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A Unique Eco-designed Process for an Innovative Natural Active Ingredient

L. Verzeaux, C. Chauprade, C. Soulié, S. Richer, L. Marchand, E. Aymard, B. Closs

Introduction

For over 35 years, Nature has been at the heart of the company business. Each SILAB active ingredient starts its history as a natural raw material, a source of active molecules capable of endogenously reactivating the skin's original resources. For each development, SILAB guarantees a perfect mastery of natural, from the raw material up to the manufactured ingredient, corresponding to the Mastering natural® program specifications. The resulting active ingredient is natural with no compromise on safety, quality and efficacy. These 3 parameters are considered very early in each active ingredient development.

The neck and décolleté area symbolizes femininity and needs to be cosseted with the application of adapted beauty care. This fragile area, exposed to the effects of time, gravity and external stresses, shows evidence of the early signs of aging. However, there is a lack of care dedicated to this body area.

The aim of this study was to develop a natural active ingredient, which is safe, of high quality, with identified active molecules and effective on biological markers involved in the appearance of sagging neck and décolleté skin.

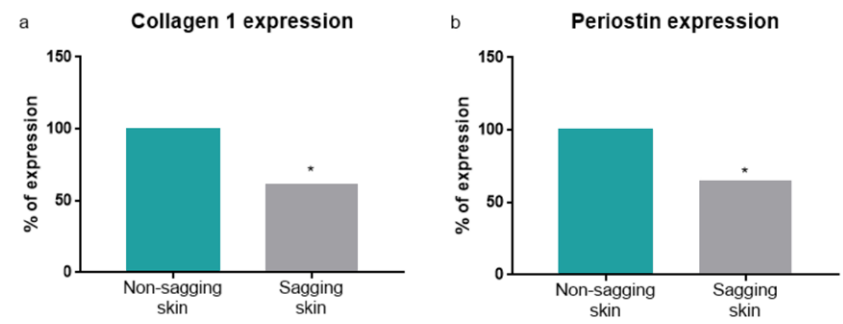


Fig. 1 Collagen 1 and periostin expression by fibroblasts isolated from non-sagging and sagging skin. a) Collagen 1 expression investigated by Q-PCR. b) Periostin expression investigated by Q-PCR.

Results

I. Identification of the Neck and Décolleté Needs through Modelling

After investigating *in vivo* sagging neck and décolleté skin with innovative tools [1], this skin area was investigated at the functional level.

The structure of the extracellular matrix (ECM) is based on a protein network governed by what is called the matrisome. The matrisome is defined by the set of genes coding for the structural components of the ECM (core matrisome), as well as those coding for proteins that can restructure or interact with the matrix.

The core matrisome is composed of collagens, proteoglycans and glycoproteins [2]. In human skin, collagen proteins are the most abundant, accounting for more than 90% of its dry weight [3]. This network of collagen fibrils ensures the skin's properties from tension and resistance to deformations [2]. Proteoglycans are proteins to which glycosaminoglycans are bound. These proteins are intercalated in the collagen network, where their physicochemical characteristics enable them to bind to water molecules and also to growth factors or secretion factors of the ECM. Finally, glycoproteins composing the core matrisome carry out a number of functions, in particular in the assembly of the ECM, binding to growth factors and promoting intercellular adhesion of fibroblasts.

The proteins associated with the ECM include growth factors and enzymes that modify the matrix network [4].

SILAB decided to investigate changes of the matrisome when neck and décolleté skin begins to sag. The expression of 30 matrisome genes was therefore determined in fibroblasts from donors with or without sagging neck and décolleté skin. This analysis showed the deregulation of expression of 11 of these genes within sagging skin. These genes code for both the matrisome of the dermis and the dermal epidermal junction (DEJ). Among them, the collagen 1 and periostin, respectively a main component of the ECM and an architectural protein favouring collagen cross-linking, are two biological markers representative of biological changes occurring within sagging neck and décolleté skin.

Results in **Fig. 1** demonstrate that fibroblasts isolated from sagging skin display a significant decrease of the expression of collagen 1 by 39% ($P < 0.05$) (Figure 1.a) and of perios-

Fig. 2 Picture of jojoba seeds on a shrub.

tin by 35% ($P < 0.05\%$) (Figure 1.b) compared to fibroblasts isolated from non-sagging skin. These two biological markers were then used to guide the process development in terms of efficacy.

II. Development of a Natural Active Ingredient

A. The Natural Raw Material: Jojoba

SILAB paid attention to jojoba *(Simmondsia chinensis),* a plant known for its multiple cosmetic properties. Jojoba, also named "desert gold" or "the plant that is never thirsty", with a lifespan of more than a century, displays remarkable adaptation properties [5,6]. Originally from Mexico, this resistant plant grows in extreme conditions, where annual rainfall are only about 200 to 250 mm with a temperature up to 54°C in the shade. Thanks to its long roots, jojoba is able to capture moisture from the lower layers of the soil. This shrub is usually thick and bushy, with elongated blue-green leaves fitted with a thick, waxy cuticle. Although leaves are renewed every 2 to 3 years, the plant remains green throughout its existence. After 5 to 10 years, the female plant produces hard brown seeds, similar to hazelnuts (**Fig. 2**). For the new plant to grow, the seed must contain a reserve of energy, in the form of proteins, carbohydrates or lipids. At the heart of these seeds, jojoba contains a precious oil rich in cerides (*i.e.* esters of fatty alcohol and a fatty acid). This is the first plant discovered to be able to produce these molecules, previously found in the blubber. Jojoba oil is currently used in cosmetics for its many benefits such as hydration, anti-seborrheic effect, anti-dandruff, etc. This oil is obtained by cold-pressure, generating the jojoba oil cake. SILAB, as expert of natural for more than 35 years and committed to its sustainable development policy, decided to add value to this co-product by isolating natural molecules capable of restoring firmness and tone to sagging neck and décolleté skin.

To ensure a perfect traceability of this raw material, SILAB selected a producer in the Negev desert. This supplier is both producer and transformer and controls the quality and traceability of the entire production process. 25 years of experience support the producer's technological prowess and policy

of sustainable management in order to use the best methods at each step of culture and transformation process. These environment-friendly agricultural practices favor renewable energies and the reuse of water, as well as minimize the use of crop protection products. In addition, the rigorous selection of varieties best suited to growth in the Negev desert contributes to the fight against desertification. This considerable know-how has been used to promote and improve the culture and transformation of jojoba.

B. Establishment of a Unique Manufacturing Process

Solvent: as water makes up 70% of the body and the skin, it is quite naturally that SILAB uses it as principal extraction solvent for its natural active ingredients.

Evaluation criteria: many eco-designed processes were developed and their evaluation were based on dedicated criteria. Among them, 4 processes were detailed in **Fig. 3** to illustrate the following experimental approach:

- *Quality:* stability of physico-chemical parameters were monitored over time to answer the SILAB's and cosmetic market quality standards: light color, odorless and efficacy of active molecules;
- *Efficacy:* each candidate was evaluated on the 2 biomarkers previously identified: collagen 1 and periostin;
- *Safety:* SILAB's toxicologist evaluated the natural raw material, innocuousness and absence of toxic molecules in the final candidate.

C. Experience Plan

With the water solubilization, the resulting candidate 1 displays no efficacy regarding neither collagen 1 nor periostin expression (data not shown). This solubilization step does not seem to be sufficient to release active molecules probably embedded in their natural structure.

Based on its long experience in enzymatic hydrolysis, SILAB decided to use enzymes in order to optimize the process and release molecules of interest. To this end, 4 enzymes were selected among an *in house* enzymatic toolbox containing more than 40 enzymes perfectly mastered. As proteins and carbohydrates (starch, pectic compounds, etc.) are the ma-

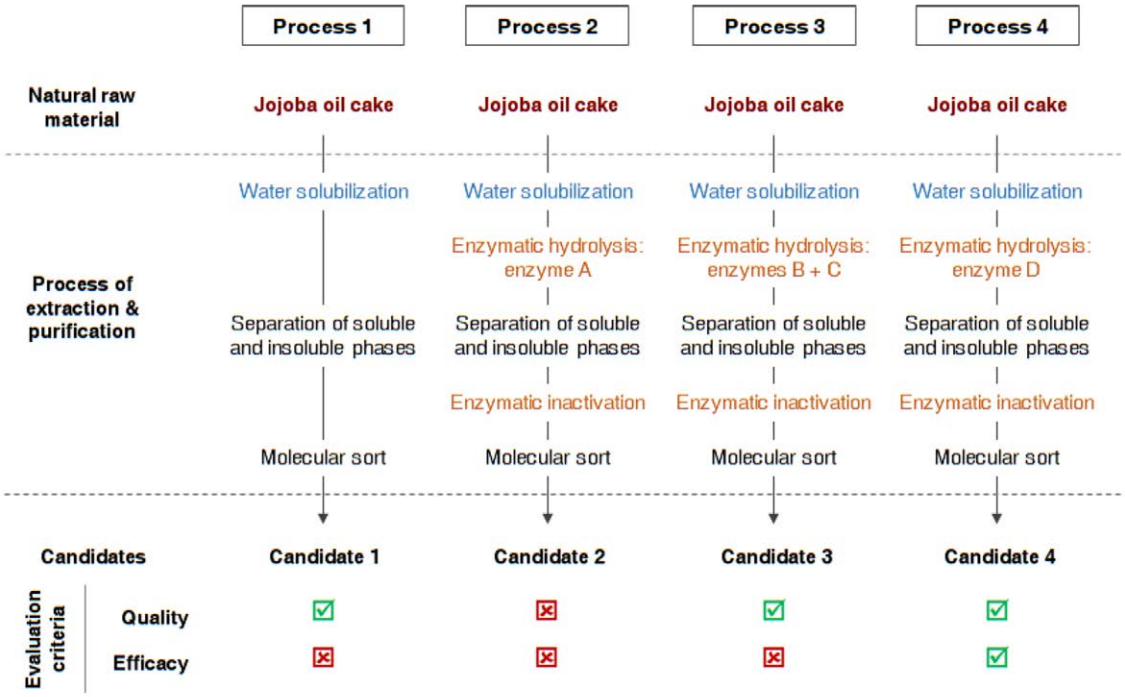
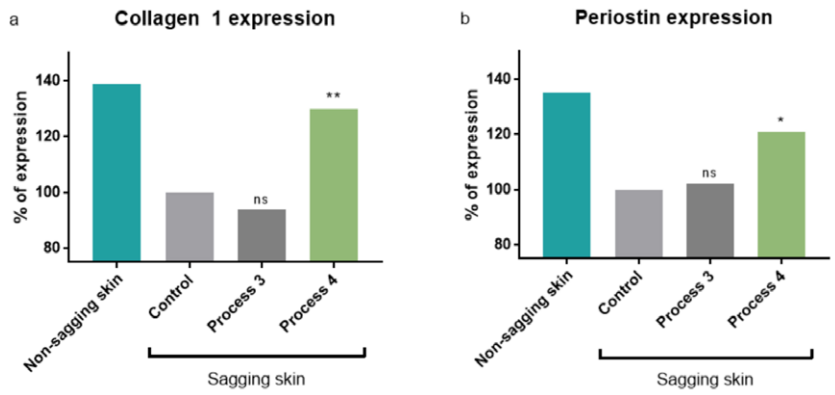


Fig. 3 Schematic representation of 4 eco-designed processes.



*: significant results according to the signed ranks test/sagging skin control (P < 0.05)
**: significant results according to the signed ranks test/sagging skin control (P < 0.01)
ns: non-significant result/sagging skin control

Fig. 4 Collagen 1 and periostin expression by fibroblasts isolated from non-sagging and sagging skin submitted or not to molecules extracted with process 3 or process 4. a) Collagen 1 expression investigated by Q-PCR. b) Periostin expression investigated by Q-PCR.

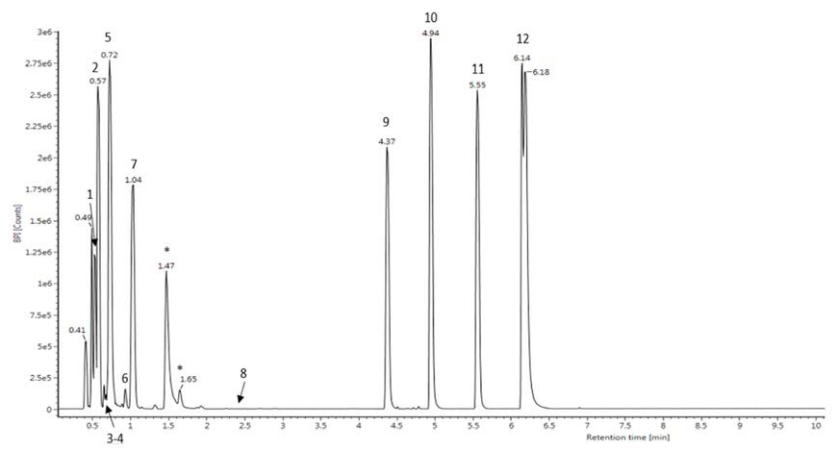


Fig. 5 Chromatographic profile of molecules isolated by process 4 and principal carbohydrate components identified. (* non-carbohydrate components)

Molecular Family	Possible Attribution	Chromatographic Peak
Uronic acid	Galacturonic acid	1
Disaccharides and derivatives	Methylgalactinol*	2
	Digalactosyl glycerol	3
	Sucrose*	5
	Methyl β-xylobioside	8
Trisaccharide	Galactotriose*	4 - 6 - 7
Simmondsine and derivatives	Didemethyl simmondsine*	9
	Demethyl simmondsine*	10 - 11
	Simmondsine*	12

Tab. 1 Possible attributions of principal species detected in the carbohydrate fraction of molecules isolated by process 4 (* compounds described in publications on *Simmondsia chinensis*).

jor components of jojoba seeds, the jojoba oil cake was submitted to an amylase (process 2), to a carbohydrase and a protease (process 3) or to another carbohydrase displaying different activity (process 4).

With the process 2, the resulting candidate is not stable over time to physicochemical parameters evaluated.

Results revealed that enzymes B and C do not allow to extract molecules increasing neither the collagen 1 (**Fig. 4a**) nor the periostin (**Fig. 4b**) expression. Processes 2 and 3 were thus stopped at this step of development.

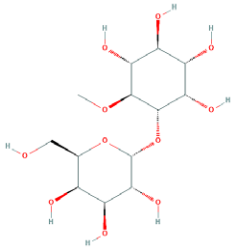
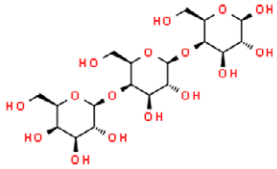
Very interestingly, enzyme D extracts molecules from jojoba oil cake that significantly stimulate the collagen 1 and periostin expression by 30% ($P < 0.01$) and 21% ($P < 0.05$) respectively, restoring the expression level to the one of non-sagging skin.

The candidate 4 was the only one to answer SILAB's requirements in terms of safety, quality and efficacy. It was thus selected for further investigations.

D. Identification of Active Molecules by Metabolomics

In order to go further in the characterization of active molecules isolated in the process 4, a special attention was paid to the carbohydrate fraction. The principal species detected by mass spectrometry (MS) were identified by comparison of precise masses of the ions detected by MS and fragments obtained by MS/MS to molecules previously identified in publications on *Simmondsia chinensis.* Other attributions were made by comparing the results to theoretical spectra of molecules in databases. **Fig. 5** shows the chromatogram obtained and all results are summarized in **Tab. 1**.

The carbohydrate fraction of molecules isolated by process 4 is composed primarily of oligosaccharides and of simmondsine and its derivatives. A statistical metabolomic study by UPLC-MS/MS was conducted for molecules isolated by process 3, displaying no efficacy, and isolated by process 4, effective on collagen 1 and periostin expression. This enabled the identification of intensity differences of certain markers between samples. The analysis showed that the concentrations of certain

Characteristics	Compound No. 1	Compound No. 2
***m/z* ion detected (-ESI)**	355.1240	503.1612
Elemental composition	C13H24O11	C18H32O16
Possible Attribution	Methylgalactinol	Galactotriose
CAS No./ reference	pubchem 10713243	chemspider 395580
Structure		

Tab. 2 Details of the principal markers of efficacy within molecules isolated from jojoba oil cake with process 4.

galacto-oligosaccharides were much lower in samples obtained with process 3. Detailed attributions are listed in **Tab. 2**. These results reveal that concentrations of galactotrioses and methylgalactinol are clearly higher in process 4. Moreover, isolated molecules are inherent to jojoba oil cake since their presence is reduced in the leaves and none in its oil.

III. Substantiation of Isolated Molecules on Sagging Neck and Décolleté Skin

As the candidate obtained by process 4 meets all SILAB's requirements in terms of safety and quality as well as displays an efficacy on two biological markers, its efficacy on the 11 matrisome genes previously detailed to be involved in sagging skin was investigated.

Very interestingly, on the 11 genes identified to be altered in sagging neck and décolleté skin, expression of 10 of them was restored with candidate 4.

Tested on fibroblasts from sagging skin, candidate 4 acts on the dermal matrisome by significantly boosting the expression of several of these constituents (collagens I and III) and also of proteins participating in the construction and stabilization of the network (periostin, HAPLN1, lumican). This action results in the improved capacity of cells to produce a functional collagen I network (+73%) and also in a significant increase of overall density of dermal fibers at the scale of the tissue (+85%). The action of this natural active ingredient on the matrisome results in positive effects on the organization and density of the dermis, two indispensable factors for firming the skin.

Tested on fibroblasts from sagging skin, candidate 4 acts on the matrisome of the DEJ by boosting the expression and synthesis of its major components. This natural active ingredient significantly induces the expression of collagen IV (COL4A1: +67% and COL4A2: +48%) and of agrin, a protein that stabilizes the network (+63%). This action also increases the production of laminin 5 at the scale of the tissue (+114%).

Hence, tested *in vivo* on 19 women, candidate 4 restarts the production of constitutive proteins of dermis and DEJ, revitalizing this skin structure and restoring lost firmness and tone to the skin (data not shown).

Conclusion

The development of the resulting natural active ingredient, which was named MATRIBUST®, was based on three pillars governed by the Mastering natural® program. The first one corresponds to the selection and traceability of jojoba, a natural raw material known for its multiple properties. In addition, SILAB decided to add value to a co-product in the framework of optimizing the use of natural products. Secondly, the development of a unique manufacturing process allows to enrich galactotrioses and methylgalactinol with biological efficacy on the skin matrisome. This latter is based on the rigorous selection of a carbohydrase-type enzyme whose activity is adapted to the extraction of galactotrioses naturally present in the raw material. All steps of the manufacturing process are mastered at the industrial level. Choosing the co-product issued from jojoba seeds combined with a specific technological process makes the resulting isolated active molecules a unique eco-designed ingredient that answers expectations of the cosmetic market to treat the sagging of décolleté area. A safety evaluation dossier proving that its use is safe for human and environment accompanies this ingredient.

