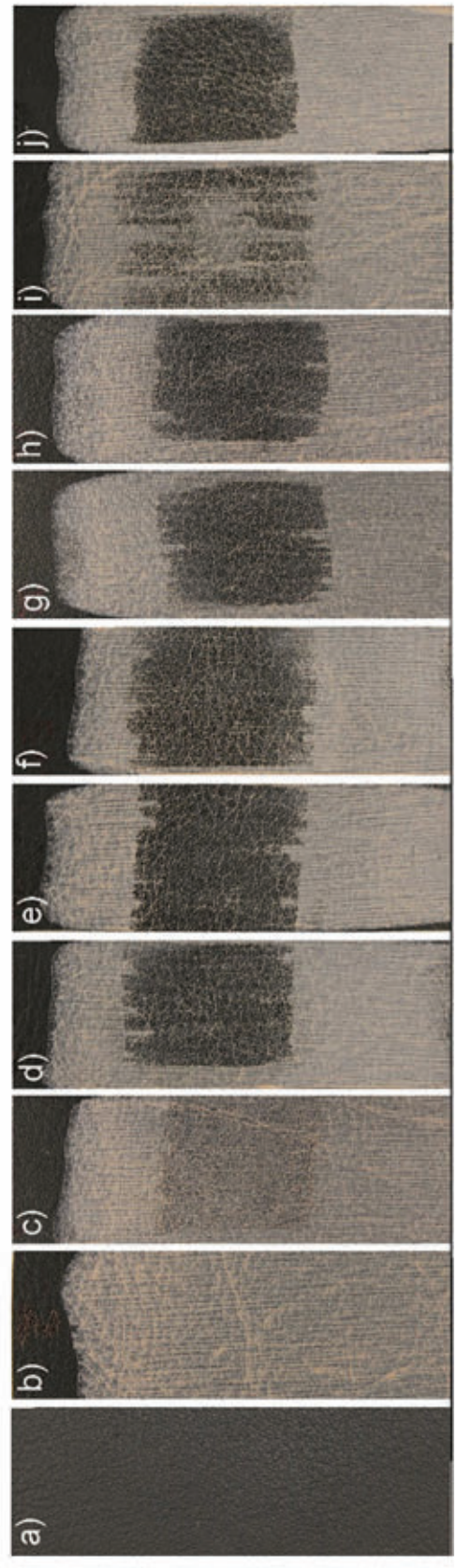

Figure 5. Visual comparison of the cleaning results on synthetic skin leather: a) initially; b) with makeup; c) with water; d) with 3% beech cellulose 1; e) with 3% beech cellulose 2; f) with 2% beech cellulose 1 + 1% beech cellulose 2; g) with 3% polyethylene; h) with 3% silica; i) with commercial product 1 and polyethylene; and j) with commercial product 2 including silica

KEY POINTS

- The need to prevent skin from UV damage is well-understood but the most effective way to achieve this is not.
- In Part I (see April 2018 issue), a novel photostabilizer was presented. Here, its efficacy and biodegradability are reported.

Didn't Catch Part I?

Part I of this series appeared in the April 2018 issue of Cosmetics & Toiletries. *Find it in your digital edition archive (click on "Back Issues") or online at:*

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Photostability Leads the Way

Formulating Safer Sunscreens

Part II: Results

Ratan K. Chaudhuri and Zoia Lascu
Sytheon Ltd., Boonton, NJ USA
Francois Marchio
Sytheon SARL, Boulogne Billancourt, France

Continued from Page 64 of C&T's April issue

As stated, the key focus in developing sunscreen products is to select a combination of UV filters that delivers the intended broad-spectrum protection with maximum efficiency. Consequently, photostability is imperative in order for the product to achieve and maintain maximum efficiency, as it has a significant effect on the SPF—particularly for the UVA region (320–400 nm).

In this work, the photostabilizer and in vivo SPF booster TMBP[28] was evaluated in sunscreen sprays containing two UVB absorbers, HMS and OS, and the UVA absorber avobenzone. A key advantage to using ethanol in the spray sunscreen is avobenzone's aforementioned stability in it;[1] furthermore, this system requires no preservation.

The efficacy of a sunscreen is

UVA-irradiated fibroblasts revealed ROS continued to increase 1 min to 6 min post-irradiation, suggesting a cascade or enzyme-mediated effect.

defined by two parameters: the SPF and UVA protection parameter. The SPF is provided by in vivo testing in a panel of human subjects, although different protocols for determining SPF are used in different countries/regions. And indeed, differences in the details of the SPF test method can make significant differences in the results. For example, the U.S. protocol tends to overestimate the SPF number versus the protocol used in Europe. The present authors used the U.S. Food and Drug Administration (FDA) protocol for determining the in vivo SPF of the test products.

Results: SPF and Critical Wavelength

The in vivo SPF and critical wavelength of variations of **Formula 1** are summarized in **Table 3**. Levels of avobenzone at 3% and TMBP at 2.5%, along with HMS at 10% and OS at 5%, were required to obtain an in vivo SPF > 50. This formulation was very effective as it provided an SPF of 56 with only 18% sunscreen, meaning an SPF yield of 3.1 to percentage of sunscreen. Also, the critical wavelengths of all four formulations were > 375 nm and hence qualified as broad-spectrum sunscreens.

Formula 1. Broad Spectrum Test Sunscreen Pump Spray

A.	Ethanol	40.00% w/w
	VA/Butyl Maleate/Isobornyl Acrylate Copolymer (Advantage Plus, Ashland)	2.00
B.	Avobenzone (Eusolex 9020, EMD)	3.00
	Trimethoxybenzylidene Pentanedione (TMBP) (Synoxyl HSS, Sytheon)*	2.50
	Homosalate (Eusolex HMS, EMD)	10.00
	Octisalate (Eusolex OS, EMD)	5.00
	Diisopropyl Adipate (Dermol DIA, Alzo)	10.00
C.	Phenethyl Benzoate (X-tend 226, Ashland)	20.50
	C12-15 Alkyl Benzoate (Finsolv TN, Innospec)	5.00
	Isosorbide Dicaprylate (HydraSynol DOI, Sytheon)	2.00
		100.00

**TMBP was replaced with OCR, EMC or DESM; Procedure: Combine A. Pre-mix B and heat to 75°C until completely free of solids. Add A to C, then AC to B at 50°C with continued mixing.*

Table 3. Comparison of In vivo SPF and Critical Wavelength of Test Formulations

Spray Sunscreen	No stabilizer	TMBP	OCR	EMC	DESM
In vivo SPF	32	56	40	43	42
% In vivo SPF boosting	-	75%	25%	34%	31%
Critical wavelength in nm	Not determined	376	377	378	377