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Eco-systemic Fermentation – Creating Bio Progression

M. Wolf

abstract

WORESANA® and AURAFIRM® ingredients are produced utilising Woresan GmbH organic, eco-systemic fermentation technology and through several mechanisms of action help a number of skin conditions. These ingredients are cereal derived natural peptides using sustainable, organic plant-substrates and a highly controlled, proprietary eco-systemic fermentation process, which produces a variety of differential small peptide fragments with very broad biological activity.
WORESANA® and AURAFIRM® ingredients are clinically proven to maintain skin integrity and positively influence the skin microbiome.

Introduction

In times of steadily growing demand for natural cosmetic ingredients, the strong growth of this market continues. This demand is no longer limited to the raw materials but increasingly covers the method of production. Today's customer is no longer satisfied with the fact that the ingredients of their care products are derived from plants. Aspects such as sustainability, environmentally friendly cultivation of the substrate and their further processing are increasingly coming into the consumer consciousness. In addition, customers expect the effectiveness of their care products not to suffer, when using these eco-friendly ingredients and indeed want improved efficacy.
This trend has resulted in considerable efforts to implement biotechnology solutions from the food industry, which has faced similar customer demands at an earlier stage.
One of the methods to produce these new ingredients is through targeted fermentation.

Fermentation

The technique of targeted denaturation of various raw materials has been valued in the food industry for centuries. Properties such as increased shelf life, nutrient content, bioavailability of nutrients, breakdown of anti-nutrients as well as various sensory changes have made fermentation the method of choice in many different biotechnology applications. Fermentation is also becoming more and more of a focus for manufacturers of cosmetic ingredients.
There is a number of different fermentation methods.
Submerged fermentation techniques, where enzymes and other reactive compounds are produced and submerged in a nutrient broth are the first choice, as this method offers many additional benefits. The species of microorganisms being used is of considerable importance as individual bacterial species deliver very different chemical profiles and also have very specific operating envelopes within their growth conditions. A key choice is whether to use a single microorganism or a mixed multi-culture.

Single Strain Fermentation

The single strain fermentation method is used predominately, as it achieves excellent results in terms of bioavailability together with the creation of new constituents. In most cases, manufacturers use individual lactobacilli cultures or yeast cultures. This is a safe method of production as it provides reliable, predicable and reproducible results. This method does however reduce the possible opportunities that multi-cultures would offer. Therefore, the range of resultant molecules is likely to be reduced. and the overall utilization of the substrate tends to be poor.

Multi-culture Fermentation

Multi-culture fermentations offer several advantages over single-culture as they are likely to include more complex multistep molecular conversions and individual microorganisms will metabolize the pre-digested compounds of another microorganism. The Process requires the manufacturer to identify the optimum starter by trial, and then develop process conditions to ensure that the resultant mixture can successfully competitive against contaminants. It has been seen that compounds made by this mixture of microorganisms often complement each other and work to the exclusion of unwanted microorganisms.
Multi-cultures improve utilization of the substrate which for fermented ingredients remains a complex mixture of carbo-

hydrates, proteins, and fats. Mixed cultures possess a wider range of enzymes and are able to attack a greater variety of natural compounds. Likewise, with proper strain selection they are better able to change or destroy toxic or noxious compounds that may be in the fermentation substrate. The addition of a symbiotic species that supplies the growth factors is a definite advantage in terms of stability.
But this method can be counterproductive in achieving reliable, constant product quality [1–5].

Eco-systemic Fermentation

The core idea is to combine the advantages of a reliable, reproducible and efficient single-strain fermentation with the strengths of better substrate utilization and resulting in a broader component spectrum of a multi-strain fermentation (**Fig. 1**).
The first prerequisite for this type of technique is a substrate-specific multi-culture which is likely to be highly complex and remain batch identical. The development of this type of culture is based on the selection of a number of specific effective cultures, which originate from an existing large culture database held by a fermentation company. Within this database original sourdough cultures have been collected over a very long period (decades) and then exactly preserved through meticulous regeneration, ensuring the profile remains unchanged. The database was initially created in the late 70's as it was recognized at an early stage that these aged multi-cultures were of significant effect and therefore valuable, although they were originally derived from spontaneous changes in the fermentation process. These selected multi cultures have been further diversified, deliberately exposing it to exogenous and endogenous challenges until the required state of stability and complexity had been achieved.
The other main advantage of this fermentation technique is the ability to extensively control environmental operating envelope during the actual production process. The complex operating envelope can only be achieved by specialized pro-

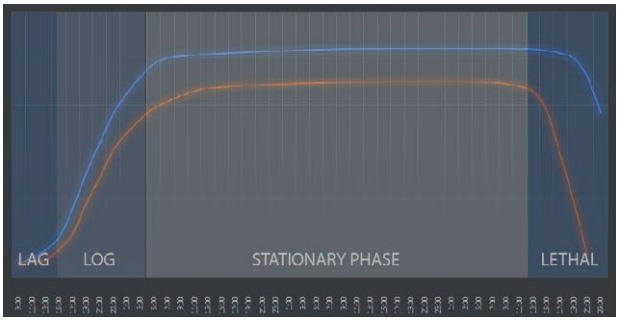

Fig. 1 Comparison of microbial development phases of a microbial multi-strain culture under the influence of 5% rye-based lactobacillus (blue) ferment to untreated microbial multi-strain culture (orange).

cess technology. The process is automatically changing certain parameters depending on the multi-culture and substrate used. By doing this the interactive behaviour of the diverse species is kept at a high level, forcing the culture to adjust the production of bio-actives to keep the balance correct within their ecosystem. For each substrate, clear analytical specifications and specific programmable production protocols exist for optimal utilization and effective production of bio-active compounds.

The last significant parameter is the control of the time frames as each individual microbial cycle takes place. Starting with the attachment phase, through the exponential growth phase, through transition to the stationary phase and finally the lethal phase, which is brought about by pasteurization, each phase has a carefully controlled timeframe.

During each phase microorganisms do metabolize certain compounds from the substrate to different metabolites. During the attachment- and exponential growing phase they mainly metabolize easy accessible nutrients to perform reproduction and environmental conditioning e.g. biofilm formation, pH-adjustments and temperature control. Compounds metabolized during this time are so called primary metabolites. The production of secondary metabolites is reasonable in depleting of easy accessible nutrient sources. Because of that microorganisms have to perform enzymatic multistep conversions of polymers to ensure adequate supply of food. To perform those effectively it becomes necessary to interact. Those interactive behaviours can be of commensal, competitive, predative, neutral, amensalism or cooperative nature. They are not exclusively direct but can also be indirect e.g. stimulation/modulation of the immune defence. These interactions are performed by numerous molecules.

Conducting the eco-systemic fermentation in the right manner results in a compound mixture of pre-, pro- and postbiotic activity, accompanied by many additionally tissue nourishing ingredients.

In the following section, the eco-systemic fermentation approach and its benefits are described in more detail. Please, note that the below-stated compounds do represent only a fraction of bio active components involved in these processes.

Prebiotics

Extracellular Polymeric Substances (EPS)

EPS are natural polymers of high molecular weight secreted by microorganisms into their environment. EPS establish the functional and structural integrity of biofilms and are considered the fundamental components that determine the physiochemical properties of a biofilm. The EPS layer acts as a nutrient trap, facilitating bacterial growth. Extracellular polymeric substances (EPS) serve as a biopolymer to protect cells from external environment and serve as energy and carbon sources for food deprived cells.

AHAs

Strains of lactobacilli can produce α-hydroxy acids (AHA's) to exhibit pH-adjustments and antibacterial activity against most dermal pathogenic bacteria. AHAs are widely used as exfoliators and are effective on desquamation of the skin. In addition, AHAs can improve the stratum corneum barrier function and enhance the production of ceramides by keratinocytes.

Ectoin

Ectoin is a natural substance which is produced by bacteria to protect against extreme conditions. It has cell-protecting, anti-inflammatory, nourishing and membrane-stabilizing properties. It is an effective long-term moisturizer that prevents dehydration of the epidermis. It can also be used at relatively low concentrations as a whitening agent because of its inhibitory effect on melanin synthesis. Ectoin also alleviates skin inflammation and is currently recommended for the treatment of moderate atopic dermatitis. In addition, ectoin strongly absorbs ultraviolet (UV) radiation and protects DNA from breaking down in some cell types.

Phenolic Compounds

Polyphenols are secondary metabolites with antioxidant, anti-inflammatory and antimicrobial activity. Phenolic acids are key class of polyphenols. They exhibit a variety of functions including growth, development, and defense mechanisms. They are precursors of other significant bioactive molecules regularly used for therapeutic purposes and cosmetics.

Bacteriocins

Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Research suggests that bacteriocins contribute to the modulation of the skin microflora, skin lipids and the immune system, leading to the preservation of natural skin homeostasis. Bacteriocins may represent an opportunity to cure infections caused by multi-resistant bacteria. They are currently used against Propionibacterium acnes and as immune modulators.

Short-Chain Fatty Acid

Lactobacilli and bifidobacteria are not only protective in many ways, they are also immunologically active. There is evidence that commensal microbes affect the immune system via expansion of regulatory T cells (Tregs) on the skin. This is mediated via short-chain fatty acids, bacterial metabolites generated during fiber fermentation. They suppress excessive inflammatory responses by stimulating the formation of regulatory T cells. Regulatory T cells form the messenger Interleukin 10, which has a balancing effect on the various immunological processes.

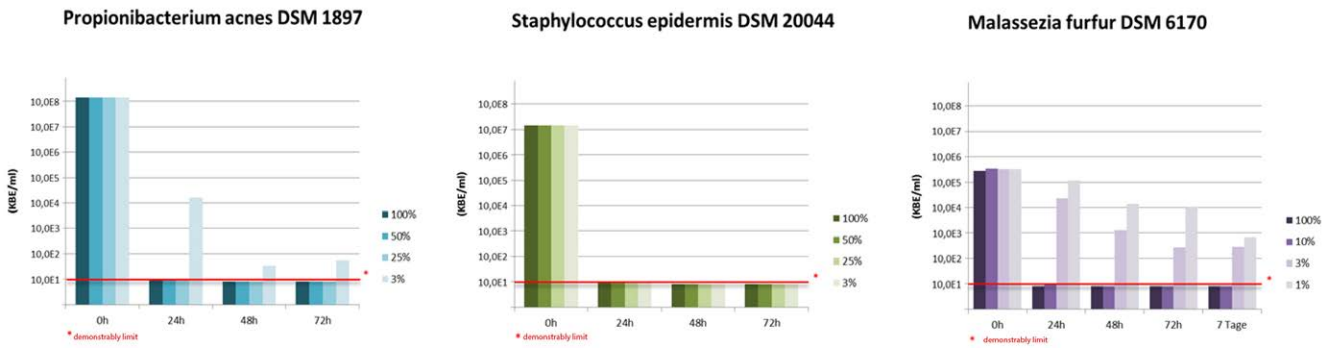

Fig. 2 Antimicrobial Activity

Lipoteichoic Acids and Peptidoglycan

Lipoteichoic acid (LTA) and Peptidoglycan (PG) are structural components of the cell walls of Gram-positive bacteria and play a vital role in the growth and physiology of the bacteria. Upon topical application, LTA and PG have been found to stimulate skin defense against microbial threats via induction of toll-like-receptors (TLR). Activation of TLR triggers the release of soluble actives such as the antimicrobial peptides which assist in the maintenance of the sterility in the dermis.

Probiotics

Microorganisms

Lactobacilli and bifidobacteria can inhibit pathogen attachment to epidermal cells of the skin, even in non-viable state by blocking attachment surface and attracting bacteria of the same or similar species (**Fig. 2**). Furthermore, data indicates that heat-killed bacteria, their fractions or purified components that have key probiotic effects, with advantages versus live probiotics (mainly their safety profile).

Sphingomyelinase

Sphingomyelinase (SMase) is an enzyme that generates a family of ceramides and phosphorylcholine from glucosylceramide and sphingomyelin precursors for the development of extracellular lipid bilayers in the stratum corneum. SMase activity has been demonstrated to be important for skin barrier function.

Diacetyl

Strains of lactobacilli and bifidobacteria can produce diacetyl suggesting its potential to exhibit dermal antimicrobial activities, with greater sensitivity against Gram-negative bacteria and fungi as compared to Gram-positive bacteria [6, 7].

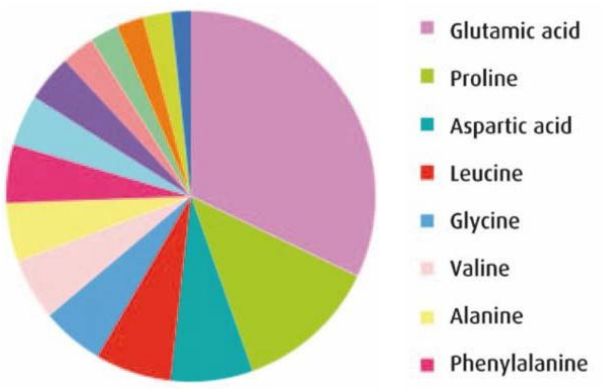

Fig. 3 Lactobacillus Rye Flour Ferment/Filtrate Amino Acid Composition (%)

Postbiotics

Amino Acids

Amino acids are the building blocks that form polypeptides and ultimately proteins. Consequently, they are fundamental components of our bodies and vital for physiological functions such as protein synthesis, tissue repair and nutrient absorption. Microorganisms must synthesize amino acids in order to grow, develop and perform all routine metabolic functions.
Microbes have a remarkable capacity to build amino acid frameworks that are not incorporated into proteins. It has been estimated that about 500 naturally occurring amino acids have been identified to date, leaving the 20 proteinogenic amino acids as the 4% minority. While some of the nonproteinogenic amino acids are utilized as intermediates in primary metabolic pathways, most of the unheralded 96% majority serve as building blocks for small bioactive peptide scaffolds. They may represent an underutilized inventory of building blocks for protein engineers, medicinal chemists, and materials scientists (**Fig. 3**) [8].

Bioactive Peptides

There is an expanding focus on bioactive peptides because of their health benefits, and they have been considered as the new generation of biologically active regulators. Peptides are involved in the modulation of cell proliferation, cell migration, inflammation, angiogenesis, melanogenesis, and protein synthesis and regulation. Its high bioavailability and heat stable nature allow its use as cosmetic ingredient.

Summary

The eco-systemic fermentation takes into account the fact that until today science does not have a rough comprehensive understanding of the complex interaction between skin, microorganisms, immune system and further participants. Therefore, we do not attempt to mimic their solutions but use natures vast head start in terms of experience and development. Through integration of natural processes into technical systems, eco-systemic fermentation is capable to produce highly effective, safe, and innovative skincare ingredients. Additionally, this technique does fulfil customer demand regarding ecology, ethics, energy saving, material saving and renewable sourcing. Thereby offering comprehensive overall sustainability. The ingredients do affect the interplay between microbial, mechanical, thermal, physical and immunological functions of the skin by using compounds and formulations developed and produced by nature.
In addition, manufacturers of cosmetic products receive ingredients that can be beneficially used in numerous applications with most different purposes and forms. They are easy to formulate, have a short INCI despite many different components, perfect for relaunches, lowering the preservative content, do have a long shelf life.
Eco-systemic solution equals natures solution.

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Submicron Particles from Lipid Mixtures – Safe, Efficient Carrier Systems for Natural Whitening Agents

S. Digel, F. Olechowski, S. M. Pyo, O. Pelikh, C. M. Keck, R. M. Müller

abstract

The global facial care market offers numerous opportunities for enhancing the outward appearance of the facial area. For this, formulators have numerous cosmetic active ingredients at their disposal. However, a powerful active ingredient can only achieve the desired effect on the skin if it has the relevant bioavailability. The active ingredient must, therefore, be able to penetrate the skin efficiently and build up a specific concentration of the active ingredient there.
Glabridin is a natural whitening agent extracted from the liquorice root *(Glycyrrhiza glabra)*. It intervenes in the biosynthesis of the skin's own pigment (melanin) and lightens the skin. Its efficacy has been confirmed in numerous publications. However, it is difficult to formulate this active ingredient as it has a very low solubility in lipids. If attempts to incorporate the active ingredient homogeneously into the facial care formulation do not succeed, it will not achieve the relevant bioavailability. This is not the desired outcome given the high price of the active ingredient (five figure amounts per kg). Efficient, controlled penetration of the skin by Glabridin can be achieved by embedding Glabridin in submicron particles made of a semi-crystalline, unordered matrix of selected liquid and solid lipids. Furthermore, the lipid particles help to repair a damaged skin barrier by adhering to the skin thanks to effective adhesion. This adhesion offers the added advantage of prolonged release. The particles are stabilised in suspension and remain in a solid state even at skin temperature.

The Success of Whitening Products

A person's face is the gateway to the world. That's why facial care products that boast advantageous results on the skin, are used for various reasons by consumers, for instance to reduce lines, to mask irregularities or to provide moisture. In many cases the users would like to lighten their skin tone – either in specific areas (age spots or hyperpigmentation caused by scars, for instance by acne) or in larger-scale areas. Particularly on the Asian and African continents skin tone has an additional social component. There a lighter skin tone is deemed to be a sign of higher social status, success, health and beauty. Consequently, facial care products with lightening properties (known as whitening, lightening or brightening products) are particularly important there and account for a large share of the skin care market. In east and south-east Asia, 40% of women regularly use whitening products. In India 60% of skin care products have a whitening component [1]. Furthermore, it is estimated that the global whitening market will generate sales revenues of USD 31.2 billion by 2024 [2].

Pigmentation, Hyperpigmentation and Depigmentation

Melanin is the natural skin pigment which determines skin colour and the skin's sensitivity to UV radiation. The type and intensity of pigmentation mainly depend on two factors: the number, shape and distribution of melanin aggregates and the distribution of the chemical types of melanin. Here, a distinction is made between eumelanins (brown to black) and pheomelanins (yellow to red). Actual skin colour depends on the ratio between eumelanins and pheomelanins and not on the number of melanocytes (pigment-forming cells). Irrespective of skin colour every single person has the same concentration of melanocytes in his skin, but their degree of activity may vary. Today, we have identified around 150 different genes which are involved in the pigmentation process [3]. These genes are responsible for the formation off melanocytes, the biogenesis of melanosomes (organelles within the melanocytes in which melanin is formed), the transport of melanosomes and the biosynthesis of the various types of melanin. The pigmentation mechanism and ways

of reducing pigmentation are the subject of active research work (**Fig. 1**). The type and intensity of pigmentation are primarily regulated by genes but may also be affected by external factors, for instance solar radiation, medication and hormonal influences or result from inflammatory processes such as acne and injuries.

The pigmentation process (melanogenesis) mainly encompasses the following steps:

- Formation/differentiation of melanocytes
- Biogenesis of melanosomes
- Biosynthesis of various types of melanin from the amino acid tyrosine with tyrosinase as the key enzyme
- Transport or transfer of the melanin from the melanosomes via dendrites to the adjacent keratinocytes (skin cells)
- Distribution of melanin by the melanosomes in the epidermis

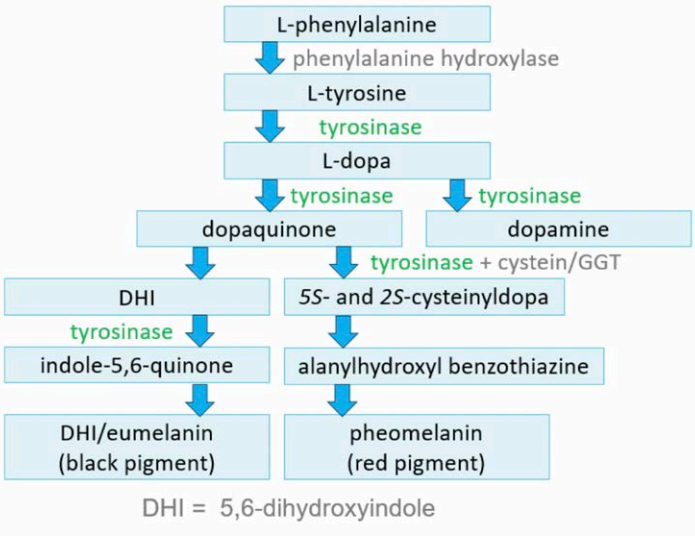

Fig. 1 Diagram of melanin synthesis. The enzyme tyrosinase plays a key role [4].

Whitening products are used either to treat pigmentation disorders or to lighten overall skin tone. The goal here is purely cosmetic. When what is known as hyperpigmentation occurs, more melanin than usual is present. Here are some of the possible causes [5]:

- **Post-inflammatory Hyperpigmentation**
  Increased pigmentation can occur as a consequence of inflammatory injuries to the skin caused by acne, ingrowing hairs, insect bites, scratches, chemical burns, etc.

- **UV Radiation**
  The well-known tanning of the skin when exposed to sunlight is probably also a consequence of inflammatory processes in the skin caused by UV damage. Pigmentation can take place via several pathways through stimulation of the melanocytes: for instance by means of inflammatory mediators such as Interleukin IL-1-α, Endothelin-1, through reactive oxygen species (ROS) and through certain hormones secreted by damaged epidermal cells.

- **Lentigo**
  Lentigo is the term used for various types of pigmented spots on the skin. It encompasses both classical moles and age spots or a condition known as lentiginosis where pigment spots occur locally and in clusters. They present primarily on areas of the skin exposed to the sun.

- **Melasma**
  Frequently, this condition manifests in the face as irregularly shaped spots and may be a consequence of chronic exposure to UV radiation or also hormonal changes (e.g. contraceptive pill or pregnancy). Consequently, it presents more in women. Furthermore, genetic predisposition also plays a major role whereas inflammatory processes are of minor importance here.

There are numerous whitening active ingredients which can reduce the concentration of melanin in the skin. The most important active ingredients are addressed in more detail below.

Hydroquinone

Given their structural similarity to melanin precursors (tyrosine or DOPA), this molecule and its derivatives have an inhibiting effect on the key enzyme tyrosinase. For a long time hydroquinone was the most popular active ingredient for the treatment of hyperpigmentation [6]. However, clinical studies have shown that it has cytotoxic activity and forms disulfide bonds with DNA and RNA resulting in cell death [7]. Since 2001 the use of hydroquinone in cosmetics has, therefore, been banned in the EU.

Vitamin C (ascorbic acid)

This active ingredient is used for numerous purposes in cosmetics [8]. Its skin-lightening properties are due to its impact on tyrosinase activity and the prevention of oxidation processes in melanin biosynthesis. Furthermore, vitamin C has other advantageous properties for the skin. It stimulates fibroblast renewal and collagen synthesis and has antioxidative properties [9]. However, the use of vitamin C is very restricted because of its extreme instability which can lead to degradation and discolouration. Stabilised derivatives such as ascorbyl palmitate have far lower activity but are used very frequently.

4-*n*-Butylresorcinol

This molecule has skin-lightening properties due to two activities: it inhibits tyrosinase activity and also TRP-1-(tyrosinase related protein) activity which plays a role in eumelanin synthesis [10].

Arbutin

It is a derivative of hydroquinone of natural origin. The whitening mechanism is described as competitive inhibition of ty-

rosinase activity. It was originally developed as an alternative to hydroquinone but still presents cytotoxic activity which is due to the release of a diphenol by means of hydrolysis. This released diphenol then oxidises directly into hydroquinone. Although it has fewer side effects than hydroquinone itself, its long term effects have not been sufficiently elucidated [11].

Kojic acid

This active ingredient is obtained from fermentation with species of *Aspergillus*. The whitening mechanism results from the inhibition of tyrosinase activity by complexing copper ions in the active site of the enzyme [12]. Its use in cosmetics is a subject of controversy as some studies have identified a risk of irritation and allergenic action. Furthermore, it acts as an endocrine disruptor and the use of this active ingredient on damaged skin is not recommended [13].

Glabridin – An Effective Whitening Agent

Glabridin (**Fig. 2**) is the main component in the extract of the liquorice root *(Glycyrrhiza glabra)*. It is used not only in skin care but also to treat non-dermatological disorders mainly because of its anti-inflammatory, anti-viral, antibiotic and anti-carcinogenic properties. On the skin, the active ingredient achieves good results in the treatment of hyperpigmentation even at low concentrations [14].

Glabridin inhibits the enzyme tyrosinase and thus lowers melanin production without having a cytotoxic effect. Furthermore, it inhibits inflammatory reactions which may be caused by solar radiation, by blocking the cyclooxygenase enzyme in the arachidonic acid cascade [15]. Glabridin therefore offers a double avantage which has been confirmed by several sources in scientific literature and is therefore undisputed [16–18]. Typical use concentrations in cosmetics are 0.05–0.14%. Moreover, one advantage of this active ingredient is that it is suitable for use in natural cosmetics because it is plant-based.

One general problem with the formulation of Glabridin is its extremely poor solubility both in oils and in water which means that only a small concentration gradient of dissolved

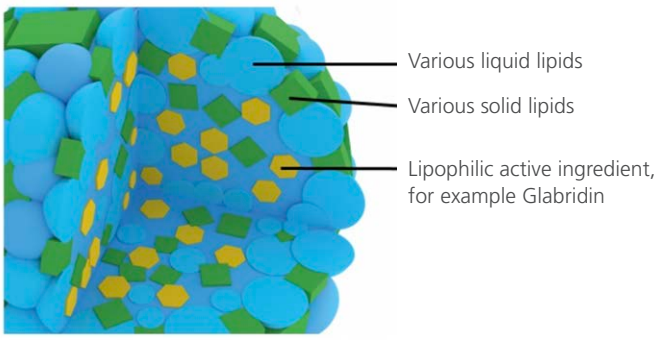

Fig. 3 Structure of a BergaBright SmartLipid, a lipid-based carrier system for active ingredients

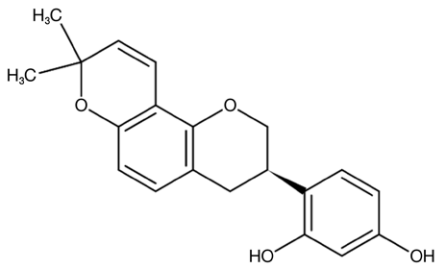

Fig. 2 Structure of Glabridin.

Glabridin can be formed on the skin. Consequently, the driving force of diffusion in the layers of the skin is very small and this limits its efficacy. Furthermore, Glabridin is a very expensive raw material (five figure amounts per kg for 95% powder). Consequently, there is considerable interest in fully exploiting the efficacy of this valuable active ingredient.

Submicron Lipid Particles (SmartLipids) – A Carrier System for Glabridin

The SmartLipids technology offers the possibility of optimising the efficacy of active ingredients that are difficult to formulate. It consists of an encapsulation system based on lipids that stabilise the active ingredients, facilitate controlled release, can normalise the skin barrier and can be easily processed.

The structure of a BergaBright SmartLipid is presented in **Fig. 3**. Various solid and liquid lipids, which are selected using comprehensive screening, form a matrix. A complex lipid mixture was chosen intentionally as it forms a chaotic structure that is unable to crystallise perfectly. The corresponding active ingredient, for example Glabridin, is embedded in this matrix. This is done during production using a patented process. BergaBright SmartLipids are very small particles with a particle size of between 0.1 and 1.0 µm – which explains the name submicron lipid particles. The disordered structure of the SmartLipids remains stable during storage and migration of Glabridin to the outer phase (water) is prevented. Thanks to this technology particularly high active ingredient loads can be achieved. These special particles were developed in collaboration with the pharmacist Prof. Dr. R. Müller from the Freie Universität Berlin. With the BergaBright SmartLipids Glabridin, Berg + Schmidt have translated an academic-scientific development into commercial use. They are available as aqueous suspensions. The individual particles are stabilised against agglomeration.

Special mention should be made here of the fact that the integrity of the particles, in contrast to other transport systems for active ingredients, can be determined both in the raw material (of the aqueous suspension) and in the formulation (e.g. serum). An easy-to-conduct DSC (differential scanning calorimetry) is used as the detection method. The presence of particles is proven through the detection of the corresponding melting enthalpy.

The melting process takes place in a range of around 50°C [19]. Furthermore, the stability of the matrix structure is proven by means of x-ray diffraction. Thanks to the combination of several liquid and solid lipids, the disordered structure is maintained over a long period whereas, in comparison, the lipid matrix with just one or two components presents a transition of the disordered α to a crystalline ordered β modification after just one month [20].

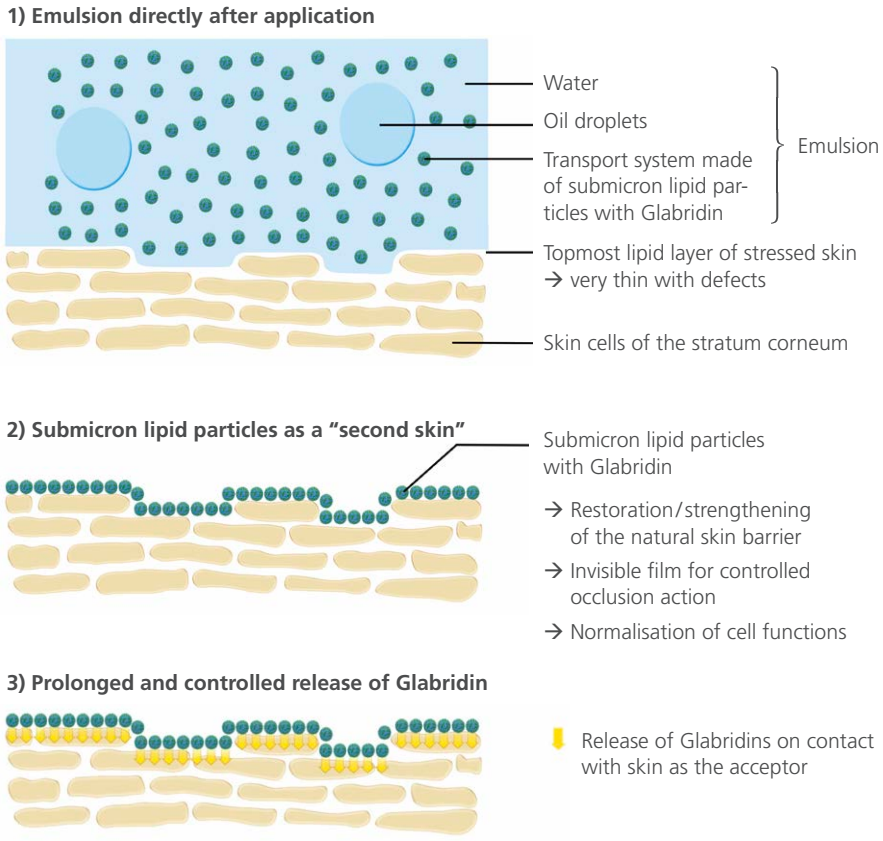

Fig. 4 Diagram of the dermal transport of Glabridin using submicron lipid particles as the transport systems.

Mode of Action of the Carrier Systems based on Submicron Lipid Particles

According to Einstein an exchange of matter between two interfaces takes place at different speeds depending on the physical state (liquid or solid) of the two systems in equilibrium with each other. At the solid-liquid interface (submicron particles in an aqueous suspension), there is a negligible exchange of matter. Glabridin is firmly protected in the matrix. Even after the lipid particles have been incorporated into a skin care product (cream, serum), the surrounding of the submicron particles is aqueous and the release of the active ingredient is negligible. However, if the product comes into contact with the skin, the particles form a thin film on the topmost layers of the skin. Like a second skin this has several benefits both for the condition of the skin and for the transport of the active ingredient.

The mode of action of the BergaBright SmartLipids loaded with the active ingredient is presented in **Fig. 4**. 1) After application of the formulation (in this case an emulsion), the aqueous phase evaporates within a few minutes. 2) The submicron particles position themselves evenly on the upper skin layers ("invisible second skin") and adhere readily to them due to their particularly small size. Any defects on the surface of the skin, which are often found in the case of stressed skin, are also covered by the particles and the skin barrier is strengthened. Furthermore, this SmartLipids layer has an occlusive effect and therefore reduces moisture loss. 3) The skin then acts as the acceptor for Glabridin. The concentration gradient acts as the driving force behind the diffusion of the Glabridin-charged particles into the skin containing little Glabridin. In the skin Glabridin develops its action and is used up which means that the concentration gradient is maintained. In this way each submicron particle gradually "empties" its active ingredient reservoir. The additional top-down occlusive effect likewise contributes to improved Glabridin penetration [21, 22].

Penetration Study and *in vivo* Effects

In order to investigate whether Glabridin from BergaBright SmartLipids really does penetrate the skin more effectively than in its original, commercially available powder form, a tape strip experiment was conducted (**Fig. 5**). For this, a suspension of Glabridin powder (95% purity) and Glabridin in encapsulated form as BergaBright SmartLipids Glabridin were used and compared. Both samples contained the same total amount of Glabridin. After a penetration time of 30 minutes, the skin was wiped and the actual tape strip experiment was conducted. The recovery rate of Glabridin was analysed for each strip. Already in strip number 4, no Glabridin from the powder suspension could be detected any longer (depicted in blue). In strip number 10, Glabridin from the BergaBright SmartLipids Glabridin (depicted in green) was still found. Consequently, the SmartLipids technology is proven to result in better and faster penetration with an elevated total penetration amount of Glabridin. Given that Glabridin must reach the melanocytes in order to have skin-lightening action, good penetration is of crucial importance.

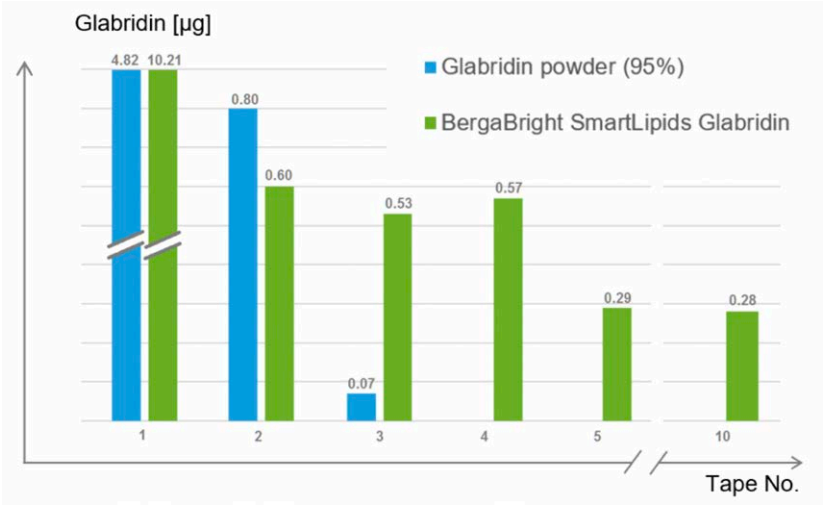

Fig. 5 Tape strip experiment with Glabridin in powder form and with encapsulated Glabridin in the form of submicron lipid particles.

The reduction of hyperpigmentation in the facial area was demonstrated in an *in vivo* experiment (**Fig. 6**). In this experiment a test person used a serum, formulated with 2% submicron lipid particles loaded with Glabridin, daily over a period of 12 weeks. At the end of the test period the pigmentation had visibly diminished.

Application

The submicron particles (BergaBright SmartLipids Glabridin) loaded with Glabridin are marketed by Berg + Schmidt GmbH & Co. KG as an aqueous, milky suspension (**Tab. 1**).

Summary

Glabridin is one of the most effective whitening active ingredients on the market. However, the extremely high price and the low bioavailability because of its poor solubility result in it being dependent on a suitable carrier system if the action of this valuable, natural active ingredient is to be optimised. This can be achieved by encapsulation in a complex mixture of liquid and solid lipids. The technology marketed as BergaBright SmartLipids consists of lipid particles of submicron size which only release the active ingredient when the particles reach the skin. They adhere very well to the skin which means they can withstand both friction and wetness. This results in the prolonged, gradual supply of the active ingredient to the skin. Thanks to its lipid nature and the good adhesion of the particles (like a "second skin"), the raw material is able to compensate for defects in the topmost skin layers, and to restore the protection barrier and the condition of the skin to their normal state. The particles are available as an aqueous suspension – BergaBright SmartLipids Glabridin and can be added into the corresponding formulation in the final production phase.

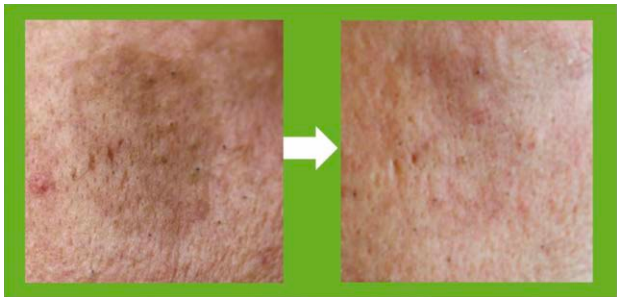

Fig. 6 Reduction of pigmentation in the facial area after 12 weeks of daily application of a serum with 2% BergaBright SmartLipids Glabridin.

Appearance
Aqueous, milky suspension with low viscosity

Incorporation
Simple admixture to the finished formulation

Recommended Dosage
Up to 3% of the suspension

Tab. 1 Technical framework data for Glabridin-loaded submicron lipid particles (BergaBright SmartLipids Glabridin).

Carrier systems with four different active ingredients are currently available on the market under the commercial names BergaCare SmartLipids Retinol, BergaCare SmartLipids Q10, BergaBright SmartLipids Glabridin and BergaBright SmartLipids NatWhite (lemongrass oil). The portfolio is also continuously being extended.