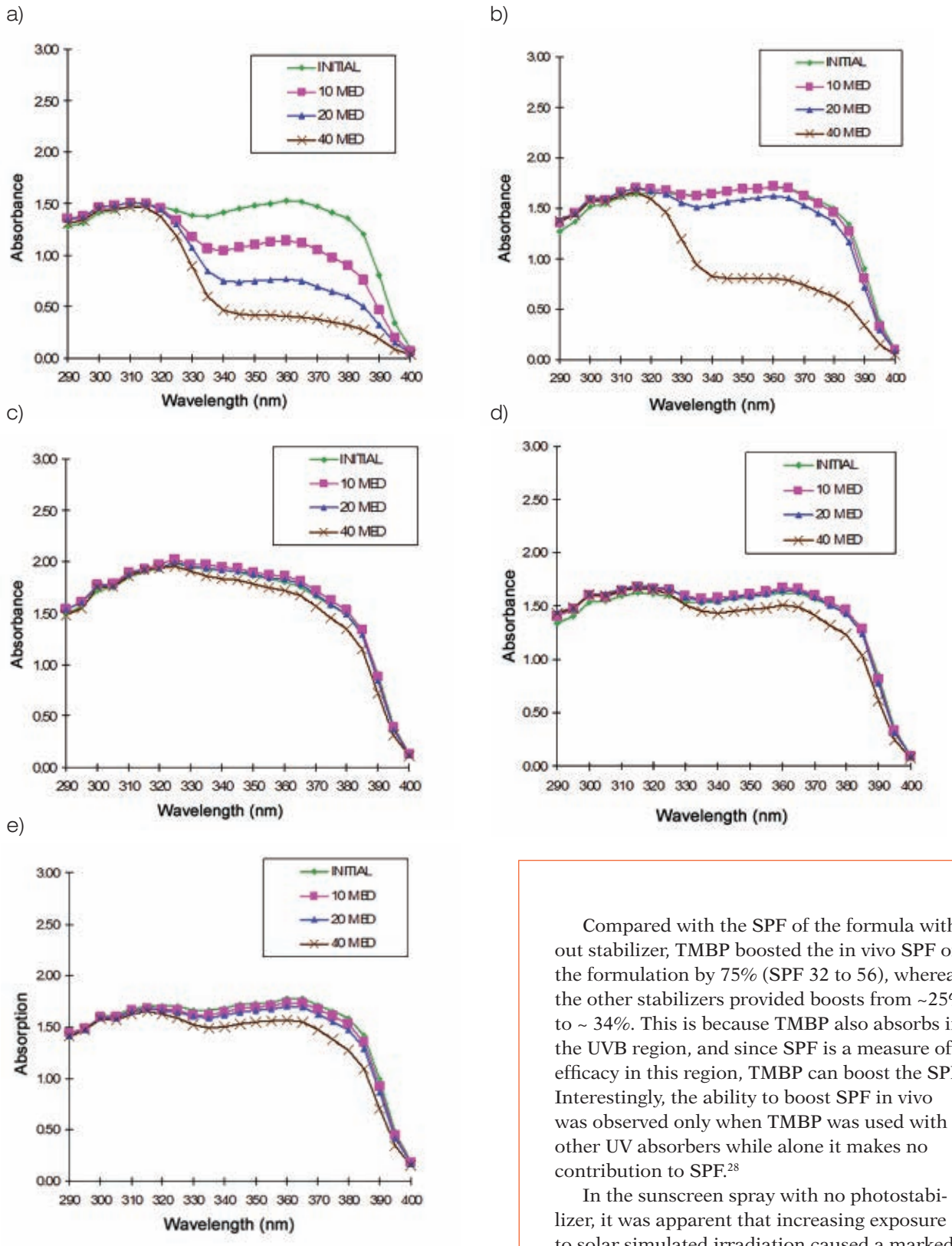


Figure 4. In vitro photostability of spray sunscreens without photostabilizers (a) and with DESM, (b) TMBP (c), OCR (d) and EMC (e)

Compared with the SPF of the formula without stabilizer, TMBP boosted the in vivo SPF of the formulation by 75% (SPF 32 to 56), whereas the other stabilizers provided boosts from ~25% to ~ 34%. This is because TMBP also absorbs in the UVB region, and since SPF is a measure of efficacy in this region, TMBP can boost the SPF. Interestingly, the ability to boost SPF in vivo was observed only when TMBP was used with other UV absorbers while alone it makes no contribution to SPF.[28]

In the sunscreen spray with no photostabilizer, it was apparent that increasing exposure to solar simulated irradiation caused a marked loss of photoprotection in the UVA region. Here, 20 MED showed a 50% loss and 40 MED, a 73% loss (see **Figure 4a**). Avobenzone is well-known to photodegrade, so this is not a surprising

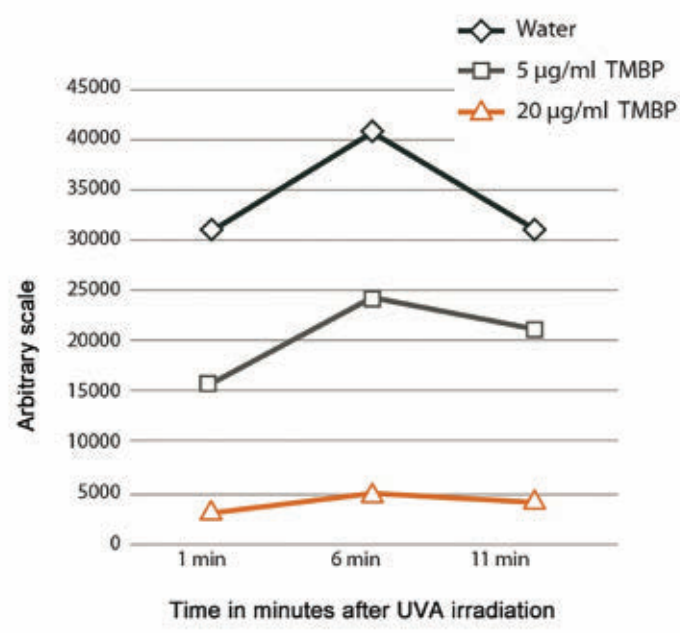


Figure 5. TMBP inhibited UVA-induced radical formation by inhibiting the NOX enzyme

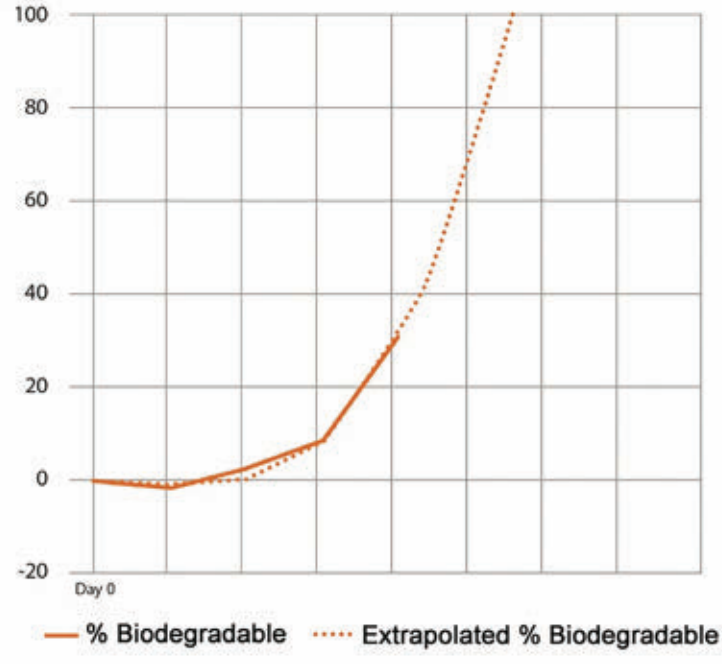


Figure 6. Biodegradability of TMBP per OECD 301D

result. And although less severe, photodegradation also was apparent with DESM; 40 MED registered a loss of 45%, compared with the control (see **Figure 4b**).

In the presence of TMBP, a marked improvement in photostability occurred in the UVA region. Here, a minimal loss of just 4% at 40 MED was observed, and no loss at 20 MED (see **Figure 4c**). TMBP was thus able to prevent the photodegradation of avobenzone.[17] TMBP was equally effective as OCR and EMC (see **Figures 4d** and **4e**, respectively).

Results: Antioxidant Activity and Human Safety

In response to free radicals, TMBP provided a key advantage in that it inhibited their excessive formation, as induced by the over-production of NOX. The NOX family includes seven unique members that are now considered the primary regulated sources of ROS.[38] These enzymes are expressed in diverse cells and tissues, and their products are essential to several physiological settings. For example, they share the capacity to transport electrons across the plasma membrane and to generate superoxide and other downstream ROS.

Knockout mouse models have been instrumental in identifying the physiological functions of NOX enzymes and the impact of their pharmacological targeting on disease progression.[39] NOX represents the first step in the oxidative stress cascade. Keratinocytes and fibroblasts both generate ROS in response to UV light,[40] which involves NOX enzymes. NOX-derived ROS are also involved in the regulation, expression and/or activation of matrix metalloproteases MMPs,[38] which are well-known to cause damage to collagen, elastin and other skin proteins.

Recently, Raad et al.[41] explored the role of NOX1 in photocarcinogenesis and its importance in the DNA damage repair network. Their work suggested a transient surge of ROS may serve as a priming signal to trigger a series of redox reactions that further stimulate cellular defense mechanisms, including the DNA damage response.

Results of the preliminary work described here using fibroblasts (UVA irradiation 3.6 J/ m^2) revealed that ROS continued to increase from 1 min to 6 min post-irradiation, suggesting a cascade/or enzyme-mediated effect (see **Figure 5**). ROS started to subside 11 min post-irradiation due to the lack of continuing stimulation. However, a nearly eightfold decrease in radical formation was observed using only 20 µg/mL of TMBP. Thus, TMBP appears to inhibit excessive radical formation induced by the over-production of NOX due to UV exposure.