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The Influence of Natural Gelling Agents on the Foaming Behaviour and Foam Structure in Surfactant Systems

G. Marin Velasquez, M. Neubauer, T. Willers, V. Vill

abstract

The use of microplastics in cosmetic surfactant formulations represents an increasing ecological problem. As a natural alternative the polymers examined in this work, guar gum, cationic guar gum and xanthan gum can be used as thickening agent or as stabiliser of emulsions. The rheology changed by the polymer-based gelling agent also influences the foam properties as well as the stability of the product. This is why the compatibility of several natural polymers were analysed with two different surfactant systems (anionic non-ionic surfactant system and amphoteric non-ionic surfactant system) for the specific application parameters such as the foaming behaviour, the foam stability and the foam structure and its aging. The influence of electrolytes was also analysed on the example of sodium chloride (NaCl) and then compared with the foam properties of the polymer-based gelling agent. The foam analytics was carried out using the *Dynamic Foam Analyzer* DFA100 (KRÜSS GmbH). In doing so, the recently released Foam Flash Method is presented and used, a method that is particularly good for the analysis of the foaming behaviour of strongly foaming surfactant solutions.
The addition of the polymer-based gelling agent to the surfactant systems had a substantial improvement to the foam formation and the foam stability, and significant influence on the foam structure. The strongest effects were shown by xanthan gum. It is also shown that the influence of individual gelling agent on the foaming behaviour depends strongly on the characteristics of the surfactant system.

Introduction

Water-soluble and insoluble polymers are being used in personal hygiene products for 50 years. They are found in skin cleansing agents such as liquid soap and shower gel, in hair care products, toothpaste and make-up products [1]. Being microplastics, they are however increasing to represent a major problem for the environment. Polysaccharide gums such as xanthan gum and guar gum belong to the natural alternatives and are frequently used as thickening agent together with complex surfactant systems such as, in shampoos and shower gels [2, 3].
The thickness and flow properties of cosmetics are described by the term viscosity. The viscosity influences the cleaning performance as well as the perception of a product by the consumer. Electrolytes such as sodium chloride have an influence on the micelle formation which is why they are able to increase the viscosity of a surfactant system. This is why electrolytes are generally used to increase the viscosity of shampoos [4]. The rheology also influences the foam properties as well as the stability of the product. Cleaning care products have the property to form foam and air bubbles during use. Consumers perceive the foam as being a confirmation if the product acts effectively as a cleaning agent. With the development of cleansing formulations, the foam volumes, foam stability, foam structure and the sensory properties of the foam generated by the formulation are important criterion that could lead to the economic success of a finished cleaning product [5].

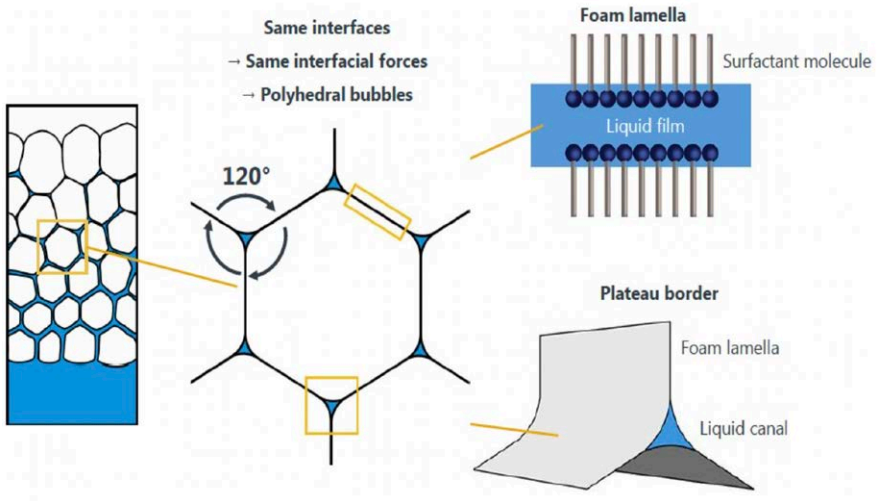

Fig. 1 Schematic structure of the ideal foam structure. Polyhedral foam structure, planar liquid lamella and plateau border according to Joseph Plateau [7].

Foam in Surfactant Solutions

Foam is the dispersion of a gas in a liquid or solid matter. This work is exclusively focused on the liquid foams. The foam structure is influenced decisively by the liquid content. With a high liquid content, the gas bubbles are circular-shaped and the liquid lamella between the bubbles is very thick (circular-shaped foam). With low liquid content, the gas bubbles are separated by thin and planar liquid lamella (polyhedral bubbles) (**Fig. 1**) [6, 7].

Materials and Methods

In the scope of this examination, two surfactant systems were analysed with 12% washing action. An anionic nonionic surfactant system (Ani-Ni-SS) based on 7% sodium laureth sulphate (SLES) and 5% coco glucoside and an amphoteric nonionic surfactant system (Amp-Ni-SS) based on 7% cocamidopropyl betaine (CAPB) and 5% coco glucoside.
The influence of the electrolyte sodium chloride (NaCl) and the influence of the polymer gelling agents xanthan gum (XG), guar gum (GG) as well as with the cationically charged guar gum (cGG) were examined on these surfactant systems. Due to the stability, the unloaded guar gum is used with Ani-Ni-SS and the cationic guar gum is used with Amp-Ni-SS. The concentration (wt/wt) of the polymer gelling agent was 0.5% and 1%, and the NaCl 0.5%. The formulations prepared in this way were diluted with bi-distilled water at a ratio of 1:10 and analysed with regards to their foaming behaviour using the *Dynamic Foam Analyzer* DFA100 (KRÜSS GmbH).

Foaming Behaviour

The formulations examined here are heavily foaming and as a result are therefore designated as "high foamer". High foamers are characterised by, amongst others, that after foaming with a controlled input of gas ("Sparging" method on the DFA100), cannot be differentiated with regards to their foaming properties. This is because each gas bubble that is generated on the frit/surfactant solution interface is occupied with surfactant molecules sufficiently and quickly enough, and thus is stabilised as foam bubble. If, e.g. 100 ml air are introduced to the solution via a frit, at least 100 ml foam volume is always created independent of the formulation of the respective high foamers. Many aqueous surfactant solutions with a concentration above the 10-fold critical micelle formation concentration are *high foamers*.
However, in order to still detect differences in the foaming properties of such high foamers, two alternative foaming methods are suitable: The Ross-Miles method and the stirring/Foam Flash method [8, 9]. The KRÜSS Foam Flash method is used here in order to determine the different foaming behaviour of the formulations. With this method, the solution is foamed on the base of a glass column by a stirrer during a

sequence of stirring intervals. In doing so, the measurement takes place in between the stirring intervals in order to eliminate the influence of the swirl formation during the stirring process to the measured foam height, see **Fig. 2**. The resulting curve (displayed in yellow) indicates a gradual increase in the foam height with each further stirring cycle.

A comparison of the slopes of the measurement curves obtained (yellow) in this manner therefore allows us to determine which high foamer represents a quicker and which a slower foaming behaviour. A comparison of the maximum (saturation) foam height also permits a statement of the foaming properties for a given stirring velocity.

The following parameters for foaming with the Foam Flash method were applied with the measurements shown here: 3,500 rpm stirring velocity, 2 seconds stirring time, 50 stirring cycles and 3 seconds measuring time between the stirring intervals.

Foam Stability and Structure

For determining the foam stability, 100 ml of the respective solution were prepared and stirred for 15 seconds at 3,500 rpm. Then the decay of the foam was observed over a time of 5 minutes. The total height, foam height as well as the liquid height were recorded by means of optical sensors. The foam structure was also observed using a prism (foam structure model of the DFA100).

Results and Discussion

Fig. 3 shows the foaming properties of both surfactant systems with the different gelling agents depending on the stirring time.

Without adding gelling agent or electrolyte, the foaming behaviour of the Ani-NI-SS is poorer than the Amp-NI-SS (see black curve in **Fig. 3**). *Bikermann et al.* show that the anionic surfactants have the tendency of the hydration of the surfactants; this can lead to a degradation of the foaming properties [10].

With Ani-Ni-SS, it can be observed that there is a large difference of the foaming properties between the samples with polymer gelling agents and the samples without polymer. After approx. 30 seconds, the samples with XG and GG achieve the maximum foam height. The Ani-Ni-SS without gelling agent, or that with NaCl show significantly lower values of the foaming properties in this time interval. They require a much longer stirring time (approx. 40 s) to achieve the maximum foam height.

With Amp-Ni-SS, the addition of polymer did not have any distinctive influence on the foaming property. After about

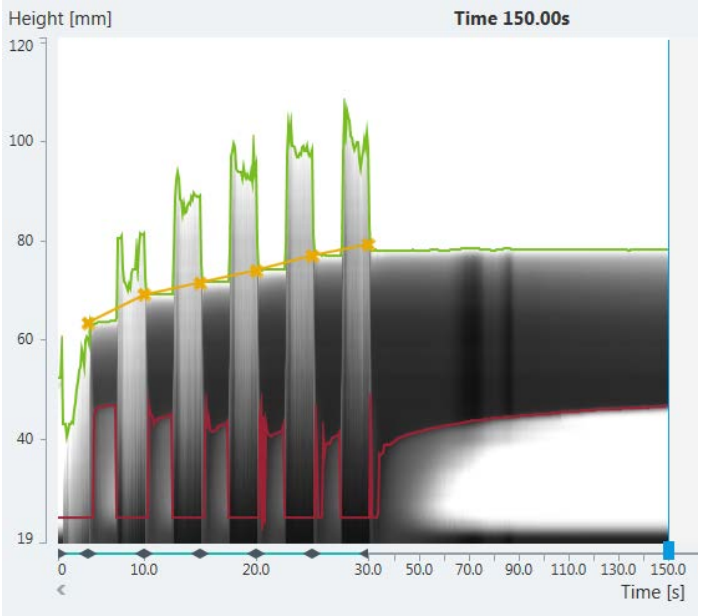

Fig. 2 Foaming behaviour using the KRÜSS Foam Flash method. The liquid foam boundary is represented in red and the foam/air boundary in green. The detected total height at the end of each stirring cycle is represented in yellow [9].

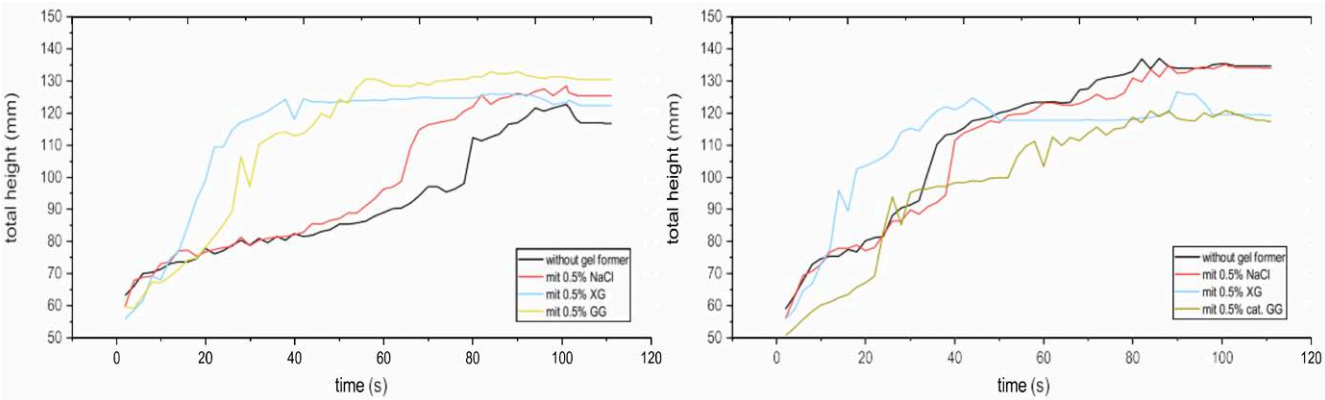

Fig. 3 Representation of the foaming properties depending on the time in a Foam Flash measurement. Left: anionic non-ionic surfactant system. Right: amphoteric non-ionic surfactant system. Black curve: without gelling agent, red curve: with 0.5% NaCl, blue curve: with 0.5% xanthan gum, yellow curve: with 0.5% guar gum, green curve: with 0.5% cationic guar gum.

40 seconds the sample with XG reached the maximum total height at approx. 120 mm. During this time, the samples without gelling agent and with NaCl reached a total height of about 118 mm up to a maximum value of 135 mm at 90 seconds stirring time.

The charging of the Ani-NI-SS in combination with the gelling agent seems to have a positive effect on the foaming behaviour. In doing so, the XG that is also charged negative shows a higher influence on the foam behaviour of the Ani-NI-SS than the uncharged GG.

Within the isoelectric point, the surfactant CAPB has a natural behaviour to the outside whereas in lower (below 3) or higher (above 6) pH values, the surfactant has an anionic, respective cationic character [11]. As the pH value of the Amp-Ni-SS is within the isoelectric range, the surfactant system does not correspond with a charged system. The polysaccharide gelling agents therefore do not have any significant influence on the foaming properties.

Foam Stability and Structure

For the analysis of the foam stability as well as the foam structure, the foam degradation was measured after the foaming process within a time interval of 5 minutes. The destabilising of the foam is accelerated by different processes, such as drainage of the liquid, coalescence of the foam bubbles and disproportionation of the bubbles [12]. **Fig. 4** shows the foam height and the two-dimensional bubble density depending on the time. Only the data after to the end of the foaming process of 20 seconds are shown.

The curve progression of the measurement of the Ani-NI-SS with the various gelling agents shows that the use of polymers has a significant influence on the foam stability and in particular, the foam aging thus, modification of the foam structure (bubble density). In contrast to the samples without gelling agent or with electrolytes where the foam aging starts immediately, the foam heights of the formulations with XG remain constant. Analogously, the bubble density behaves in the samples affected. Whereas the bubble density in the samples without gelling agent and with NaCl show a significant reduction of approx. 70%, the samples with XG behave stable. With the Amp-Ni-SS, with an exception of those with XG, all samples showed a quickly acting foam aging, thus a reduction of the bubble density and thus a post following decay of the foam. The sample with cGG thereby shows a foam stability at the beginning compared with the samples without gelling agent and with NaCl.

Noteworthy with this measurement is the significant difference of the foaming property between the XG samples where the sample with 0.5% XG has produced almost twice the foam volumes as with the samples with 1% XG.

After a greater proportion of the liquid in the foam has drained, the reduction of the bubble density is described by the typical aging processes coalescence and Ostwald ripening.

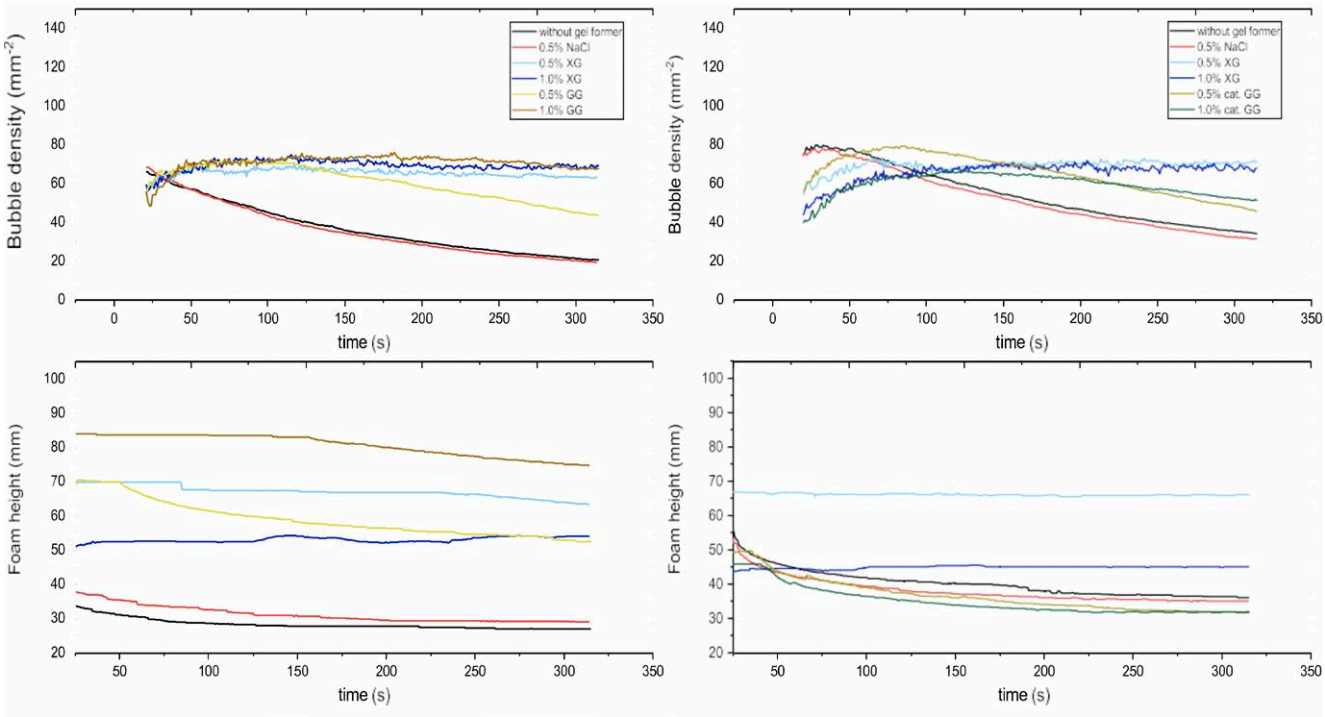

Fig. 4 Representation of the foam structure and the foam stability after foaming. Top: bubble density depending on the time. Bottom: foam decay depending on the time. Left: anionic non-ionic surfactant system. Right: amphoteric non-ionic surfactant system. Black curve: without gelling agent, red curve: with 0.5% NaCl, light blue curve: with 0.5% xanthan gum, dark blue curve: with 1.0% xanthan gum, yellow curve: with 0.5% guar gum, brown curve: with 1.0% guar gum, bright green curve: with 0.5% cationic guar gum, dark green curve: with 1.0% cationic guar gum.

In addition to the speed aging of the foam structure, a comparison of the initial foam structure is also interesting. Whereas the Amp-Ni-SS samples differ significantly with regards to their initial foam structure, the Ani-Ni-SS formulations show an almost identical initial foam structure (see also **Fig. 4** and **Fig. 5**)

Fig. 5 shows the time-dependent visual change of the foam structures of Ani-Ni-SS or Amp-Ni-SS. The foam stability of the samples can be explained by the results of the foam structure. It can be noticed that the results from Ani-NI-SS without gelling agent and with NaCl have shown the lowest stability and the lowest bubble density. The Amp-Ni-SS on the other hand shows a better foam stability and more stable foam structure with the sample without gelling agent and with NaCl. **Fig. 6** shows the bubble area of the respective samples. The bubble area of the Ani-Ni-SS without gelling agent and with NaCl increased significantly faster than with the other samples. This foam aging running much quicker also explains the must quicker decay (due to the large bubbles bursting) of the foam with NaCl and without gelling agent.

All samples with XG exhibit a particular foam stability originating from a large initial bubble density with corresponding small mean bubble areas and a slow aging (i.e. change) of this foam structure. In the mixture with *Xanthan Gum,* a mass aggregate could possibly have formed that thicken in the plateau boundaries of the foam and prevent drainage. The foam lamellae that are then thicker slow down the transport of gas through the liquid and thus, the Ostwald ripening, i.e. the aging of the foam structure.