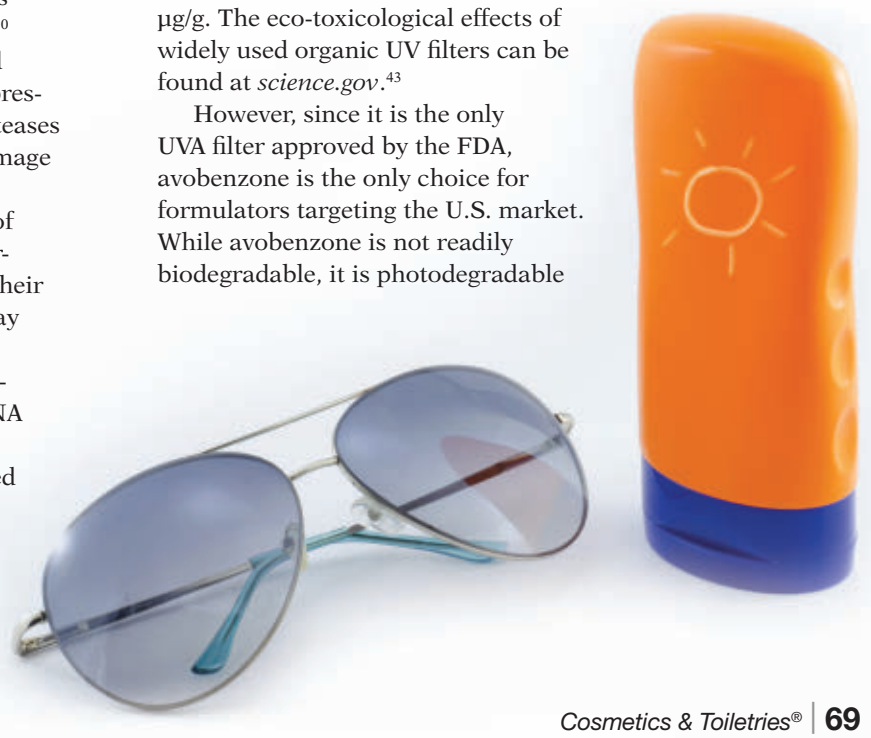
In terms of human safety, as noted, HIRPT was performed in 103 subjects using 2% and 5% dilutions of TMBP in corn oil and isosorbide dicaprylate, respectively. Results showed TMBP to be a non-primary skin irritant and non-primary skin sensitizer, and no adverse effects or reactions were observed. The material also was found to be non-phototoxic and non-mutagenic.[37]

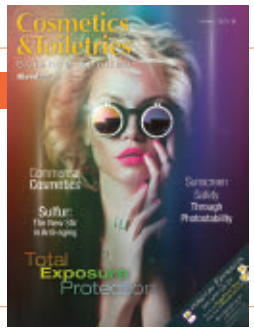
In comparison, OCR, while an effective avobenzone stabilizer, is emerging as a potential photo-allergen as it has been connected with increasing reports of positive patch and photopatch test reactions.[42] EMC is a methoxy analog of OCR, and while not explicitly identified as a sensitizer, could also pose similar safety concerns.

Results: Ecological Safety

Many UV filters represent some risk to the environment due to their lipophilicity and hence, have poor biodegradability. They can end up in sewage sludge during waste water treatment and accumulate in river sediments and biota at concentrations ranging from nano to µg/g. The eco-toxicological effects of widely used organic UV filters can be found at *science.gov*.[43]

However, since it is the only UVA filter approved by the FDA, avobenzone is the only choice for formulators targeting the U.S. market. While avobenzone is not readily biodegradable, it is photodegradable





Didn't Catch Part I?

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and it is neither bio-accumulative nor toxic to fish or algae.[44] Likewise, the UV filter HMS is not readily biodegradable, bio-accumulative or toxic to fish or algae. The UV filter OS, however, is readily biodegradable and not toxic to fish or algae.[45] Interestingly, avobenzone and OS have not been found to bleach coral, whereas BP-3 and OMC have.[46]

For the present study, as noted, the biodegradability of TMBP was determined following the OECD protocol 3011D. The biodegradability of TMBP was found to be -2.04%, 2.30%, 8.18% and 30.0% (based on ThOD, i.e., 1.957 mg O_2/g) on days 7, 14, 21 and 28, respectively. Extrapolation of this data revealed TMBP is estimated to degrade fully in approx. six weeks (see **Figure 6**).

In relation to safety for aquatic vertebrates, after 48 hr, TMBP registered an EC_{50} > 25 mg/L, deeming it non-toxic to *Daphnia* sp. Toxicity to aquatic algae was assessed by OECD 201. After 72 hr, it registered an EC_{50} >25 mg/L, indicating TMBP is non-toxic to algae (*Pseudokirchnerella subcapitata*).

Conclusion

The present two-part series in no way attempts to provide a comprehensive review of all the safety and eco-toxicity data available for sunscreens in the literature. However, authors have analyzed key data available to pinpoint the challenges formulators face to develop safe and effective sunscreen products for the U.S. market.

Based on the presented in vitro photostability and in vivo SPF data, one can conclude that TMBP can provide safe, eco-friendly and photostable broad spectrum protection (SPF 50+), as demonstrated in a sunscreen spray and earlier, in a lotion.[28] It is worth noting the ethanol content in the test spray should be maintained in the range 40–45% in order to achieve the in vivo SPF of 50+. In relation, since ethanol sprays tend to make skin feel dry, the addition of ingredients such as isosorbide dicaprylate can provide a soft and smooth skin feel.[36]

TMBP also was found to reduce the upstream oxidative stress cascade, presumably via the NOX family. Finally, a significant synergistic boost (75%) to the in vivo SPF was observed using TMBP (2.5%) with the globally approved UV absorbers avobenzone (3%), HMS (10%) and OS (5%). Taken together, these facets offer a complete sunscreen protection strategy array.

References (Part II)
All websites accessed on March 16, 2018

1. Colipa method for in vitro determination of UVA protection, *European Cosmetics Association* (2009)
17. H Gonzalez et al, Photostability of commercial sunscreens upon sun exposure and irradiation by ultraviolet lamps, *BMC Dermatology* 7 1-9 (2007)
28. RK Chaudhuri, MA Ollengo, P Singh and BS Martincigh, 3-(3,4,5-Trimethoxybenzylidene)-2,4-pentanedione: Design of a novel photostabilizer with in vivo SPF boosting properties and its use in developing broad-spectrum sunscreen formulations, *Int J Cosmet Sci* 39 25-35 (2017)
37. *https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/10239*
38. K Bedard and KH Krause, The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology, *Physiol Rev* 87(1) 245-313 (2007)
39. G Sirokmány, Á Donkó and M Geiszt, Nox/Duox family of NADPH oxidases: Lessons from knockout mouse models, *Trend in Pharmacol Sci* 37(4) 318–327 (2016)]
40. SM Beak, YS Lee and JA Kim, NADPH oxidase and cyclooxygenase mediate the ultraviolet B-induced generation of reactive oxygen species and activation of nuclear factor-kappaB in HaCaT human keratinocytes, *Biochimie* 86 425–429 (2004)
41. H Raad et al, NADPH oxidase-1 plays a key role in keratinocyte respsonses to UV radiation and UVB-induced skin carcinogenesis, *J Invest Dermatol* 137 1311-1321 (2017)
42. M Avenel-Audran, H Dutartre, A Goossens, Octocrylene, and emerging photoallergen, *Arch Dermatol* 146(7) 753-757 (2010)
43. *science.gov/topicpages/o/organic+uv+filters.html*
44. M Schlabach et al, Environmental screening of selected organic compounds 2008, Norwegian Pollution Control Authority, SPFO-rapport 1046/2009
45. *https://echa.europa.eu//registrationdossier/-/registered-dossier*
46. ncbi.nlm.nih.gov/pmc/articles/PMC2291018/

C&T

Natural/Botanical Formulary

ECOCERT/COSMOS-COMPLIANT NATURAL BODY WASH

(DuPont Tate & Lyle Bio Products)

A. Water (*aqua*)	48.73% w/w
Propanediol (Zemea Propanediol, DuPont Tate & Lyle Bio Products)	3.00
B. Coco-Glucoside (and) Sodium Coco-sulfate (Surfacare AGS, Galaxy Surfactants Limited)	46.20
Dicaprylyl Ether (and) Lauryl Alcohol (Cetiol LDO, BASF SE)	0.50
C. Benzyl Alcohol	0.50
D. Sodium Phytate	0.25
E. Citric Acid	0.82
	100.00

Procedure: Mix A. Mix B and add to A. Add C to AB. Add D to ABC. Adjust pH to 5.0–5.5 with E. Add sodium chloride as needed for a viscosity of 4,000 cps.

SUPERFRUIT SHAMPOO

(Bio-Botanica Inc.)