The samples with XG showed a significantly higher viscosity, which was displayed in the master thesis of Marin, Hamburg University [13]. This, in combination with a reduced transport of gas through the liquid could explain why this sample is almost completely stable against disproportionation and coalescence [14, 15].

As the foams of the SS with XG almost do not age at all, this means that no Ostwald ripening and no coalescence takes place and thus, these foams will most likely also be the most stable ones over the long-term. The foam structure of Ani-NI-SS with GG shows a better stability than Amp-NI-SS with cGG.

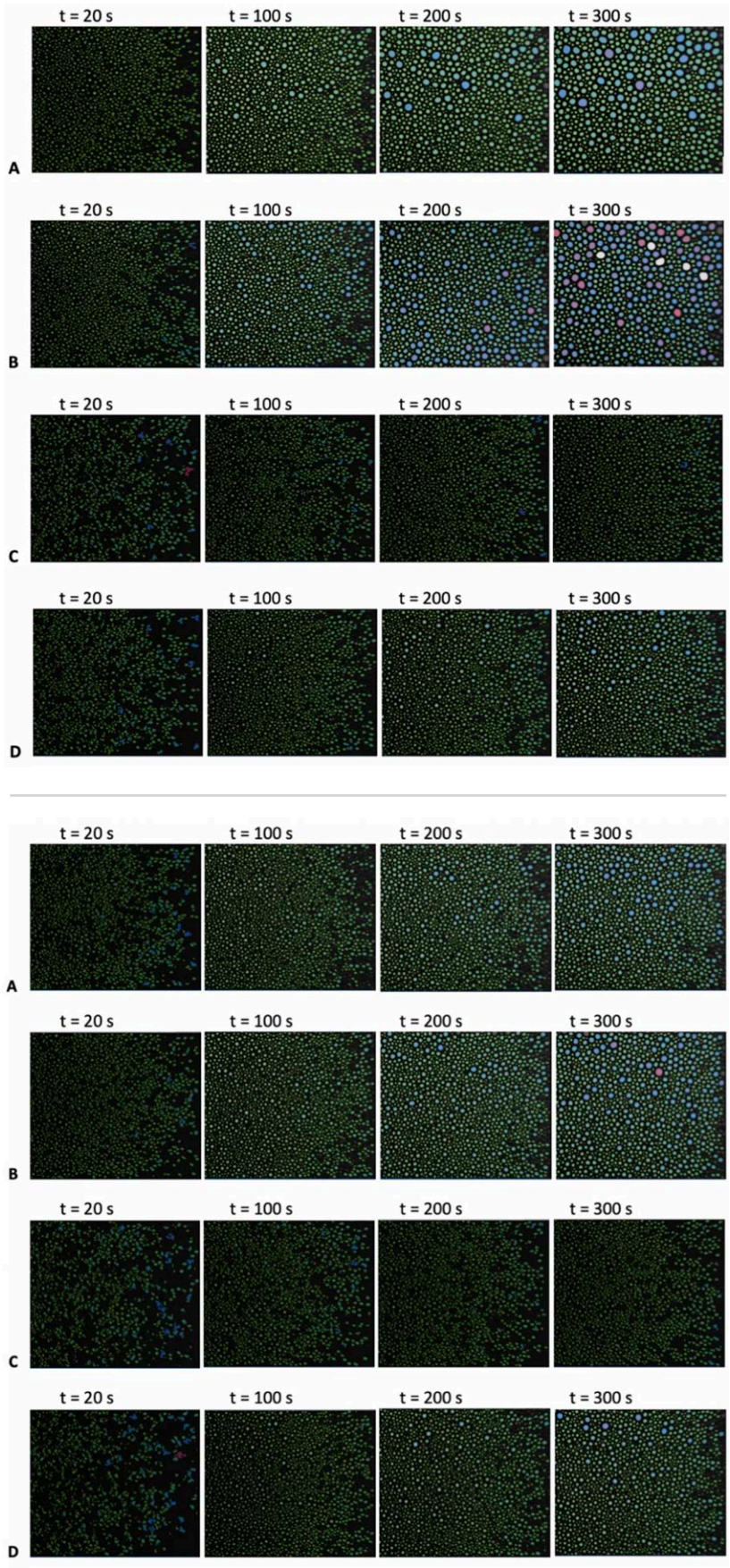

Fig. 5 Changing of the foam structure within 300 seconds. Top: anionic/non-ionic surfactant system. Bottom: amphoteric non-ionic surfactant system. A: without gelling agent, B: with 0.5% NaCl, C: with 0.5% xanthan gum, D: with 0.5% guar gum or cationic guar gum.

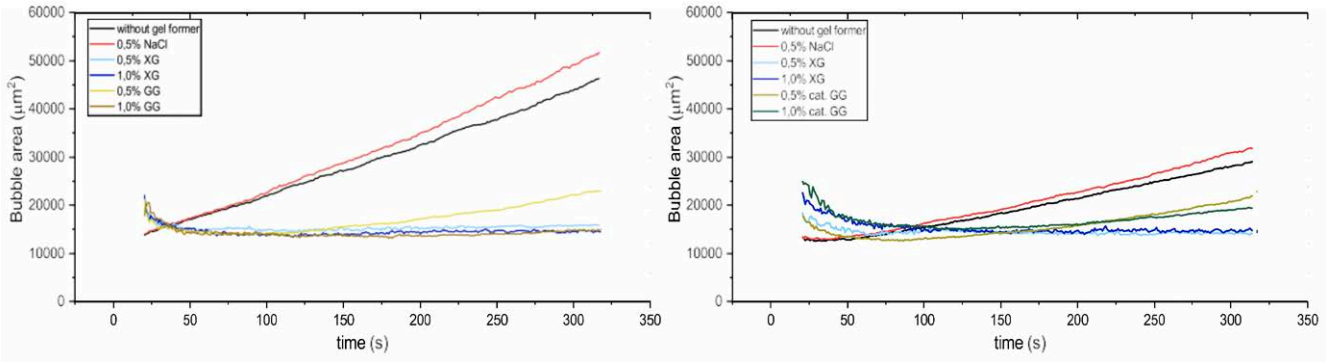

Fig. 6 Representation of the bubble area. Left: anionic non-ionic surfactant system. Right: amphoteric non-ionic surfactant system. Black curve: without gelling agent, red curve: with 0.5% NaCl, light blue curve: with 0.5% xanthan gum, dark blue curve: with 1.0% xanthan gum, yellow curve: with 0.5% guar gum, brown curve: with 1.0% guar gum, bright green curve: with 0.5% cationic guar gum, dark green curve: with 1.0% cationic guar gum.

Summary

Generally, the surfactant systems analysed in this work show a good foaming behaviour where the Amp-Ni-SS without gelling agent and with NaCl foam more than the respective samples of the Ani-NI-SS. For Amp-Ni-SS the foaming behaviour is largely independent from the selection of the gelling agent added. In contrast to this, it has been observed that with the Ani-Ni-SS, the selection of the gelling agent has a significant influence on the foaming behaviour.

It could also be shown that the polysaccharide polymers have a greater positive influence on the foam stability as well as the foam structure. In-particular, the samples with XG have shown the best foam stability compared to all samples.

Finally, it could be shown that for the foam stability, there is a direct dependency of the polymer gelling agent selected. The surfactant systems without gelling agent and with NaCl age quicker with regards to their structure and thus decay much quicker as hose with gelling agent.

The results presented in this thesis underline the significance of the optimum combination of surfactant system and gelling agent. By a careful selection and reconciliation of both components, it becomes clear that a positive effect on the foam behaviour, foam structure, foam aging and stability can be achieved in surfactant based cosmetic products.

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High Wavelength Blue Light Induced Damages in Human Skin

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abstract

In our hyper-connected world, we spend countless hours in front of digital screens emitting blue light (BL) with the general perception that this is safe for our skin, but the infiltration of electronic devices in our daily lives does not come without consequence. There is scarce data that touches on the effects of BL on human skin. Moreover, the data available so far has been performed on basic cellular models that are far from the complexity of human skin. This study focuses on the identification of the BL induced damage using 3D models. We found that direct BL exposure to the skin generates ROS leading to oxidative stress marker generation including protein carbonylation, nitrotyrosine, 4-hydroxynonenal. In addition, the expression of matrix metalloproteinase-1 which is responsible for extracellular matrix degradation during chronological aging and photoaging processes is increased. These data show that blue light emitted by electronic devices affect skin health and may be involved in premature aging.

Introduction

In our hyper-connected world, we spend countless hours in front of digital screens (TVs, computers, smart phones and tablets). The infiltration of electronic devices in our daily lives does not come without consequence even though there is the general perception that they are safe, LED exposure creates deleterious effects with measurable damage to our skin. Unfortunately, all current digital devices emit blue light in a spectral range from 400 to 490 nm with a peak around 450–453 nm (Rascalou *et al.*, 2018; Renard and Leid, 2016; Hamza and Sayadi, 2016). In a physiological context, the beneficial role of blue light exposure from the sun (visible light) is well known and is understood to regulate the normal circadian rhythm through successive interactions between the eye and nervous system. Indeed, the retina contains photoreceptors sensitive to blue light which are responsible for the entrainment of circadian rhythms. Unfortunately, the rise in populations subjected to daily artificial blue light (ranging from a few minutes to several hours) combined with the abnormal exposure during nocturnal hours is creating maladjustments in circadian rhythm that contribute to increases in sleep and metabolic dysfunctions (Gianluca Tosini, Ian Ferguson, Kazuo Tsubota, 2016). Further, the over exposure to the retina by artificial blue light at this emission spectra leads to deleterious damage. Specifically, it creates oxidative stress, alters the expression of inflammatory genes and contributes to photoreceptor death (Boulton *et al.*, 2001; Marek *et al.*, 2018).

The commonly referred to as light induced damage (Gianluca Tosini, Ian Ferguson, Kazuo Tsubota, 2016), this damage can be induced after exposure to lower doses of blue light as well (32 to 63 J/cm²) (Chu *et al.*, 2006). For example, Okuno *et al.* evaluated blue light hazards from a variety of different light sources and reports that blue light is hazardous to the retina even when exposure is less than one minute (Okuno *et al.*, 2002).

In contrast to the well described blue light induced eye damage, there is very little and only partial data that touches on the effects of blue light exposure to human skin or human skin cells. Indeed, the data only references blue light triggering the production of reactive oxygen species (ROS) in cells and modulating the expression of aryl hydrocarbon receptor target genes. Literature references conclude that blue light does not lead to cell apoptosis or necrosis and suggests that blue light emission by LEDs is safe for human skin cells (Becker *et al.*, 2016; Austin *et al.*, 2018; Avola *et al.*, 2019; Nakashima *et al.*, 2017). In contrast, at the tissue level, when an overproduction of ROS occurs after stress with UVB, the majority expect biological consequences to occur which include the damage of macromolecules such as proteins and lipids that lead to the impairment of signaling pathways which have important metabolic consequences for tissue homeostasis. Yet so far, similar damage is not reported or anticipated after skin exposure to blue light.

The data available so far has been performed on basic cellular models that are far from the complexity of human skin. This study focuses on the identification of the blue light induced damage using relevant 3D models. To simulate real life conditions and habits in populations regarding the use of digital screens, we have developed two experimental models which are based on modern assumptions for daily LED usage. The first model, referred to as the chronic model is intended to mimic frequent short periods of exposure to digital devices (less than 5 minutes) which are used to read and write text messages or e-mails. The second model, referred to as the

acute model is intended to mimic longer periods of blue light exposure to the skin by LEDs such as when spending hours in front digital screens to watch movies and read books (more than 6 hours). As previously mentioned, digital screens generate blue light with an emission peak around 450–453 nm. The spectral irradiance is defined as the power received per area around 36 µW/cm² for 100% brightness. Accordingly, the conditions of irradiation that we applied to human cells and skin explants with a light emitting diode (LED) have a similar spectral emission to reproduce the conditions that mimic daily exposure to screens (**Fig. 1**).

Here we report that direct blue light exposure to the skin generates ROS leading to oxidative stress marker generation including protein carbonylation, nitrotyrosine, 4-hydroxy-nonenal (4-HNE). These modifications dramatically impair biomolecular functions and signaling. In addition, the expression of matrix metalloproteinase-1 (MMP-1) which is responsible for extracellular matrix degradation during chronological aging and photoaging processes is increased. Altogether, the results of this study show that blue light emitted by electronic devices affect skin health and may be involved in premature aging.

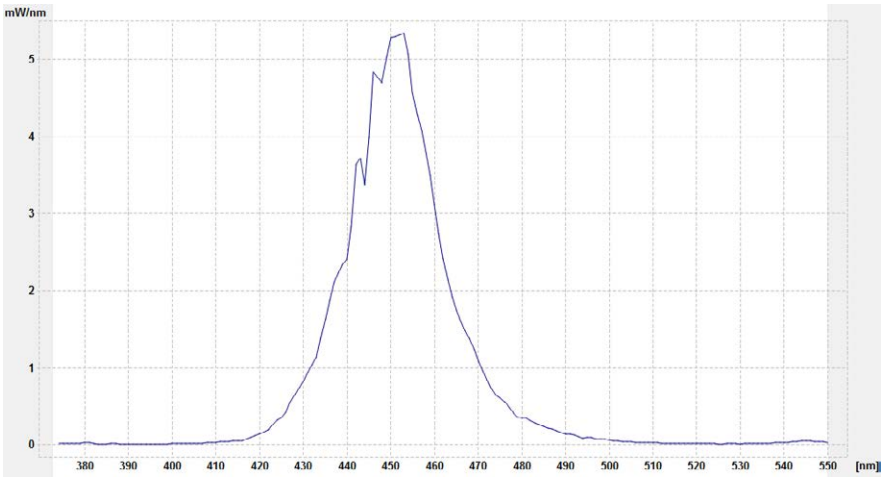

Fig. 1 Light emitting diode emission spectra.

Results and Discussion

LED-BL Induces Intracellular Reactive Oxygen Species Generation in Human Keratinocytes

Although ROS are recognized as by-products of physiological metabolism, the additional generation of these reactive species in cells by exogenous stress (e.g. UVB, pollution, etc.) increases their intracellular concentration over the typical threshold thereby causing cellular and tissue injury. The harmful effects of ROS include impairment in structure and function of proteins and lipids in addition to interference with signaling pathways that are involved in cell metabolism. This results in chronic inflammation, deregulation of immune function and degradation of tissue after induction of matrix metalloproteinases (Ristow, 2014). These reactive species also play a major role in aging processes (Wölfe *et al.*, 2014).

In this experimental assay, we measured the quantity of intracellular ROS found in keratinocytes after exposure to blue light. The quantification was determined by using a cell permeant probe to confirm the presence of singlet oxygen, superoxide, hydroxyl radical and various peroxide and hydroperoxides when becoming fluorescent.

Hydrogen peroxide (H_2O_2 at 1 mM) served as the positive control and validates the assay. As evidenced in **Fig. 2a**, exposure of normal human keratinocytes with LED-BL at 5 and 20 J/cm² induced a dramatic and significant increase in fluorescence by +376% and +2172% respectively. The results demonstrate that LED-BL significantly induces intracellular ROS formation in a dose-dependent manner. This data, confirms those published by Avola *et al.* even though in their *in vitro* model the applied BL doses were higher (15 and 45 J/cm²) than in our experimental conditions (Avola *et al.*, 2019). Mamalis *et al.* also show that blue light induces

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ROS in human dermal cells. They found that exposure of normal human fibroblasts to blue light at 5 and 30 J/cm² results in statistically significant increases in ROS of 110.4% and 127.5%. Moreover, Austin *et al.* have exposed a cell line of human fibroblasts for 1 hour to electronic devices generating blue light and also found an increase in ROS (Austin *et al.*, 2018). We noticed under our experimental conditions that the level of ROS present in normal human keratinocytes after blue light irradiation is surprisingly greater than that found in fibroblasts which suggests that keratinocytes are more sensitive to oxidative stress than fibroblasts and require protection against blue light damage. LCE plant extract applied at 1% on human keratinocytes for 24 hours before blue light irradiation clearly protects cells from ROS generation (**Fig. 2b**). The protective effects of LCE observed on keratinocytes might be attributable to the activation of endogenous defense systems separate from the antioxidant properties inherent from the extracts polyphenol content. Indeed, following the topical application of plant extract LCE on human skin, we see it can significantly stimulate transcription factor nrf-2 protein expression (data not shown). Nrf-2, also called nuclear factor (erythroid-derived 2)-like 2 is a transcription factor regulating the adaptive response to oxidants and has a role in the resistance to oxidative stress.

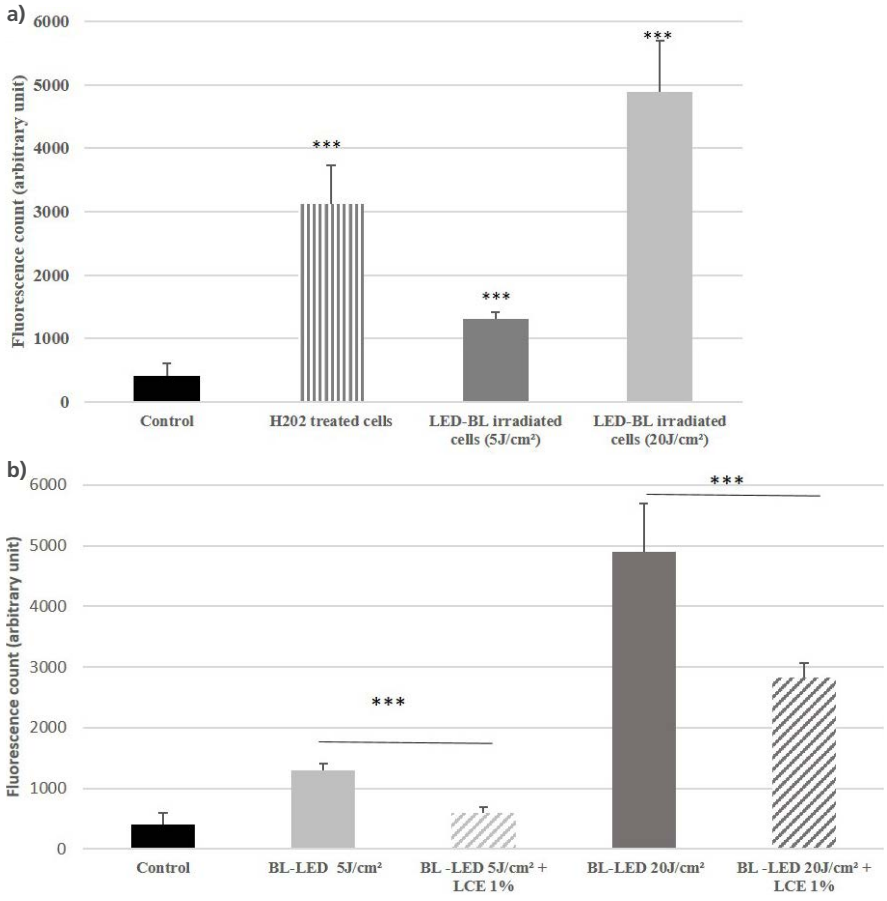

Fig. 2 Measurement of intracellular reactive oxygen species generated using H2DCF-DA fluorescent probe.

LED-BL Induces Oxidative Damage in Human Skin via the Release of 4-Hydroxy-2-Nonenal (4-HNE)

LED-BL induces oxidative stress in human keratinocytes, thus one expects lipid peroxidation to occur as a result of this type of light exposure. Therefore, 4-HNE, a toxic lipid peroxidation byproduct and potent inducer of oxidative stress signaling may be formed. 4-HNE is an easily diffusible substance in tissue making it a very toxic compound. This biomarker of oxidative stress triggers its harmful biological effects by modifying covalently important biomolecules including proteins, DNA and phospholipids (Dalleau *et al.*, 2013; Breitzig *et al.*, 2016). Additionally, Siddiqui *et al.*, demonstrate that 4-HNE induce alterations in the protein expression of mitochondria-mediated apoptosis markers (Bax, Bcl-2 and Caspase-3) (Siddiqui *et al.*, 2010). Further, recent papers demonstrate that one of the biological targets damaged by blue light is mitochondria (Rascalou *et al.*, 2018), a major reactive oxygen species generator (Yang *et al.*, 2016), therefore supporting a vicious cycle of oxidative stress generation. These results suggest that blue light may induce damage to mitochondria concomitantly to the generation of 4-HNE and through the accumulation of 4-HNE create and maintain a vicious and harmful cycle in tissue. Indeed, we observed a statistically significant increase in 4-HNE formation in the epidermis in both exposed models (**Fig. 3a** and **3b** for acute model and **Fig. 3c** and **3d** for chronic model). The strong basal level of 4-HNE observed in control conditions may be explained by the fact that the skin models used are skin maintained in survival and are understood to have some limitations (accelerated degradation) (Lebonvallet *et al.*, 2010). Despite these limitations, skin explants are an acceptable and convenient model for dermatological studies. As this aldehyde is found to accumulate in numerous pathologies (neurodegenerative disease, cancers and metabolic diseases), in UVB exposed skin (Kang *et al.*, 2017) and also in normal aged skin (Jørgensen *et al.*, 2014), we can assume that exposing our skin to devices emitting blue light at this high wavelength represents a real hazard for its safety or at minimum can accelerate the rate of the skin aging process. In consequence, protecting the skin from blue light by limiting the release of 4-HNE, seems to be a reasonable strategy. In our experimental conditions, we observed that the plant extract LCE when topically applied at 3% before and after blue light exposure efficiently reduces the quantity of 4-HNE (**Fig. 3a** and **3b** for acute model and **Fig. 3c** and **3d** for chronic model).

Oxidative Stress Induced by LED-BL Leads to Protein Damage in Dermis and Epidermis

Here we measured carbonylated proteins and nitrotyrosine as biomarkers for oxidative stress and nitrosative stress respectively which also serve as indicators of tissue damage. It is understood that the level of carbonylated proteins present in tissue is a reliable marker of oxidative damage (Fedorova *et al.*, 2014). Nitrotyrosine is a marker involved in nitrosative-oxidative stress and is generated when tyrosine and tyrosine residues in proteins are attacked by reactive nitrogen (peroxynitrite) and oxygen species (Köse *et al.*, 2011; Ahsan, 2013). Peroxynitrite can form as a result of reactions between superoxide and nitric oxide (NO).

In **Fig. 1**, we showed that LED-BL dramatically increases the level of carbonylated proteins in the dermis and nitrotyrosine in the epidermis. It is widely described that photo-damage from UV exposure contributes to protein oxidation in skin and leads to premature aging (Sander *et al.*, 2002) but to the best of our knowledge, this is the first time that photo-damage has been imputed to LED-BL exposure.

After using cellular models to demonstrate that reactive oxygen species are produced in skin following irradiation with blue light, we also observed an accumulation in nitrotyrosine under these conditions. We suspected blue light may trigger NO production, which may explain the accumulation of nitrotyrosine in the skin. NO is produced in skin following the induction of host-defense mechanisms that upregulate inducible nitric oxide synthase iNOS. Yet iNOS expression was not observed under our experimental conditions following exposure to blue light (data not shown). Based on these findings, the increase in nitrotyrosine content observed may be attributed to an increase in iNOS activity rather than an increase in iNOS expression.

To summarize, we demonstrate that when skin is exposed to devices emitting blue light, it is exposed to both oxidative and nitrosative stress as confirmed by the elevated levels of carbonylated protein and nitrotyrosine found in the dermis and epidermis. Moreover, we can conclude that blue light emitted by LED screens may contribute the same damage as UVB irradiation. Indeed, following UVB irradiation accumulations of nitrotyrosine (Kang *et al.*, 2017) and carbonylated

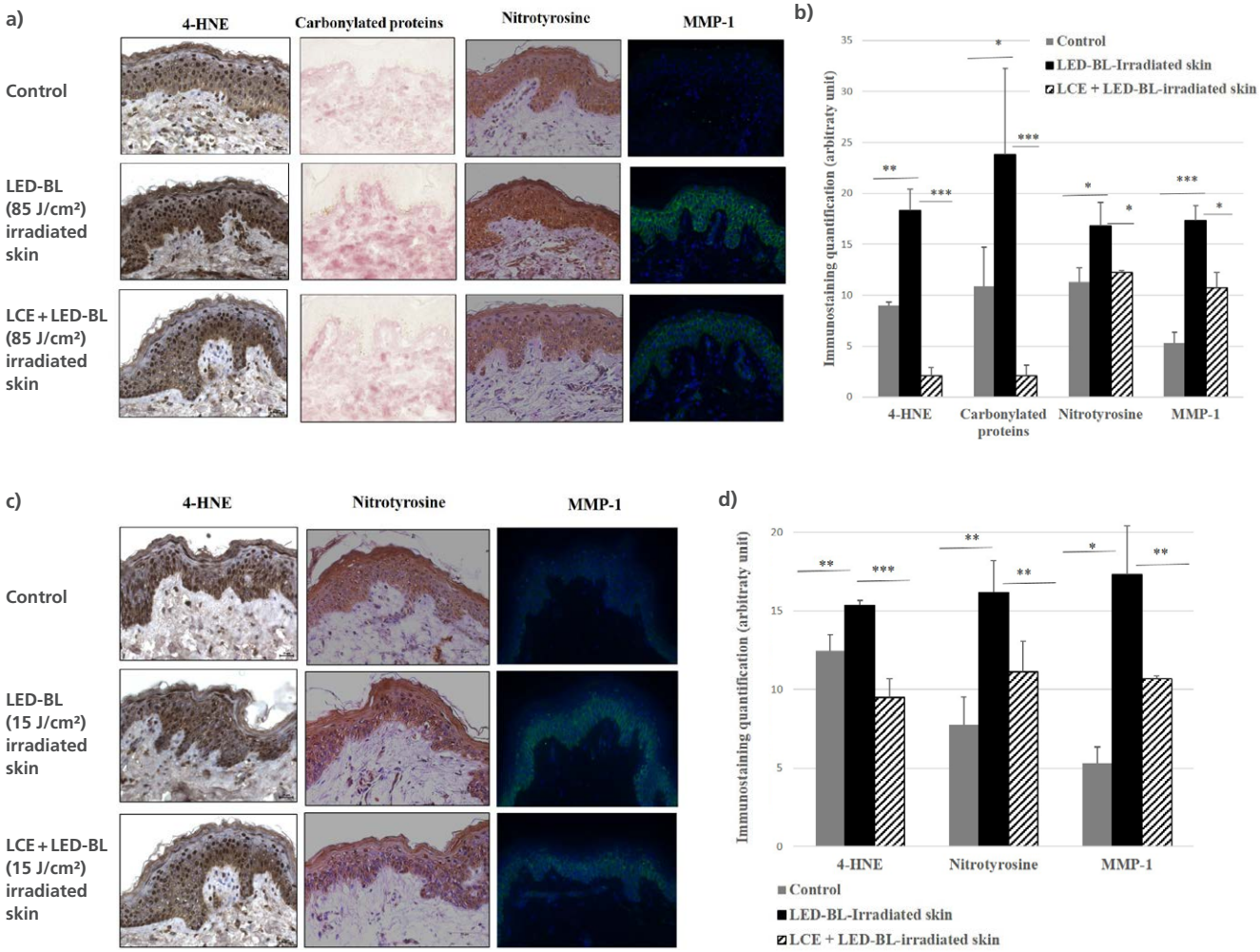

Fig. 3 Visualization and quantification of oxidative stress markers in human skin explants exposed or not to blue light.

proteins (Perluigi *et al.*, 2010; Sander *et al.*, 2002) have been observed. We should consider protecting our skin from artificial blue light emitted by electronic devices with the same regard as we do for protecting our skin against UV light. In our experimental models, we demonstrate that plant extract LCE at 3% can limit the accumulation of two damaging oxidative and nitrosative markers in skin (**Fig. 1**).

LED-BL is a Potent Inducer of Skin Damage through MMP-1 Overexpression and Protein Oxidation

MMP-1 (collagenase type I) is a member of the collagenase subgroup of Matrix metallo proteinase enzymes (MMPs). The MMPs are zinc-containing endopeptidases classified in the subgroup according to their structure and substrate specificity. MMP-1 in skin is mainly expressed by keratinocytes and fibroblasts with substrates consisting of collagens type I and III. Both substrates are major components of the human dermis. Some papers have shown the role of MMP-1 in the progress of skin aging and carcinogenesis (BCC, SCC or melanoma). Indeed, most of the data related to photo-aging and photo-carcinogenesis demonstrate that UV light (UVA and UVB) is responsible for the production of MMP-1 in skin. Our data in both experimental conditions (**Fig. 3**) demonstrate that blue light emitted by LED is also an inducer of MMP-1 expression and suggests the harmful effects of blue light on the skin. While some data published in 2010 states that irradiation of normal skin for five consecutive days on eight healthy volunteers with blue light does not induce MMP-1 expression, here we demonstrate the opposite of these findings (Kleinpenning *et al.*, 2010). These contradictory results could be explained by the different devices used. The device used in the previous publication is a tool dedicated to dermatological application with an emission spectrum from 380 to 480 nm with a peak at 420 nm, whereas the device used in our study has a similar blue light emission spectrum to smartphones and TVs (from 420 nm to 480 nm with a peak at 450–453 nm) which corresponds to a higher wavelength (**Fig. 1**). Moreover, in their experimental study, they applied 20 J/cm² each day to reach a cumulative dose of 100 J/cm² on the skin, which is higher than the cumulative dose used for the skin models in this study. Thus, we confirm that using both chronic and acute models with higher blue light wavelengths (450–453 nm) damage the skin even with exposure to lower doses. Further, the over expression of MMP-1 observed in the epidermis following blue light irradiation in both models can be diminished with topical treatment of LCE before and after blue light exposure (**Fig. 3**).

LED-BL Induces Similar Effects as UVB Irradiation in Skin Suggesting a Potential Role in Triggering Premature Aging

Dermatological practice has shown that blue light emitted by LED does not cause damage to normal skin (Kleinpenning *et al.*, 2010; Ramaswamy *et al.*, 2014). Yet our data demonstrates that when following the normal context of use in a modern lifestyle, exposure to artificial blue light emitted by LEDs induces adverse effects in skin with dramatic increases in ROS leading to lipid and protein oxidation in addition to the overexpression of MMP-1, an enzyme involved in extracellular matrix degradation. As in photo aged skin, we observed the accumulation of 4-HNE, carbonylated proteins, nitrotyrosine and MMP-1 (Kang *et al.*, 2017). Taken together, these results highlight the damage observed in skin exposed to blue light irradiation from LED devices for short or long durations which is comparable to photoaging damage induced by UVB irradiation.

Skin Damage Induced by Blue Light Can Be Easily Prevented or Limited by the Use of Cosmetic Products

The experimental data suggests that blue light emitted by LED-devices is a non-negligible contributor to premature skin aging. Although the intent of this study is to determine if blue light emitted by electronic devices such as smartphones and tablets is safe for our skin, the unexpected findings demonstrate skin damage with several signs of premature aging leading us to question if natural blue light emitted from the sun is damaging. The irradiance of the sun (≈ 50 mW/cm²) is stronger than the irradiance from electronic devices (≈ 36 µW/cm²) (Rascalou *et al.*, 2018), suggesting that damage can be created from sun exposure. Moreover, even if less than half of sun radiation reached the earth (Ben Yehuda Greenwald *et al.*, 2017). The remaining radiation reaching the earth is composed by 44% of visible light including blue light detected by our eyes (400–700 nm), 53% infrared radiation and only 3–7% is UV light (290–400 nm) (Frederick *et al.*, 1989). Surprisingly, the potential harm to the skin by natural blue light irradiation is rarely described and the effects are currently unknown. Most of the damage attributed from the sun is linked to UV rays (UVA and UVB) (Sanches Silveira and Myaki Pedroso, 2014; Rittié and Fisher, 2015). Yet so far only a few findings suggest that other parts of the solar spectrum, particularly visible light contribute to signs of premature photoaging of the skin and these do not reference blue light (Leibel e. al., 2012). Recently published papers report blue light at 20 J/cm² to 90 J/cm² stimulate melanogenesis which can contribute to hyperpigmentation disorders (Regazzetti *et al.*, 2018); Mahmoud *et al.*, 2010; Sondenheimer and Krutmann, 2018). Here we have shown that skin exposed to realistic doses of blue light are highly deleterious for skin and generate oxidative stress which ultimately leads to irreversible damage consisting of lipid peroxidation, protein oxidation and the overproduction of MMP-1.

The data highlights the need to protect skin from artificial blue light and more importantly to protect against visible light. We demonstrate that cosmetic products can efficiently prevent adverse effects from visible light. After screening several plant extracts, we found an extract derived from *Lespedeza capitata* leaves to be an effective means of protection from blue light damage. When keratinocytes and skin

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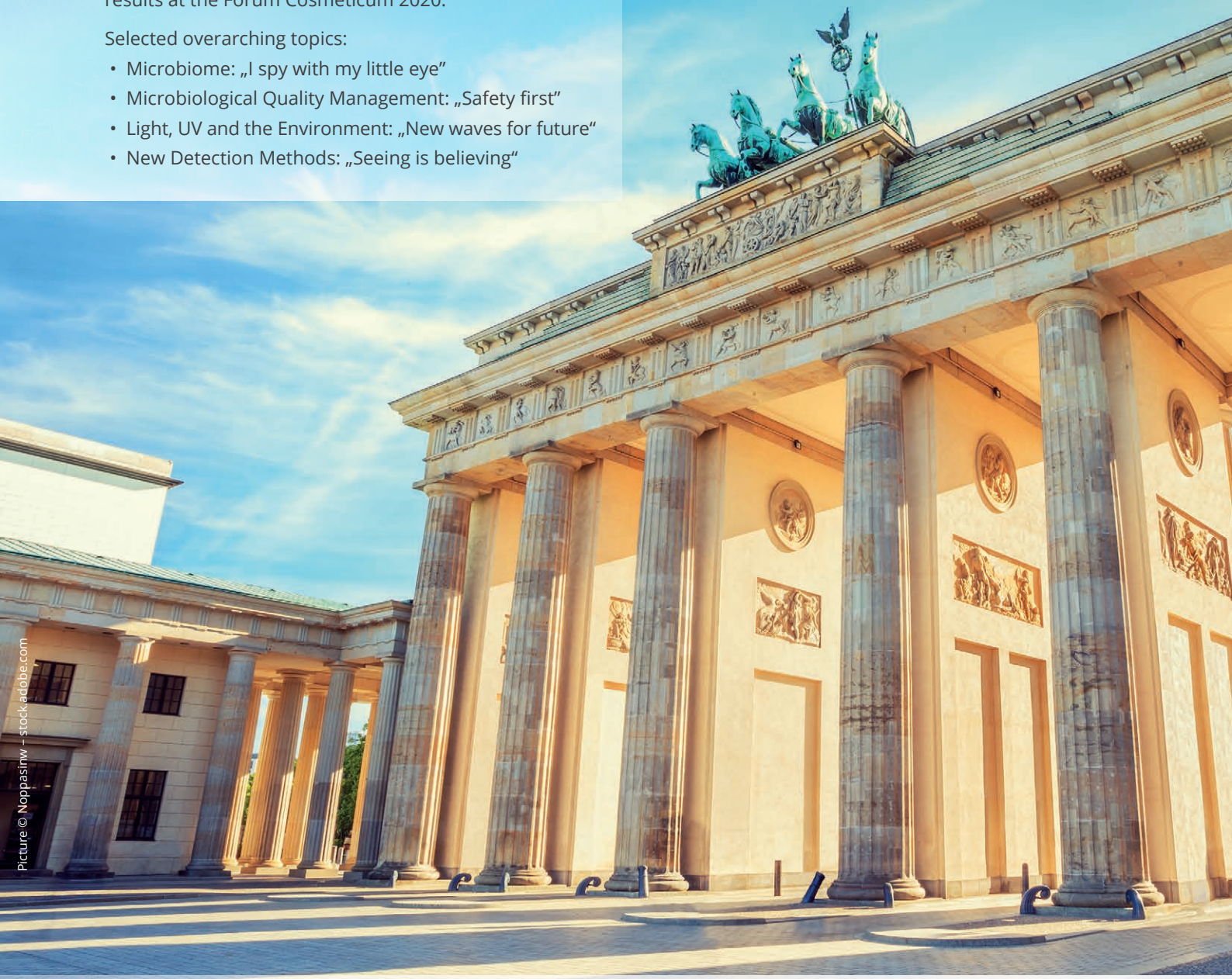

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explants are treated with LCE, there is less accumulation of ROS, 4-HNE, carbonylated proteins, nitrotyrosine and MMP-1 which as previously mentioned contribute to premature skin aging.

Materials and Methods

Description of Light-Emitting Diode Generated Blue Light (LED-BL) Device Used in the Experimental Studies

The LED-BL with an emission spectrum from 420 to 490 nm centered around 450–453 nm was used (**Fig. 1**). The emission spectra shown in **Fig. 1** validates the calibration of the device. The exposure time to the device was modulated in different experimental studies to deliver 5 J/cm², 20 J/cm² or 85 J/cm².

Plant Extract Description

The *Lespedeza capitata* plant originated in Korea. An ethanolic extraction using 70% ethanol was carried out with dried leaves. After, a vacuum evaporator was used to remove ethanol to yield a powder. 2% powder was suspended in a propanediol/water solution to make LCE. 1% LCE was used to treat cells and 3% was topically applied to human skin explants.