A. Water (*aqua*)	qs to 100.00% w/w
PEG-7 Glyceryl Cocoate	0.20
Punica Granatum Seed Oil	0.60
Lycium Barbarum (Goji) Fruit Extract (and) *Coffea Arabica* (Coffee) Fruit Extract (and) *Euterpe Oleracea* Fruit Extract (and) *Morinda Citrifolia* Fruit Extract (and) *Punica Granatum* Extract (and) *Garcinia Mangostana* Fruit Extract (and) *Camellia Sinensis* Leaf Extract (and) Propanediol (Fruitanical Superfruit Blend, Bio-Botanica Inc.)	2.00
Lavandula Angustifolia (Lavender) Extract	2.00
Chamomilla Recutita (Matricaria) Flower Extract	2.00
Rubus Idaeus (Raspberry) Fruit Extract	2.00
Rosmarinus Officinalis (Rosemary) Extract (Rosemary (*Rosmarinus officinalis*) extract, Bio-Botanica Inc.)	2.00
Cocamidopropyl Hydroxysultaine	8.50
Cocamidopropyl Betaine	8.00
Decyl Glucoside	7.75
Panthenol	0.60
Hydrolyzed Soy Protein	0.50
Phenoxyethanol	0.50
Polyquaternium-7	0.50
Citric Acid	0.20
B. Red 33	0.20
Yellow 6	0.40
	100.00

Procedure: In main beaker, mix A under light mixer agitation and create a vortex. Heat to 60°C. Begin cooling under agitation. When batch reaches 40°C, add B; *appearance: medium viscosity, pink color; pH = 4.50-6.50.*

REPLENISHING DEEP CONDITIONER FOR AFRICAN HAIR

(Croda)

This moisturizing conditioner hydrates hair and scalp, and reduces breakage. Crodazoquat MCC restores hydrophobicity, improves hair esthetics and delivers a consumer-perceivable feel improvement. Keravis PE penetrates the hair cuticle, building strength from within for optimal anti-breakage benefits. DuraQuench IQ SA delivers effective moisturization to the scalp while also providing conditioning benefits. Arlasilk PLN imparts a lasting silky feel to the hair. The formula is also enriched with Cropure Almond and Cropure Apricot Kernel. On both relaxed and natural African hair, this formulation delivers improved dry attributes, including less breakage.

A. Water (*aqua*)	87.50% w/w
Linoleamidopropyl PG-Dimonium Chloride Phosphate Dimethicone (Arlasilk PLN, Croda)	1.00
B. Behentrimonium Methosulfate (and) Quaternium-87 (and) Cetearyl Alcohol (Crodazoquat MCC, Croda)	2.00
Cetearyl Alcohol (Crodacol CS50, Croda)	6.00
Cetyl Alcohol (and) Isostearyl Isostearate (and) Potassium Cetyl Phosphate (and) Cetyl Stearate (and) Stearic Acid (Duraquench IQ SA, Croda)	1.00
Prunus Amygdalus Dulcis (Sweet Almond) Oil (Cropure Almond, Croda)	0.50
Prunus Armeniaca (Apricot) Kernel Oil (Cropure Apricot Kernel, Croda)	0.50
C. Water (*aqua*) (and) Hydrolyzed Vegetable Protein PG-Propyl Silanetriol (Keravis PE, Croda)	1.00
Water (*aqua*) (and) Sodium Benzoate (and) Potassium Sorbate (euxyl K 712, schülke)	0.50
	100.00

Procedure: Heat A to 75-80°C while mixing. Separately, heat B to 75-80°C. Add B to A while mixing. Cool to 40°C and add C. Cool to RT. Adjust pH to 4.5-5.0, if necessary; *viscosity = 20,000 cPs ± 10%, (RVT Spindle #TC @10rpm @ 25°C @24 hr).*

Want More Formulas?

Check out page DE1 of the July/August digital edition for the expanded formulary.

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BLOOMING SPA FOOT BATH OIL

(Bio-Botanica Inc.)

A. *Helianthus Annuus* (Sunflower) Seed Oil	36.00% w/w
Caprylic/Capric Triglyceride	19.10
Olea Europaea (Olive) Oil	7.70
Tocopherol	0.50
B. Polysorbate 80	34.20
Rosmarinus Officinalis (Rosemary) Leaf Oil (Certified Organic Rosemary Essential Oil #25670EO, Bio-Botanica Inc.)	1.00
Mentha Piperita (Peppermint) Oil (Certified Organic Peppermint Essential Oil #25665EO, Bio-Botanica Inc.)	1.00
Cymbopogon Schoenanthus Extract (Certified Organic Lemongrass Essential Oil # 25650EO, Bio-Botanica Inc.)	0.50
	100.00

Procedure: Mix A. Premix B and add to A. Mix well.

SOOTHING ANTIPERSPIRANT FOR SENSITIVE SKIN

(Floratech)

This moisturizing antiperspirant reduces irritation and sensitivity caused by typical antiperspirants. Floraesters K-100 Jojoba and Floraesters 60 provide natural jojoba emolliency, which hydrates and soothes skin. Floraesters and Floramac 10 are gentle ingredients, making them an ideal choice for delicate underarm skin.

A. Stearyl Alcohol	17.00% w/w
Hydrogenated Castor Oil	4.00
Jojoba Esters (Floraesters 60, Floratech)	0.50
Ethyl Macadamiate (Floramac 10, Floratech)	1.50
B. Hydrolyzed Jojoba Esters (and) Jojoba Esters (and) Water (*aqua*) (Floraesters K-100 Jojoba, Floratech)	1.00
Lactic Acid (Purac HiPure 90, Purac)	0.10
C. Cyclopentasiloxane	20.00
D. Aluminum Starch Octenylsuccinate (Dry-Flo PC, AkzoNobel, Personal Care)	3.00
Talc (Supra H, Luzenac Group)	0.50
Aluminum Zirconium Tetrachlorohydrate	22.00
E. Cyclopentasiloxane	qs
C12-15 Alkyl Benzoate (and) Stearalkonium Hectorite (and) Propylene Carbonate (Bentone Gel TN V, Elementis PLC)	1.00
F. Fragrance (*parfum*)	qs
	100.00

Procedure: Combine A in a vessel and heat. Start mixing with propeller agitation at 60-65°C. Continue heating to 70-80°C. In a separate vessel, mix B at RT by stirring. Once B becomes a clear liquid, add C to B at RT. Begin heating to 70-80°C. Add BC to A at 70-80°C with medium propeller agitation. When mixture becomes uniform, cool to 65-70°C. Add D to ABC in the order with medium propeller agitation at 65-70°C. The aluminum zirconium tetrachlorohydrate should be added at 65°C. In a separate vessel, combine E gradually to avoid making clumps and mix at RT with medium propeller agitation. When mixture becomes uniform, add F to E. When EF becomes uniform, begin heating to 60-65°C. Add EF to ABCD with medium propeller agitation at 60-65°C. Transfer mixture into a container at 55-60°C.

GINGER CITY LIFE CLEANSER

(The Hallstar Company)

Ginger City Life Cleanser is recommended as the first step of any skin care regimen. It contains Olivem 2020 and Florasolvs Macadamia-16 to gently remove dirt, oil and makeup without leaving skin feeling dry. It has a lightweight, non-greasy, whipped texture that won't clog pores or cause breakouts. Blue Oléoactif blocks pollutants from penetrating the skin. Opuntia Oléoactif moisturizes and leaves skin soft and glowing.

A. Glycerin	qs to 100.00% w/w
Ethylhexyl Olivate/Sodium Acrylates Copolymer/ Polyglyceryl-4 Olivate (Olivem 2020, The Hallstar Company)	0.50
B. PEG-16 Macadamia Glycerides (Florasolvs Macadamia-16, The Hallstar Company)	5.00
Brassica Campestris (Rapeseed) Seed Oil (and) *Camelina Sativa* Seed Oil (and) *Opuntia Ficus-Indica* Flower Extract (Opuntia Oléoactif, The Hallstar Company)	1.00
Glycine Soja (Soybean) Oil (and) Polyglyceryl-3 Diisostearate (and) *Oryza Sativa* (Rice) Extract (Blue Oléoactif, The Hallstar Company)	1.00
Propylene Glycol	43.00
C. Benzyl Alcohol (and) Ethylhexylglycerin (and) Tocopherol (euxyl K 900, schulke)	qs
Fragrance (*parfum*)	qs

Procedure: Prepare A and mix for 1 hr. Add B and mix for a few minutes. Add C and mix; *properties (@25°C): appearance = yellow liquid; viscosity (10 rpm, Brk, RVDV-E, T-B, after 24 hr at RT, mPa·s) = 500–3,000.*

ORGANIC FLORAL SERUM

(The Hallstar Company)

This Organic Floral Serum blends natural hydrators and antioxidants to balance oily and dry skin zones and maintain a healthy, smooth complexion. The natural and organic daily moisturizer has a lightweight texture and is quickly absorbed. Olivem 1000 and Oliwax LC create liquid crystals that prevent signs of aging and give tonicity and elasticity to the skin.