Monolayer Keratinocyte's Model Exposed to Blue Light

Normal human keratinocytes were seeded in 96-well microplates at 10000 cells/well for 24 h in complete medium, then the plant extract LCE at 1% was added to the cells for 24 h. Before proceeding to blue light irradiation, the culture medium was removed, cells were washed and the probe H2DCF-DA probe (D399, Invitrogen, Carlsbad, CA, USA) diluted at 50 µM in non-supplemented medium was applied and the plates were incubated for 45 minutes at 37°C/5% CO_2. After that, cells were irradiated without culture medium with blue light at 5 or 20 J/cm². Control wells were not irradiated and not treated with LCE. Five minutes after irradiation, fluorescence is measured at 490 nm (excitation) and 525 nm (emission) wavelengths (Tecan Safire II plates reader). This assay is based on the use of a detection system, a probe (H2DCF-D), which is degraded into DCF which become fluorescent in presence of reactive oxygen species. Each condition was done in triplicate.

Human skin explants

Skin samples were supplied by Biopredic International (Saint Gregoire, France). The skin samples were obtained from patients undergoing cosmetic surgery according to French law L.1245 CSP "product and element of human body taken during surgical procedure and used for scientific research". Each patient was fully informed and provided written consent before donating their tissue.

Chronic Blue Light Exposure Skin Model: Skin explants were obtained from a 40-year old Caucasian woman. The control conditions correspond to non-irradiated skin. Samples were cultured for 24 h between each irradiation at 5 J/cm². Explants were irradiated three times daily for three consecutive days. The cumulative dose was 15 J/cm². Each condition was performed in triplicate. 3% LCE was applied topically on skin explants daily.

Acute Blue Light Exposure Skin Model: Skin explants were obtained from a 43-year old Caucasian woman. Explants were irradiated using blue light with an irradiation dose of 85 J/cm². Non-irradiated explants served as control conditions. The skin samples were cultured for 24 hours prior to histological processing. Each condition was performed in triplicate. 3% LCE was applied 4 hours prior to blue light exposure and 24 hours following blue light exposure. After the culture period, tissue samples were fixed in alcohol, formol acetic acid (AFA) over night before further histological processing.

Histological Processing of Skin Samples

After overnight fixation in AFA solution (55781-29, MM France, Brignais, France), samples were dehydrated and impregnated in paraffin using a dehydration automat (STP120 ThermoScientific™ Waltham, MA, USA). The samples were embedded using an embedding station (EC350, ThermoScientific™, Waltham, MA, USA), and the sections were mounted on Superfrost® histological glass slides. Deparaffinization and heat-induced epitope retrieval were performed simultaneously in PT Module® (ThermoScientific™, Waltham, MA, USA).

4-HNE and nitrotyrosine immunostaining were realized on paraffin sections with mouse monoclonal anti-4HNE antibody (ab48506, Abcam™, Cambridge, UK) or with rabbit polyclonal anti-Nitrotyrosine (ab42789, Abcam™, Cambridge, UK), both diluted to 1 : 100 in PBS-BSA 3% overnight at 4°C. Endogenous peroxidases were inhibited by Quanto Peroxide Block (TA-125-H2O2, ThermoScientific™, Waltham, MA, USA) before incubation with anti-mouse labelled polymer N-Histofine® (414131F, Nichirei Biosciences™, Tokyo, Japan) for 4-HNE expression and with anti-rabbit labelled polymer N-Histofine® (414141F Nichirei Biosciences™, Tokyo, Japan) for nitrotyrosine, both during 1 h. Expression was revealed by DAB, a peroxidase substrate (TA-125-QHDX, ThermoScientific™, Waltham, MA, USA). Counterstain with Mayer's Hematoxylin was performed for 5 minutes. Slides were dehydrated and mounted with Diamount® mounting medium (0304000, Diapath™, Martinengo, Italy).

Carbonylated protein immunostaining was performed on frozen sections with an anti-DNP antibody (S7150, Merc, Darmstadt, Germany) diluted at 1/250 in PBS-BSA 0.3%, overnight at 37°C, using a Vectastain Kit Vector amplifier system avidin/biotin and revealed by VIP, a peroxidase substrate (SK-4600, Vector Labs™, Burlingame, CA, USA).

MMP-1 immunostaining was carried out on paraffin sections with a rabbit monoclonal anti-MMP1 antibody (ab52631

Abcam™, Cambridge, UK) diluted at 1:100 in PBS-BSA 3% overnight at 4°C. After washing with PBS, slices were incubated two hours at room temperature with an Alexa 488 coupled secondary antibody (A-11009, Thermo Fischer Scientific™, Waltham, MA, USA). Counterstain of nucleus was performed at room temperature for 5 minutes using a Hoechst 33342 (H3570, Invitrogen™, Waltham, MA, USA) solution diluted in PBS at 1/10000. Slides were mounted using fluorescent mounting medium (S302380-2, Agilent Technologies™, Santa Clara, CA, USA).

Image Acquisition and Quantification

Microscopic observations were realized using a photonic DM2000 microscope (Leica Microsystems™, Buffalo Grove, IL, USA). Pictures were digitized with a numeric DFC450C Leica camera and LAS X software. The quantification was done with Fiji software (National Institute of Health, USA).
Statistical analysis was performed using Student t-test. P values < 0.05 were considered significant. A single asterisk (*) denotes $0.01 < p < 0.05$, double asterisk (**) $0.001 < p < 0.01$, triple stars (***) $p < 0.001$.

Conflict of Interest

The researchers were funded by Clariant Production France.

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The Future of In-can Preservation: How is the Industry Moving away from Chemical Biocides?

E. Lansdaal

Synthetically produced biocides are effective and widely used in low-pH, rinse-off household and personal care applications to preserve products. Isothiazolinones, such as benzisothiazolinone (BIT) and methylisothiazolinone (MIT), are an important biocide family, commonly used as a preservative in in-can, wash-off household and personal care applications. But growing concerns over the years about their safety, particularly in fabric conditioning and cosmetic applications where allergenic chemicals stay in contact with people's skin for prolonged periods of time, has led to tighter regulatory restriction on their use. New attitudes towards chemicals has also sparked consumer demand for more sustainable, eco-friendly products. This has placed pressure on formulators to find safer and more sustainable biocide alternatives.

Increasing Restrictions on Biocide Use

While inexpensive, easy to formulate and effective at a broad pH range (between pH 2–12), dermatologists have expressed specific concerns regarding the use of MIT as a preservative. In 2018, the EU Commission completed its evaluation of MIT, concluding that it was extremely allergenic, with long-term, consistent exposure to the biocide being associated with skin allergy. Therefore, as of May 2020, MIT will be classified as a skin sensitizer and limited to concentrations of 0.0015% (or 15 ppm). Previously, it was allowed in concentrations of up to 1,000 ppm in various products. Further to it being less effective at this lower dose, MIT-based preservation systems also require a warning symbol on labels when present in the formula and must be labelled as H317 (formerly R43) [1].

BIT is another widely used biocide preservative. Thanks to its microbicide and fungicide mode of action, it is also commonly used in home cleaning and personal care products. However, virtually all biocides, especially those in the isothiazolinone family, are skin sensitizers. In fact, the Scientific Committee on Consumer Safety in Europe has already found BIT's 'sensitizing potential' a concern [2].

The Formulation Challenge

Looking at the way the market and regulations are moving, BIT is likely to be the next isothiazolinone to be assessed and concentration limited to 15 ppm – a dosage too low to be effective in wash-off household and personal care products. And, since it is likely that all isothiazolinones will be restricted to lower concentration limits in the near future, now is the time to replace any isothiazolinone that is used in products with an alternative preservative ingredient(s).

Consumers are increasingly conscious of their impact on the planet too – eager to reduce their purchase of products that cause environmental degradation. As such, shoppers worldwide are showing a preference for eco-friendly and green products more than ever before, even within the household and personal care categories. This is causing a shift in demand away from products containing chemical-based, non-biodegradable ingredients to those derived from a natural source. Since many wash-off products often contain toxic chemicals to effectively preserve them, this is creating significant challenges for formulators, who are now seeking biocide alternatives.

There is a low number of effective alternatives that are able to fulfil the technical and safety requirements crucial for in-can preservation. Preservatives, such as benzoic and sorbic acids are widely available, although they are also synthetic and can be difficult to formulate, as they come in powder form and exhibit low water solubility. As such, few natural, safe and cost-effective options currently exist.

Lactic Acid: A Safe, yet Powerful Alternative

Lactic acid is a safe, biobased and biodegradable option for in-can preservation with a proven broad range efficacy inhibiting bacteria and fungi (**Tab. 1** shows the effect of lactic acid on fungi reduction). Research shows that high purity L(+)-lactic acid can be employed as an active ingredient for antimicrobial cleaning and personal care formulations. Working best in low pH (acidic) conditions, once inside bacterial cells, lactic acid inhibits the micro-organisms via four broad-acting mechanisms (**Fig. 1–2**):

1. Acid stress disrupts cell regulation on a general level
2. Microbes spend energy to maintain pH, by pumping out acid
3. Microbes change their metabolism to produce alkaline metabolites
4. Glycolysis block

In this respect, lactic acid is different from other chemical preservatives, like biocides, that rely on singular mechanisms

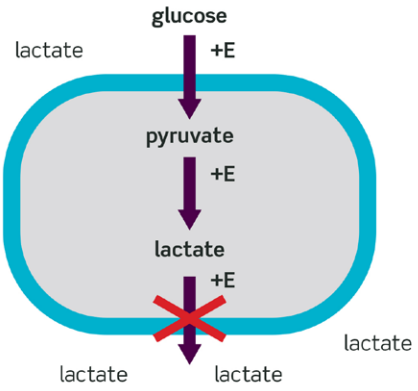

Fig. 1 Preservation mechanism: glycolysis block. Here, lactic acid inhibits sugar fermentation to lactate, preventing bacterial growth.

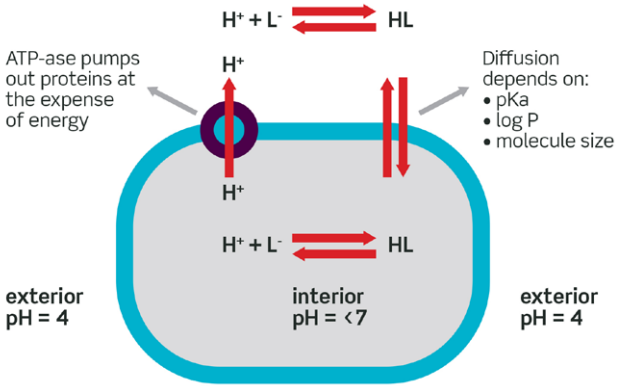

Fig. 2 Preservation mechanism: proton shuttle. Lactic acid increases the H^+ concentration inside the cell. If H^+ supply exceeds energy supply, the bacteria cannot grow.

Samples pH 3.5	Log reduction on fungi				
	24 hrs	48 hrs	7 days	14 days	28 days
Fabric softener base formula	1.3	growth	growth	growth	growth
Fabric softener + 0.75% lactic acid	3.2	3.8	4.7	4.7	4.7
Fabric softener + 15 ppm BIT	1.9	2.2	2.8	3.3	4.7
Fabric softener + 15 ppm MIT	1.5	2.3	3.2	4.7	4.7

KEY
- High
- Medium
- Low

Tab. 1 Lactic acid provides sufficient preservation against fungi – demonstrating a higher log reduction in fungi after 24 hrs and 7 days compared to 15 ppm BIT and MIT.

to inhibit microbes. In the absence of surfactants, lactic acid is especially effective against gram-negative bacteria. Meanwhile, gram-positive bacteria are generally less sensitive to lactic acid. Although, in the presence of surfactants – which weaken bacterial cell walls – lactic acid can enter to have a positive antimicrobial effect. Other secondary ingredients, such as chelating agents and alcohol, may also help to boost the bactericidal action of lactic acid.

Meeting Regulatory and Consumer Demands

Corbion's PURAC© Sanilac is an antimicrobial, biodegradable agent consisting of L(+)-lactic acid that is considered a safe, powerful alternative to traditional biocides. The ingredient is considered acceptable by consumers, as it is non-toxic to humans and the environment, therefore meeting the latest demand for eco-friendly products. In fact, with lactic acid being produced by our skin, Corbion's biocide alternative is skin-friendly and does not cause sensitization, like traditional isothiazolinone biocides. It also offers additional skin-moisture in dish wash and all-purpose cleaners, while acting as a lubricant in fabric conditioners and offering descaling properties in toilet and bathroom cleaners.

As a preservative, PURAC© Sanilac offers compatibility with other common detergent ingredients, such as surfactants and alcohols. It works in such a complementary fashion with chelating agents and alcohols (IPA) that its performance as a preservative is boosted significantly. As a result, it can be used in combination with such ingredients to offer potent efficacy.

The Future of Preservation

Consumer expectations and regulatory policies are moving the household and personal care arena toward safer, healthier and more sustainable product formulations. Manufacturers, in turn, are looking for the kind of expertise and creativity that can help them deliver quality, cost-effective solutions that meet those demands. Lactic acid, such as PURAC© Sanilac, is an emerging ingredient for preservation that offers an easy-to-use, label-friendly and safe alternative for formulators.

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GLDA as a Performance Booster for Liquid Laundry Detergents

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abstract

Laundry detergents consumers are adopting more sustainable washing habits, such as low temperature wash and use of concentrated liquid detergents. This creates new challenges for the performance of this type of detergents, in particular for stain removal.

We will show in this article the importance of water quality in laundry stain removal process and how green chelate GLDA enhances stain removal and boosts performance of concentrated liquid detergents.

Chelating Agents

Chelating agents are key ingredients in detergent formulations, as they soften the wash water and control transition metals, improving the detergency power and shelf life of the formulations.

Traditional aminopolycarboxylates chelating agents such as NTA (nitrilotriacetic acid) and DTPA (diethylenetriaminepentaacetic acid) have toxicological issues and consequently they are not frequently used in household applications. EDTA (ethylenediaminetetraacetic acid) is still the most widely used chelate but due to inherently biodegradability the household market is showing some concerns.

Phosphates (e.g. sodium tripolyphosphate – STPP) are also chelating agents, but as they cause eutrophication, they are banned from laundry and dishwashing detergents in Europe and USA. Another category of components also containing phosphorous are phosphonates, which are sequestrants (co-builders). These are currently used in detergents applications, however in limited amounts (in Europe for example only <0.5 wt% P per wash in laundry and <0.3 wt% P per wash in ADW) are allowed. Phosphonates are not readily biodegradable.

Examples of the more modern class of aminopolycarboxylates chelating agents are GLDA (glutamic acid *N,N*-diacetic acid) and MGDA (methylglycinediacetic acid), see **Fig. 1**. These chelates are bio-based products, as they are produced from natural aminoacids of biological origin. They are readily biodegradable, ecologically harmless and are also not health hazard labelled. This excellent environmental and toxicological profile makes them very suitable for use in household applications.

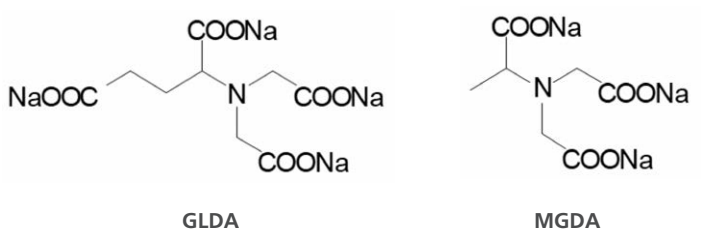

Fig. 1 Chemical formulas of GLDA and MGDA.

Laundry

Laundry is the biggest market within Home Care, with ca 80 billion USD retail market size, more than 4× the market for dishwashing. Laundry detergents accounts for the biggest share of laundry care.

In terms of formats, over the last years, there was a shift from standard detergents to concentrated ones. In Europe, concentrated liquid detergents and liquid tablet detergents accounted for most of the innovations. Powder detergents are becoming out-dated.

Moreover, consumers are getting more aware about sustainability factors, therefore washing at low temperature is becoming more common nowadays.

These two factors together bring new challenges to the efficiency of laundry detergents: washing at low temperature with liquid detergents (which do not contain bleach) makes stain removal much more challenging.

Role of Chelating Agents in Laundry

Chelating agents will have a role not only during the washing process, but also in helping to stabilize the detergent formulation.

In terms of formulation stability, modern chelating agents, such as GLDA, will boost the performance of preservatives. For liquid products less preserva-

tives are required when just a small amount of chelates are added (ca. 0.2 wt%). This is because microorganisms are deprived of multivalent cations by chelates, weakening the outer surface, and are thus more vulnerable for the action of biocides. Moreover, strong chelating agents can chelate not only calcium and magnesium, but also transition metals, such as iron and copper. This will prevent any unwanted side effects from these metals and consequently improve the shelf life of the formula (retard turbidity, improve color stability of the liquid formula and retard oxidative breakdown of oils and perfumes). In order to have these benefits, only a small percentage of chelate is required (0.2 to 0.3 wt%).

Chelating agents also provide many advantages in the laundry cleaning process. The strong chelating agents GLDA and MGDA break the calcium bonds between surface scale, soil and anionic surfactants fast and efficiently. Therefore, the detergency power at lower temperatures and shorter wash cycles can be greatly improved. In this way, not only will dirt removal become easier, but also the inactivation of anionic surfactants which are sensitive to water hardness, like LAS and soap, are prevented by chelating agents. As a result, other undesirable phenomena like turbidity and flocculation of soap (soap scum) can also be prevented. When a high amount of chelate is added, there will be less calcium carbonate incrustation, resulting in less fabric greying and improved whiteness. Chelating agents also bind transition metal cations forming water soluble metal chelates, this has a positive impact on stain removal, as explained below in the article. Moreover the color protection / color retention of garments will be improved.

However, currently no high amounts of chelating agents are used in liquid laundry detergents. Several recipes from major brands in the EU still contain phosphonates (mostly DTPMP – sodium diethylenetriamine pentamethylene phosphonate) at low concentration, sometimes in combination with citrates. Some more modern detergents contain small amounts of GLDA but most likely for stability of the liquid formulation and/or for boosting the preservatives. Some detergents with a greener profile contain higher amounts of GLDA.

Ingredient	wt%
Sodium Dodecylbenzenesulfonate	20
Sodium Laureth Sulfate	2.9
C12-15 Pareth 7	5.0
Cocoate fatty acid	3.0
Polymer	1.3
Propylene glycol	3.0
Triethanolamine	0.5
Protease	1.0
Amylase	0.5
Mannanase	0.5
Water (demin)	to 100%

Tab. 1 Recipe of base detergent.

How GLDA can Boost Stain Removal of Liquid Laundry Formulas

Effect of Water Quality in Stain Removal

To determine the advantages of using GLDA in liquid laundry application, the first step is to have a base line and a good understanding of the impact of water quality in the cleaning process.

For that, stain removal tests were done at 20°C, using different water qualities using a base detergent formulation.

Different water qualities were used: demineralized water; hard water with 17 dGH and hard water with 17 dGH with transition metals (0.3 ppm Fe, 1 ppm Cu, 0.05 ppm Mn and 5 ppm of Zn). To choose the relevant concentrations of transition metals, national guidelines for drinking water were checked and maximum levels were used (e.g. National Secondary Drinking Water Regulations guidelines from EPA).