A. Water (*aqua*)	qs to 100.00% w/w
Glycerin	1.50
Bentonite	2.50
Xanthan Gum	0.15
Aloe Barbadensis Leaf Juice (Biochemica BioVera 200X Aloe, The Hallstar Company)	0.50
B. Cetearyl Olivate (and) Sorbitan Olivate (Olivem 1000, The Hallstar Company)	3.00
Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Olivate (Oliwax LC, The Hallstar Company)	0.50
Cetearyl Alcohol	1.00
Butyrospermum Parkii (Shea) Butter (and) *Elaeis Guineensis* (Palm) Butter (and) *Simmondsia Chinensis* (Jojoba) Seed Oil (and) *Punica Granatum* Extract (Biochemica Pomegranate Butter Organic, The Hallstar Company)	5.00
Persea Gratissima (Avocado) Oil (Biochemica Avocado Oil Natural Organic, The Hallstar Company)	1.00
Coco-Caprylate	2.00
Lecithin (and) Tocopherol (and) Ascorbyl Palmitate (and) Citric Acid (Aperoxid TLA, Biochim Srl)	0.05
C. *Thymus Vulgaris* (Thyme) Leaf Water	4.00
Rosa Centifolia Flower Water	10.00
Opuntia Ficus Indica Stem Extract (and) Glycerin (and) Phenoxyethanol (and) Water (*aqua*) (AquaCacteen, Mibelle Biochemistry)	1.00
D. Preservatives	qs

Procedure: Prepare A and disperse thickeners using a suitable dispersion unit (e.g., Silverson, Ultra-Turrax). Prepare B. Warm A and B separately to 75–80°C. Add B to A at 70-75°C while homogenizing using the suitable dispersion unit. Cool to 40°C under gentle agitation. Add C and homogenize. Adjust pH to 5.0–5.5. Add D and mix until uniform; *properties (@25°C): appearance = shiny ivory cream; viscosity (Brk, RVDV-E, T-B, 10 rpm, after 24 hr at RT; mPa·s) = 4,000–7,000; pH = 5.0–5.5.*

WINTER COMFORT ECO-CONSCIOUS HAND CREAM

(AAK)

Formulated with a rich, natural emollient base featuring Lipex SheaSoft, this cream rebalances softness and acts like a glove, sealing in moisture to rehydrate skin. It leaves even the driest hands looking and feeling soothed, softened and restored. Natural ingredients (Chemically Processed Agro-Ingredients, CPAI) = 97%.

A. Xanthan Gum	0.50% w/w
Glycerin	5.00
B. Water (*aqua*)	qs to 100.00
C. Citric Acid	0.10
Benzyl Alcohol	0.50
Potassium Sorbate	0.15
Sodium Benzoate	0.20
Sodium Stearoyl Lactylate	0.75
D. Glyceryl Stearate	1.00
Stearic Acid (and) Palmitic Acid (Cutina FS 45, BASF SE)	2.00
Cetearyl Alcohol	3.50
Shea Butter Ethyl Esters (Lipex SheaLight, AAK)	7.00
Butyrospermum Parkii (Shea) Butter (Lipex SheaSoft, AAK)	11.00
C15-19 Alkane	3.00
E. *Zea Mays* (Corn) Starch	3.50
Fragrance (*parfum*)	0.60

Procedure: Blend A and add to B. Heat AB and C together, and separately heat D to 75°C. Merge ABCD and start mixing with medium speed. Homogenize. Cool to 30°C while stirring. Add E to ABCD and mix well.

MOISTURE RESCUE ECO-CONSCIOUS BODY CREAM

(AAK)

This fresh gel-cream has a light texture and is designed to soften the entire body. Instead of low-spreading mineral oil, it is formulated with our highly stable, rapeseed-based Lipex Bassol C. Easy to apply, this balm is quickly absorbed by the skin to replenish moisture and deliver softness with no tacky feel. Consumers can use it in the morning to kick-start their day, or to rebalance skin after a workout. Best of all, it's as kind to the planet as it is to skin; natural ingredients (CPAI = 95%).

A. Water (*aqua*)	qs to 100.00% w/w
Glycerin	7.00
Lysolecithin (and) Sclerotium Gum (and) Xanthan Gum (and) Pullulan (Ecogel, Lucas Meyer Cosmetics)	2.50
B. Citric Acid	0.10
Benzyl Alcohol	0.50
Potassium Sorbate	0.15
Sodium Benzoate	0.20
Distarch Phosphate	3.00
C. *Olus* Oil (Lipex Bassol C, AAK)	7.00
Shea Butter Ethyl Esters (Lipex SheaLight, AAK)	6.00
Hydrogenated Vegetable Oil	3.00
Glyceryl Stearate Citrate	2.00
D. Fragrance (*parfum*)	0.75

Procedure: Premix A vigorously. Add A to B. Prepare C. Heat AB and C separately to 75°C. Merge AB with C and start mixing with medium speed. Mix continuously, maintaining heat for ~25 min, to ensure hydration of the phospholipids. Homogenize. Cool to 30°C while stirring. Add D to ABC. Homogenize.

FACIAL FIRMING BOOSTER

(BASF Corporation)

This beauty booster reverses the signs of aging and protects against lifestyle damage. New botanical bioactive Dermagenist rejuvenates skin cells to deliver firming action and replenish skin density. A must-have in consumers' skin care regimens, this power-packed formula awakens skin's inner "sleeping beauty" for a noticeably younger look.

A. Water (*aqua*)	61.60% w/w
Glycerin (and) Glyceryl Polyacrylate (Hispagel 200, BASF Corporation)	12.00
Sodium Stearoyl Glutamate (Eumulgin SG, BASF Corporation)	0.65
Water (*aqua*)/Dimethicone/Dimethicone Crosspolymer/ Butylene Glycol/Hydrogenated Lecithin/ Polyphosphorylcholine Glycol Acrylate (AM 600 PF, BASF Corporation)	10.00
Phenoxyethanol (and) Ethylhexylglycerin (euxyl PE 9010, schulke)	1.00
B. Dicaprylyl Carbonate (Cetiol CC, BASF SE)	4.00
Undecane (and) Tridecane (Cetiol Ultimate, BASF SE)	2.00
Sucrose Polystearate (and) Cetyl Palmitate (Emulgade Sucro Plus, BASF Corporation)	1.25
C. Water (*aqua*)	7.10
Origanum Majorana Leaf Extract (and) Maltodextrin (Dermagenist, BASF Corporation)	0.40
	100.00

Procedure: Combine A and begin heating to 75°C ± 3°C. Combine B and begin heating to 75°C ± 3°C. Add B to A and homogenize until uniform. Switch to prop mixing and begin cooling. Add premixed C below 40°C and continue prop mixing. Continue mixing to RT.

SULFATE-FREE COLOR CARE ECONOMY SHAMPOO

(Stepan Co.)

This formulation provides good lather and color-retention properties.

A. Water (*aqua*)	qs to 100.00% w/w
B. Disodium Laureth Sulfosuccinate (and) Sodium Lauryl Sulfoacetate (Stepan-Mild LSB , Stepan Co.)	46.87
Cetyl Betaine (Amphosol CDB Special, Stepan Co.)	9.73%
C. Glyceryl Caprylate/Caprate (Stepan-Mild GCC , Stepan Co.)	1.25%
D. Sodium Chloride	qs
Citric Acid	qs
Fragrance (*parfum*)	qs
Dyes	qs
Preservatives	qs

Procedure: Add A to a vessel equipped with mixing capabilities. Start mixing. Add B in order to A. While mixing, add C to batch. Continue mixing until homogenous. Adjust viscosity and pH, if necessary. Add fragrance, dye and preservative.

KEY POINTS

- Anthocyanins are flavonoid compounds found naturally in plants with promising potential for skin health and protection benefits.
- Sustaining their natural supply for industry has become increasingly important; as such, microbial routes for their production have been explored, such as those described here.

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Editor's note: The cosmetic industry's knowledge of microbes and their potential applications has grown tremendously. One area of recent interest is the skin microbiome, which can be manipulated for desired effects. Another is the utilization of microbes for the sustainable and economical production of natural materials; such as the upstream production of cosmetic pigments described here. The processes presented here are at the forefront of innovation and their use holds potential for future marketing claims to appeal to environmentally conscious consumers.

Biocatalyst for Change

Part II: Strategies to Engineer Pigment Production

Continued from Page 39

Anthocyanins can be accumulated in different plant tissues when plants are subjected to biotic or abiotic stress, and several elicitors such as jasmonate.[19, 20] Anthocyanins are synthesized via the general flavonoid pathway in plants, whereby three molecules of malonyl-CoA and one molecule of 4-coumaroyl-CoA derived from the general phenylpropanoid pathway are condensed to form naringenin chalcone by chalcone synthase (CHS), which then undergoes isomerization, hydroxylation and oxidation to form anthocyanidins (see Figure 1). Anthocyanidins are then glycosylated at C3 or other positions by flavonoid glucosyltransferases (FGTs) with the incorporation of glucose or other sugar units such as rhamnose, galactose, xylose, etc., giving rise to anthocyanins. Beyond glycosylation, other modifications, such as acylation and methylation of the hydroxyl groups have also been reported.[21]

As noted, the prevailing way to industrially produce anthocyanin is by extraction from plants, which is typically performed using solvents; most commonly ethanol, due to its

environmentally friendly nature, safety profile and minimal interference with anthocyanin recovery.[22] Other extraction methods such as pressurized liquid extraction and novel extraction tools/agents, e.g., ultrasound, subcritical water and polymeric absorber resins, also have been reported as effective for obtaining anthocyanins from crops and fruits.[23-26]

Plant suspension cell culture is another possible way to produce anthocyanins, which introduces the bioreactor concept to cultivate plant cells for anthocyanin production. This allows for tight control over the fermentation process and involves the development of suitable cell lines, optimization of operating conditions, and scale-up of fermentation.[27, 28]

For some time, plants such as grapes, Cleome rose, sweet potatoes, aspen, wild carrots, etc., have been used to develop anthocyanin-producing cell lines.[29, 30] Currently, however, no systems that are commercially feasible are apparently available. This is due to the instability of cell lines, causing anthocyanin production to decrease drastically over time and other problems such as high production cost, low consumer acceptance and strict biosafety regulations on plant suspension cell cultures.[31-33] Future efforts on the development of robust cell lines and efficient fermentation processes, in terms of light irradiation, temperature and medium composition, will drive the application of suspension cell culture in the production of anthocyanins or related products.

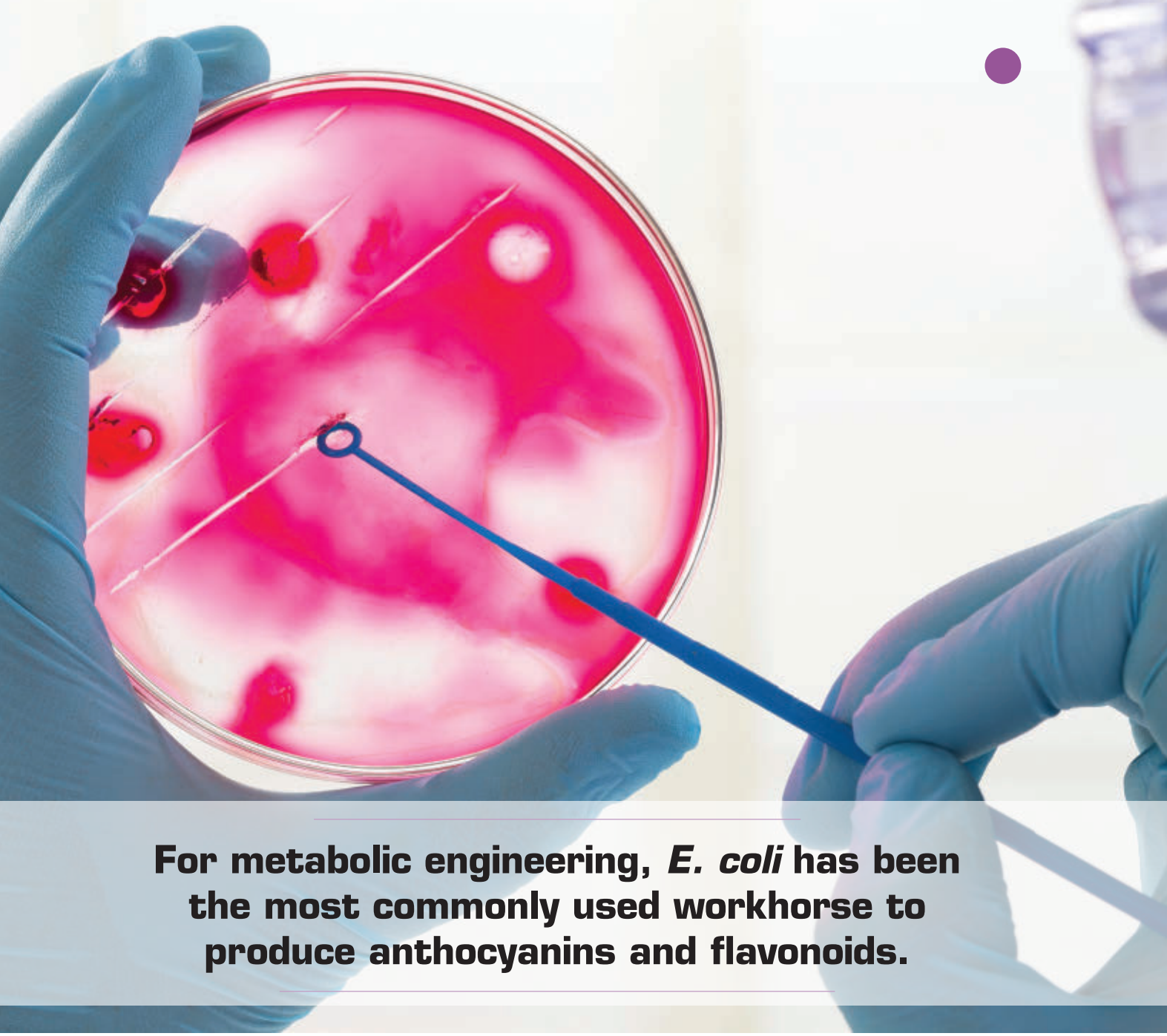
Anthocyanins from Microorganisms

Engineering microorganisms, especially model microorganisms such as *E. coli* and *Saccharomyces cerevisiae*, for the production of natural chemicals is a promising and sustainable way to satisfy production demands, owing to several distinct advantages of microorganisms: fast growth and easy cultivation, sophisticated engineering strategies and readily available metabolic network databases.

As the most commonly used workhorse in metabolic engineering, *E. coli* has been engineered for the production of anthocyanins as well as other flavonoid molecules (see **Figure 2**).[21, 34-36] As early as 2005, the genes of F3H and ANS from *Malus domestica*; DFR from *Anthurium andraeanum*; and flavonoid 3-glucosyltransferase (F3GT) from *Petunia hybrida* were successfully expressed in *E. coli*, producing 6.0 µg/L of cyanidin 3-*O*-glucoside and 5.6 µg/L of pelargonidin 3-*O*-glucoside when fed with naringenin and eriodictyol as precursors.[37] The subsequent optimization of enzyme sources and UDP-glucose pool, regulation of precursor uptake and optimization of the production process greatly increased final product titers.[38-40]

The highest production of cyanidin 3-*O*-glucoside and

Figure 2. Three different anthocyanins produced via *E. coli* (from L): peonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside



For metabolic engineering, *E. coli* has been the most commonly used workhorse to produce anthocyanins and flavonoids.

pelargonidin 3-*O*-glucoside achieved was 350 mg/L and 113 mg/L, based on catechin and afzelechin as the respective precursors. To date, the reported microbial hosts of anthocyanin biosynthesis are still limited to *E. coli*, although the heterologous production of other flavonoids has been extended to *S. cerevisiae, Streptomyces venezuelae*,[21, 37] and *Corynebacterium glutamicum*.[41-43] It remains to be seen if other microorganisms are better host strains in terms of anthocyanin production.

In the next sections, common strategies used for the development of anthocyanin-producing *E. coli* strains, as well as strategies for optimizing the fermentation process, are described.

Engineering Pathway Enzymes

The microbial production of anthocyanins involves the co-expression of enzymes from plants in the anthocyanin biosynthesis pathway, which is challenging; typically, plant-derived genes must be engineered prior to their functional expression. For example, to achieve the functional expression of a plant P450 F3'5'H from *Catharanthus roseus* in the biosynthesis of the flavonoid quercetin, a new F3'5'H was fused to a shortened P450-reductase from *C. roseus* to form a chimeric protein. To engineer F3'5'H, the first four amino acids at N-terminus were removed and the sixth amino acid was changed to alanine from leucine, followed by the replacement of the

The microbial production of natural chemicals has distinct advantages: fast growth and cultivation, sophisticated engineering and readily available metabolic network databases.

fifth codon with ATG as the new start codon.[44]

Translational fusion of multiple enzymes in successive steps is also a popular strategy for improving anthocyanin production. Such fusions can increase the local concentrations of substrates for each enzyme in enzyme complex while minimizing the degradation of unstable intermediates, achieving a high overall metabolic flux and titer.[45] For example, the translational fusion of F3GT from *Arabidopsis* to the N-terminus of ANS from *P. hybrida* was shown to produce 16.9% more cyanidin 3-*O*-glucoside than the tandem expression of ANS and F3GT.[38]

Beyond the aforementioned enzyme engineering, selection of natural enzymes from diverse species is another approach to improve anthocyanin production.[46] The orthologous enzymes usually exhibit diverse kinetic and thermodynamic properties, resulting in different metabolic behaviors and varied levels of production. For example, in one study, ANS from *P. hybrida* produced 0.47- to 4.9-fold more cyanidin 3-*O*-glucoside than from *Antirrhinum majus, Gerbera* hybrid and *M. domestica* in *E. coli*.[36] In another study, different combinations of three 4-coumaroyl CoA ligases, two chalcone synthases and two chalcone isomerases led to a diverse landscape of naringenin production and a threefold increase of naringenin production in *E. coli*.[47]

Controlling Cofactors

Besides pathway enzymes, the biosynthesis of anthocyanins relies on cofactors. For example, the enzyme ANS uses ferrous ions and sodium ascorbate as cofactors, and 2-oxoglutarate as a cosubstrate to carry out the two-electron oxidation of its substrates.[38, 48] The glycosylation of cyanidin at the C3 position requires an equimolar amount of UDP-glucose and feeding trials have demonstrated the UDP-glucose supply is a limiting factor in anthocyanin production.[38]

However, in an *E. coli* strain that produced cyanidin 3-*O*-glucoside, its production could be increased 20-fold through the regulation of the UDP-glucose pool. Here, genes responsible for the biosynthesis of 3-*O*-glucoside from orotic acid, pyrE, pyrR, cmk, ndk, pgm and galU, were overexpressed while blocking the competitive UDP-glucose consumption pathways.[38, 39]

Also, S-adenosyl-L-methionine (SAM) provides methyl groups in the production of methylated anthocyanins. Its supply can be boosted by supplementing methionine and/or up-regulating genes associated with SAM biosynthesis. However, the accumulation of SAM is inhibited owing to feedback repression by the methionine biosynthesis regulator MetJ

While challenges remain, microbial-produced anthocyanins are expected to be applied to commercial cosmetics in the near future.

in response to the intracellular concentration of SAM.[49] As another example, to increase the supply of SAM for the production of peonidin 3-*O*-glucoside—a purple-colored methylated anthocyanin—CRISPRi-mediated silencing of MetJ was employed in *E. coli*. Results showed a twofold increase in the production titer.[50]

Managing Anthocyanin Efflux

Natural product biosynthesis can result in the production of molecules that are toxic to cells, limiting their production in high yields. Therefore, a feasible solution is to continuously pump out the toxic products to extracellular media during the biosynthesis process.

For example, in an *E. coli* strain that converted catechin to cyanidin 3-*O*-glucoside, the overexpression of the product-associated efflux pump YadH increased the titer by 15%, and the deletion of another efflux pump TolC, which most likely expelled catechin inside cells, resulted in a 55% higher production. Further, the combined modification resulted in a 63% increase in cyanidin 3-*O*-glucoside production.[40]

Anthocyanins in plants are transported to vacuoles after their synthesis, and this process is accomplished by both cytoplasmic transporters and transmembrane transporters. Here, the most commonly studied plant-based transporters are glutathione S-transferase and ATP-binding cassette (ABC) transporters. It would be interesting to assess the performance of plant-based transporters in microorganisms for anthocyanin delivery and anthocyanin production.

Optimizing Biocatalysis

The instability of anthocyanins at a neutral pH is a major issue in their production by

E. coli. Unlike plants, which can stabilize the synthesized anthocyanins by storing them in vacuoles, *E. coli* does not have this protection mechanism.[51, 52] Instead, the produced anthocyanins are inclined to degrade, given that the intracellular and extracellular pH is around 7 under normal growth conditions.

To address this problem, a two-step biocatalysis strategy was developed, in which cells are first cultured in a medium at pH 7 to support cell growth and enzyme expression, and then are transferred to fresh medium at pH 5.0 to initiate anthocyanin production and accumulation in the second step.[38] Protective agents such as glutamate can be added to minimize acid-induced cell lysis. Using this approach, the production of cyanidin 3-*O*-glucoside was increased by ~15-fold from the traditional single-step production.[38]

Besides pH, induction points in time and temperature have remarkable effects on recombinant anthocyanin production.[40, 47] Induction time-points are correlated with the growth state and conditions of producing cells, which can cause differential enzyme expressions and significantly different production efficiencies. Likewise, temperature generally imposes a direct impact on cell viability and protein expression or folding, and hence influences anthocyanin bioconversion indirectly.

Coculturing to Produce Anthocyanins De novo

Low-cost production is key to the commercialization of recombinant anthocyanins. To this end, increasing the conversion yield of precursor molecules such as catechin to C3G is one focus, whereas achieving efficient C3G production directly from cheaper precursors, such as glycerol and glucose, is another focus.

As such, a polyculture strategy was recently proposed[53] that allows the metabolic burden to be divided into individual host strains to optimize each strain for the efficient conversion of a precursor molecule to an anthocyanin intermediate—and finally, to the end product of interest.[54, 55]

For example, the whole synthetic pathway from simple carbon sources such as glucose or glycerol to pelargonidin 3-*O*-glucoside was split into four modules and placed into four different *E. coli* strains. The modulation of the four-strain consortia successfully achieved the de novo production of pelargonidin 3-*O*-glucoside from glucose at 9.5 mg/L for the first time.

Conclusions and Future Perspectives

The preference for natural compounds such as anthocyanins with potential skin-protection benefits has inspired their application as cosmetic ingredients. The microbial production of anthocyanins has attracted considerable attention due to distinct advantages over traditional production methods. The present review summarizes successful strategies to improve the production of anthocyanins in *E. coli*; including engineering pathway enzymes, improving the availability of cofactors, engineering anthocyanin efflux pumps and optimizing the fermentation process.

Several challenges remain to be addressed prior to the commercialization of anthocyanin-producing bacteria; such as the poor expression of anthocyanin biosynthetic genes, an imbalance of genes in the pathway, the delivery and stabilization of final products, and safety assessments for the potency of microbial anthocyanins. However, with solutions to these issues, it can be expected that microbial anthocyanins will be applied in commercial cosmetics in the near future.

References

1. A Lopaciuk and M Curie-Sklodowska, Global beauty industry trends in the 21st century, in (conference) *Global beauty and personal care: The year in review and winning strategies for the future*, *Milan* (2013)
2. A Ribeiro, M Estanqueiro, M Oliveira and J Sousa Lobo, Main benefits and applicability of plant extracts in skin care products, *Cosmetics* 2(2) 48 (2015)
3. T Aburjai and FM Natsheh, Plants used in cosmetics, *Phytother Res* 17(9) 987–1000 (2003)
4. J Zha and MAG Koffas, Production of anthocyanins in metabolically engineered microorganisms: Current status and perspectives, *Synth Syst Biotechnol* 2(4) 259–266 (2017)
5. S Chouhan, K Sharma, J Zha, S Guleria and MAG Koffas, Recent advances in the recombinant biosynthesis of polyphenols *Front Microbiol* 16(8) 2259 (2017)
6. CF Chan, CY Lien, YC Lai, CL Huang and WC Liao, Influence of purple sweet potato extracts on the UV absorption properties of a cosmetic cream, *J Cosmet Sci* 61(5) 333–341 (2010)
7. K Tsoyi, HB Park et al, Protective effect of anthocyanins from black soybean seed coats on UVB-induced apoptotic cell death in vitro and in vivo, *J Agric Food Chem* 56(22) 10600–10605 (2008)

Increasing the supply of SAM increased the production titer of 3-O-glucoside, a purple-colored methylated anthocyanin.

8. A Tarozzi, A Marchesi et al, Protective effects of cyanidin-3-*O*-beta-glucopyranoside against UVA-induced oxidative stress in human keratinocytes, *Photochem Photobiol* 81(3) 623–629 (2005)
9. K Tsoyi, HB Park et al, Anthocyanins from black soybean seed coats inhibit UVB-induced inflammatory cylooxygenase-2 gene expression and PGE2 production through regulation of the nuclear factor-kappaB and phosphatidylinositol 3-kinase/Akt pathway, *J Agric Food Chem 56*(19) 8969–8974 (2008)
10. A Westfall, Evaluation of the efficacy of anthocyanins as biologically active ingredients in lipstick formulations, Master thesis, *Food Science and Technology*, The Ohio State University, Columbus, OH (2015)
11. AK Ghimeray, US Jung, HY Lee, YH Kim, EK Ryu and MS Chang, In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing *Punica granatum*, *Ginkgo biloba*, *Ficus carica* and *Morus alba* fruits extract, Clin, *Cosmet Invest Dermatol* 8 389–396 (2015)
12. PK Mukherjee, N Maity, NK Nema and BK Sarkar, Bioactive compounds from natural resources against skin aging, *Phytomedicine* 19(1) 64–73 (2011)
13. L Zhu, Y Huang, Y Zhang, C Xu, J Lu and Y Wang, The growing season impacts the accumulation and composition of flavonoids in grape skins in two-crop-a-year viticulture, *J Food Sci Technol* 54(9) 2861–2870 (2017)
14. P-J Tsai, S-C Wu and Y-K Cheng, Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons, *Food Chem* 106(1) 27–32 (2008)
15. Q Zhang, NP Botting and C Kay, A gram scale synthesis of a multi-13C-labelled anthocyanin, [6,8,10,3',5'-13C5] cyanidin-3-glucoside, for use in oral tracer studies in humans, *Chem Commun* 47(38) 10596–10598 (2011)
16. L Cruz, N Mateus and V de Freitas, First chemical synthesis report of an anthocyanin metabolite with in vivo occurrence: Cyanidin-4'-O-methyl-3-glucoside, *Tetrahedron Lett* 54(22) 2865–2869 (2013)
17. M Saniewski, A Miszczak et al, Effects of methyl jasmonate on anthocyanin accumulation, ethylene production and CO_2 evolution in uncooled and cooled tulip bulbs, *J Plant Growth Regul* 17(1) 33–37 (1998)
18. R Akula and GA Ravishankar, Influence of abiotic stress signals on secondary metabolites in plants, *Plant Signaling Behav* 6(11) 1720–1731 (2011)
19. RP Pandey, P Parajuli, MAG Koffas and JK Sohng, Microbial production of natural and non-natural flavonoids: Pathway engineering, directed evolution and systems/synthetic biology, *Biotechnol Adv* 34(5) 634–662 (2016)
20. F Delgado-Vargas and O Paredes-Lopez, *Natural Colorants for Food and Nutraceutical Uses*, CRC Press, Boca Raton, FL USA (2002)
21. M Corrales, S Toepfl, P Butz, D Knorr and B Tauscher, Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison, *Innovative Food Sci Emerging Technol* 9(1) 85–91 (2008)
22. Z Ju and LR Howard, Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin, *J Food Sci* 70(4) S270–S276 (2005)

23. D Kammerer, J Gajdos Kljusuric, R Carle and A Schieber, Recovery of anthocyanins from grape pomace extracts (*Vitis vinifera* L. cv. Cabernet Mitos) using a polymeric adsorber resin, *Eur Food Res Technol* 220(3) 431–437 (2005)
24. ZY Ju and LR Howard, Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin, *J Agric Food Chem* 51(18) 5207-5213 (2003)
25. A Matkowski, Plant in vitro culture for the production of antioxidants—A review, *Biotechnol Adv* 26(6) 548–560 (2008)
26. S Deroles, Anthocyanin biosynthesis in plant cell cultures: A potential souce of natural colorants, in *Anthocyanins,* Springer, New York (2008) pp 108–167
27. C Simões, N Albarello, TC de Castro and E Mansur, Production of anthocyanins by plant cell and tissue culture strategies, in *Biotechnological Production of Plant Secondary Metabolites*, Bentham Science Publishers, Sharjah, UAE (2012) pp 67–86
28. SR Rao and GA Ravishankar, Plant cell cultures: Chemical factories of secondary metabolites, *Biotechnol Adv* 20(2) 101–153 (2002)
29. J Zha and MAG Koffas, Anthocyanin production in engineered microorganisms, in *Biotechnology of Natural Products*, Springer, New York (2018) pp 81–97
30. A Angelov and V Gotcheva, Safety assessment and regulations for food ingredients derived from plant in vitro systems, in *Bioprocessing of Plant In vitro Systems*, Springer, New York (2017) pp 1–17
31. A Ananga, V Georgiev, J Ochieng, B Phills and V Tsolova, Production of anthocyanins in grape cell cultures: A potential source of raw material for pharmaceutical, food, and cosmetic industries, in *The Mediterranean Genetic Code—Grapevine and Olive*, InTech (2013)
32. JA Chemler, E Leonard and MAG Koffas, Flavonoid biotransformations in microorganisms, in *Anthocyanins: Biosynthesis, Functions and Applications*, Springer, New York (2009) pp 191–255
33. EA Trantas, MA Koffas, P Xu and F Ververidis, When plants produce not enough or at all: Metabolic engineering of flavonoids in microbial hosts, *Front Plant Sci* 6 7 (2015)
34. JA Chemler and MAG Koffas, Metabolic engineering for plant natural product biosynthesis in microbes, *Curr Opin Biotechnol* 19(6) 597–605 (2008)
35. Y Yan, J Chemler, L Huang, S Martens and MAG Koffas, Metabolic engineering of anthocyanin biosynthesis in *Escherichia coli*, *Appl Environ Microbiol* 71(7) 3617–3623 (2005)
36. Y Yan, Z Li and MA Koffas, High-yield anthocyanin biosynthesis in engineered *Escherichia coli*, *Biotechnol Bioeng* 100(1) 126–140 (2008)
37. E Leonard, Y Yan, ZL Fowler, Z Li, C-G Lim, K-H Lim and MAG Koffas, Strain improvement of recombinant *Escherichia coli* for efficient production of plant flavonoids, *Mol Pharmaceutics* 5(2) 257–265 (2008)
38. CG Lim, L Wong et al, Development of a recombinant *Escherichia coli* strain for overproduction of plant pigment, anthocyanin, *Appl Environ Microbiol* 81(18) 6276–6284 (2015)
39. N Kallscheuer, M Vogt, A Stenzel, J Gatgens, M Bott and J Marienhagen, Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2S)-flavanones, *Metab Eng* 38 47–55 (2016)
40. N Kallscheuer, M Vogt and J Marienhagen, A novel synthetic pathway enables microbial production of polyphenols independent from the endogenous aromatic amino acid metabolism, *ACS Synth Biol* 6(3) 410–415 (2017)
41. PV van Summeren-Wesenhagen and J Marienhagen, Putting bugs to the blush: Metabolic engineering for phenylpropanoid-derived products in microorganisms, *Bioengineered* 4(6) 355–362 (2013)
42. E Leonard, Y Yan and MAG Koffas, Functional expression of a P450 flavonoid hydroxylase for the biosynthesis of plant-specific hydroxylated flavonols in *Escherichia coli*, *Metab Eng* 8(2) 172–181 (2006)
43. K Springob, J Nakajima, M Yamazaki and K Saito, Recent advances in the biosynthesis and accumulation of anthocyanins, *Nat Prod Rep* 20(3) 288–303 (2003)
44. S Zhao, JA Jones, DM Lachance, N Bhan, O Khalidi, S Venkataraman, Z Wang and MAG Koffas, Improvement of catechin production in *Escherichia coli* through combinatorial metabolic engineering, *Metab Eng* 28 43–53 (2015)
45. JA Jones, VR Vernacchio, AL Sinkoe, SM Collins, MHA Ibrahim, DM Lachance, J Hahn and MAG Koffas, Experimental and computational optimization of an *Escherichia coli* co-culture for the efficient production of flavonoids, *Metab Eng* 35 55–63 (2016)
46. JJ Turnbull, J-i Nakajima, RWD Welford, M Yamazaki, K Saito and CJ Schofield, Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: Anthocyanidin synthase, flavonol synthase and flavanone 3β-hydroxylase, *J Biol Chem* 279(2) 1206–1216 (2004)
47. R Martí-Arbona, M Teshima et al, Identification of new ligands for the methionine biosynthesis transcriptional regulator (MetJ) by FAC-MS, *J Mol Microbiol Biotechnol* 22(4) 205–214 (2012)
48. BF Cress, QD Leitz et al, CRISPRi-mediated metabolic engineering of *E. coli* for O-methylated anthocyanin production, *Microb Cell Fact* 16(1) 10 (2017)
49. Y Zhang, E Butelli and C Martin, Engineering anthocyanin biosynthesis in plants, *Curr Opin Plant Biol* 19 81–90 (2014)
50. V Passeri, R Koes and FM Quattrocchio, New challenges for the design of high value plant products: Stabilization of anthocyanins in plant vacuoles, *Front Plant Sci* 7 153 (2016)
51. JA Jones, VR Vernacchio et al, Complete biosynthesis of anthocyanins using *E. coli* polycultures, *mBio* 8(3) (2017)
52. H Zhang and X Wang, Modular co-culture engineering, a new approach for metabolic engineering, *Metab Eng* 37 114–121 (2016)
53. Z Fang, JA Jones, J Zhou and MAG Koffas, Engineering *Escherichia coli* co-cultures for production of curcuminoids from glucose, *Biotechnol J* 13(5) e1700576 (2018)
54. *http://nutrasorb.com/downloads/Product-Information-Rutgers-Scarlet-Lettuce-Extract-CS.pdf* (Accessed Jun 12, 2018)
55. *https://phys.org/news/2017-06-strains-bacteria-pigment-food-cosmetics.html* (Accessed Jun 12, 2018)

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