A base detergent was used, with composition in **Tab. 1**. The concentration used was 2 ml detergent per L of washing water. Standard stains were used, in particular bleachable stains and protease sensitive stains. Before and after testing, the color of the stains was measured using Mach 5 equipment (from Center for Testmaterials). The Y value was extracted from these measurements and a ΔY value (difference of Y value before

and after washing) was used for comparison. The higher this value, the better the stain removal. The results of the stain removal can be seen in **Fig. 2**.

It can be clearly seen that the presence of hard water and transition metals have a negative impact in stain removal of all stains tested. Within the fruit juice stains, transition metals have a higher negative impact than hard water. Most likely transition metals will form colored and/or insoluble complexes when in contact with these types of stains, which makes the cleaning process more difficult.

On the other hand, in protease sensitive stains, hard water has a more negative impact than transition metals.

This effect is independent of the substrate that is used (cotton, polyester cotton or polyester, or more natural substrates such as wool and silk).

Effect of Addition of GLDA

GLDA (Dissolvine® GL-47-S) was then added to the base detergent in different concentrations. Tests were done always using hard water with transition metals. The results can be found in **Fig. 3**.

A small amount of GLDA (5 wt% of Dissolvine® GL-47-S) was already sufficient to have a substantial positive effect in

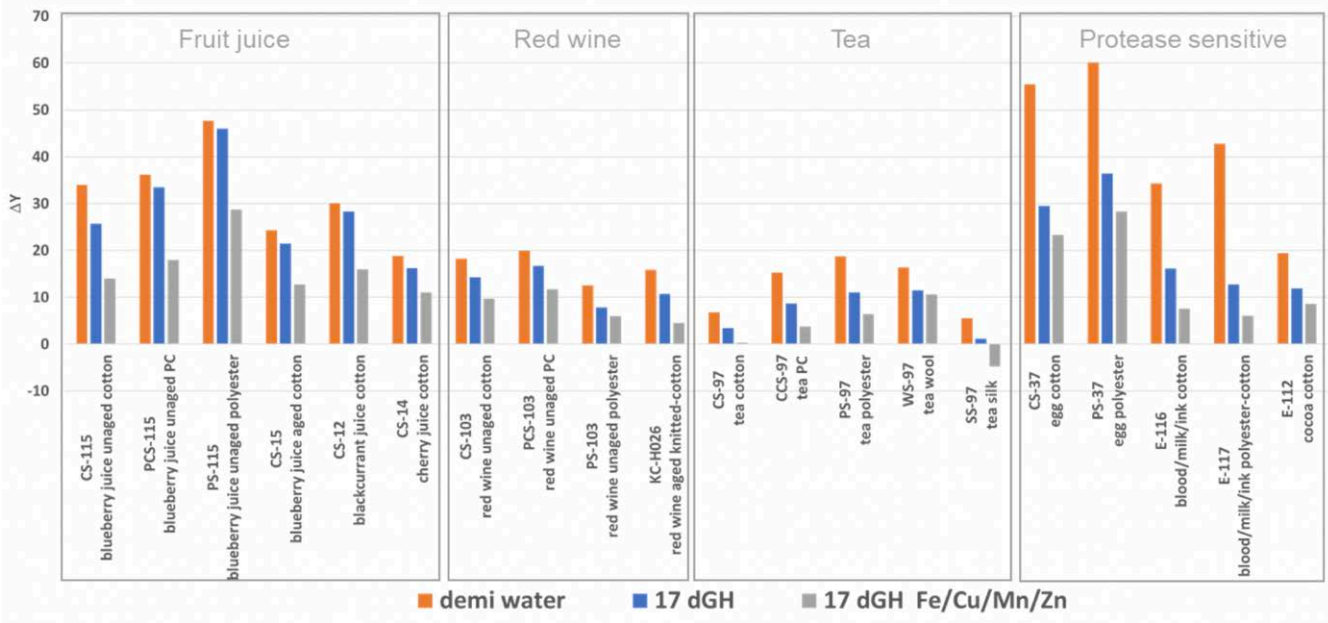

Fig. 2 Color difference before and after washing for the different stains using different water qualities for base detergent.

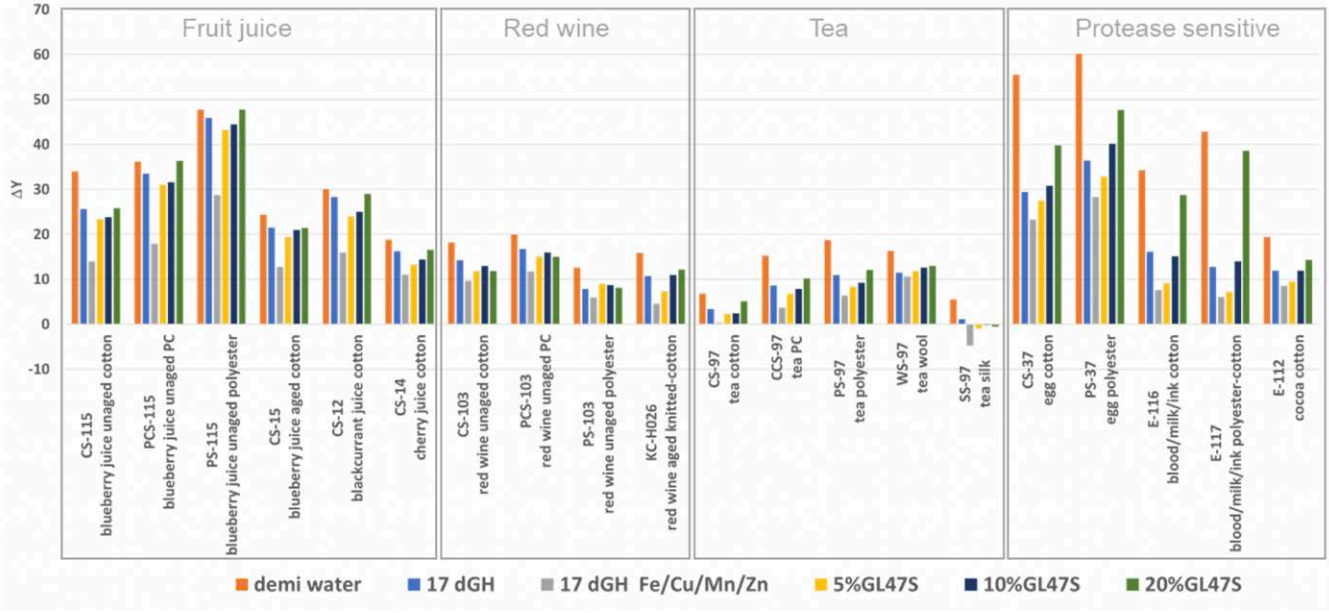

Fig. 3 Color difference before and after washing for the different stains using different water qualities on base detergent (first 3 bars) and different amounts of GLDA on base detergent with hard water containing transition metals (last 3 bars).

the stain removal of bleachable stains (fruit juice, red wine and tea). Adding higher amounts bring some additional performance benefit with these stains. This can also be seen visually in **Fig. 4**.

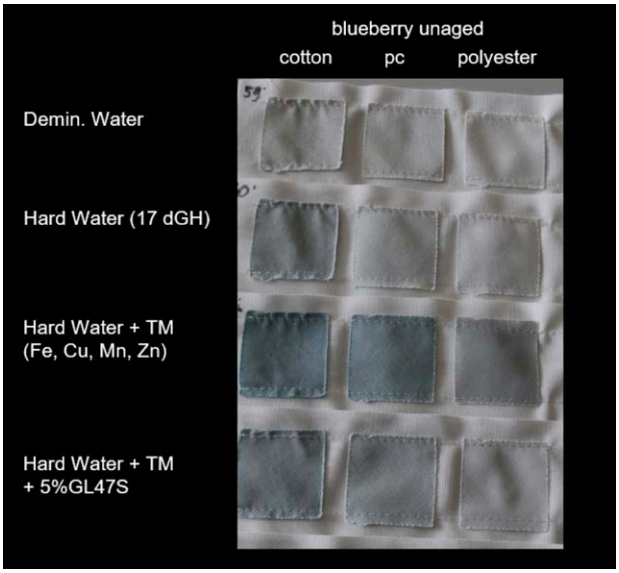

Fig. 4 Photo of the blueberry swatches after washing with base detergent at different water qualities and base detergent containing GLDA.

In the case of protease sensitive stains, addition of higher amounts of GLDA (10 wt% and 20 wt% Dissolvine® GL-47-S) continued to bring substantial performance increase in stain removal. This was also very visible on the swatches after washing, as depicted in **Fig. 5**.

The level of transition metals in the wash water corresponds to: 0.005 mmol/L Fe, 0.016 mmol/L Cu, 0.001 mmol/L Mn

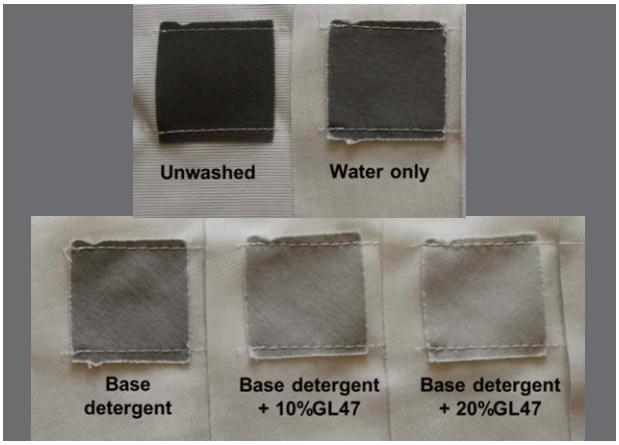

Fig. 5 Photo of the EMPA-117 swatches before and after washing without detergent, with base detergent and with base detergent with GLDA.

and 0.077 mmol/L Zn (total level of 0.098 mmol/L). Whereas the level of Ca is much higher in 17 dGH water, corresponding to a molar concentration of 3.0 mmol/L.

5 wt% Dissolvine® GL-47-S (2.35 wt% GLDA) corresponds to a molar concentration of 0.135 mmol/L. This amount is sufficient to chelate the transition metals present, but not Ca.

At the pH of the washing process (between 7 and 8), GLDA will preferentially chelate transition metals. Therefore, even with an excess of Ca, it is possible to control transition metals during the washing process and improve the cleaning of bleachable stains. This is the reason why the addition of the low amount of Dissolvine® GL-47-S (5 wt%) was enough to have such positive effect on stain removal.

In the case of protease sensitive stains, it is visible from **Fig. 2** that these are more sensitive to Ca rather than transition metals. This is the reason why when adding higher amounts of GLDA the stain removal performance was constantly better, as shown in **Fig. 3**. This could give some room to decrease protease level in the formulation.

Nevertheless, in order to chelate all calcium in the wash water, a very high amount of chelating agents would be required and that would not be not feasible from an economic view.

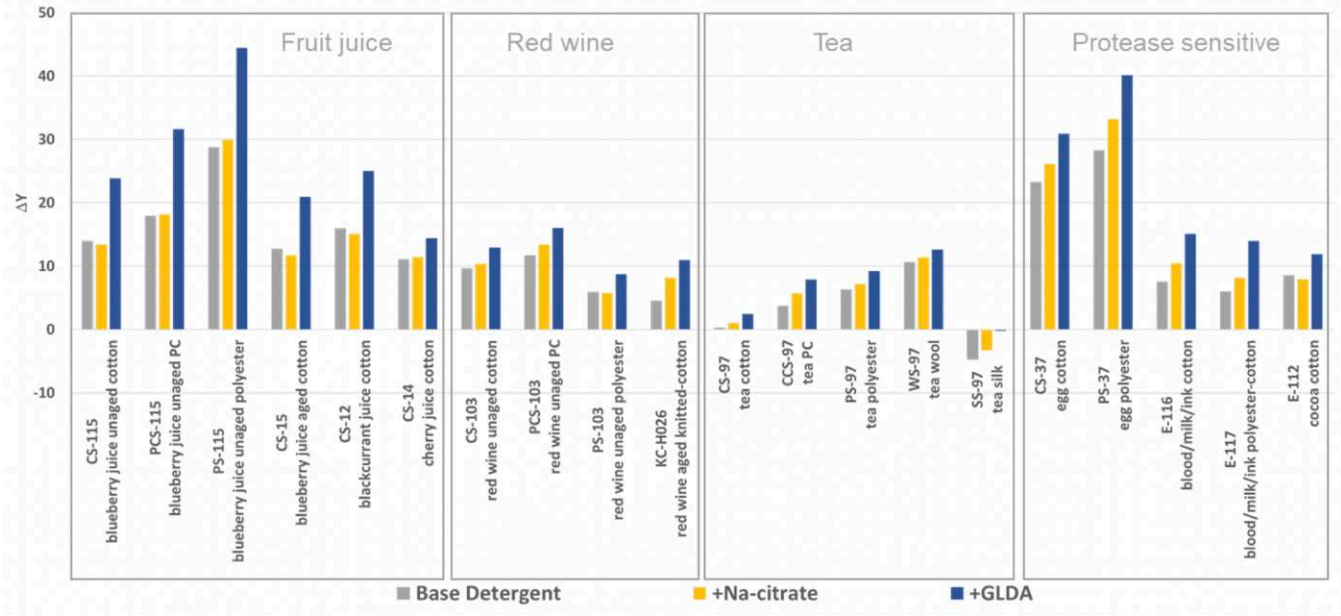

Fig. 6 Color difference before and after washing for the different stains using the base detergent alone and with addition of citrate (4,7 wt% of Na-citrate) or GLDA (4,7 wt% GLDA) all using hard water containing transition metals.

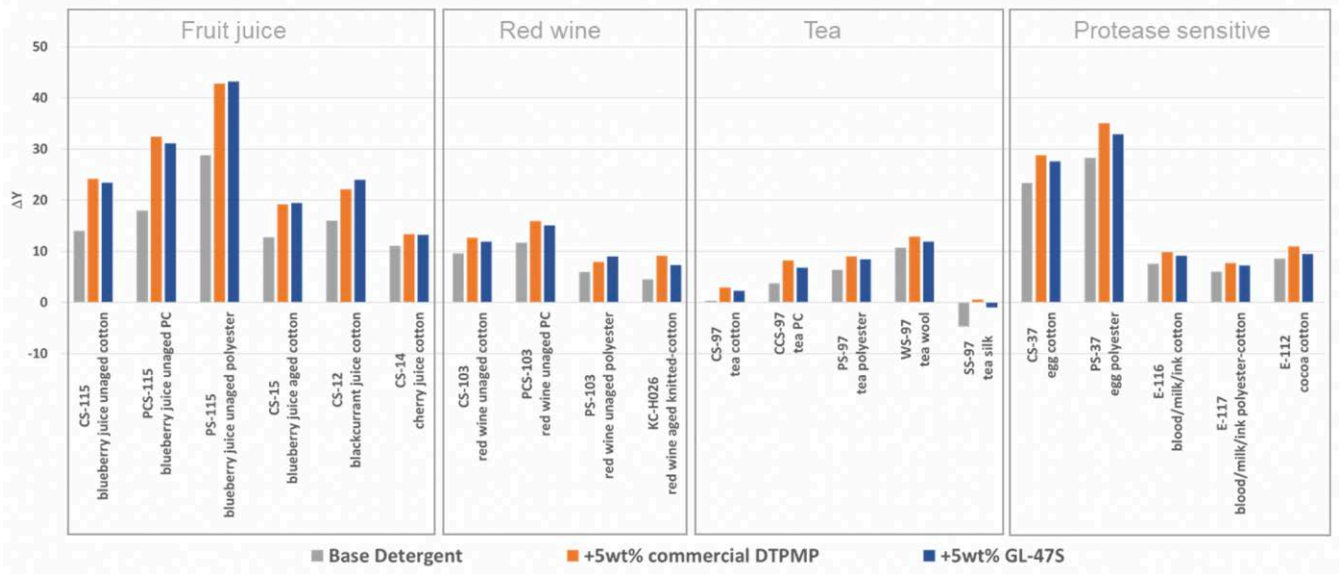

Fig. 7 Color difference before and after washing for the different stains using the base detergent alone and with addition of 2.1 wt% DTPMP (5 wt% of commercial DTPMP product) and 2.4 wt% GLDA (5 wt% Dissolvine® GL-47-S) all using hard water containing transition metals.

Transition metals are present at very low concentration levels, therefore the sequestering of those metals would require lower amounts of chelating agents and would be an economical feasible option for detergent producers to boost their detergents for stain removal.

Comparison between GLDA and other Products (Citrate and Phosphonates)

Sodium citrate is also used as a chelating agent in detergents. This is a weak chelating agent which can soften the water when used in large amounts, however it is not able to chelate transition metals. The results of stain removal tests done using either citrate or GLDA are depicted in **Fig. 6**.
Citrate is clearly not able to control the transition metals. The slight positive effect on protease sensitive stains is most likely due to Ca chelation.
In EU laundry liquid detergents, phosphonates are often used. One of the most commonly used phosphonates is DTPMP. Therefore, a comparison was done using the same concentration of Dissolvine® GL-47-S and a commercially available phosphonate DTPMP (41 wt% assay as sodium salt) (**Fig. 7**).

There are only slight differences between the two products in the stain removal test results. Therefore, it would be possible to substitute phosphonates by GLDA without compromising on stain removal performance.
Moreover, this switch will bring additional environmental benefits, as GLDA is bio-based and readily biodegradable, which is not the case for phosphonates.

Conclusion

Hard water and transition metals both have a negative impact on stain removal. In order to completely soften hard water in laundry application, a very high amount of chelating agent would be required (due to the large excess of calcium). On the other hand, transition metals can be controlled using low concentration of chelating agents (<2.5 wt%).
GLDA shows excellent performance in controlling transition metals which is shown by enhanced stain removal for liquid detergents. It largely exceeds the performance of citrate and has comparable performance to the most used phosphonate (DTPMP), but with a much better environmental footprint (bio-based and biodegradable).

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Safeguarding Microbiological Quality and Safety of Cosmetic Products through a System Approach

U. Eigener

abstract

The EC-cosmetics regulation (1223/2009) defines requirements for the adequate quality and safety of cosmetic products. These requirements include microbiological aspects. Accordingly, microbiological objectives have to be fulfilled in various processes. These objectives can only reliably be reached by means of quality management systems (QMS), which cover all relevant processes. Such a system is presented by the GMP-guidelines (ISO 22716) which is part of the cosmetics regulation. Since microbiological objectives are also located in processes which are not in the scope of the GMP-guidelines (development, up-scaling, safety assessment) the system approach must be enlarged to include all microbiological activities: the MQM (microbiological quality management) is used as QMS. In this way, general system targets, which are covered for instance in the GMP-guidelines, can also be also applied for other processes, and thereby, microbiological quality and safety targets can be controlled in a holistic way. The system application (GMP and MQM) also allows for the systematic microbiological risk management which provides the basis for the safety statement. The MQM-system is used in the microbiological area as weight-of-evidence approach for the data used for the safety statement. The text describes how system elements of GMP and then also of the MQM-system are employed in practice as well as their importance for the microbiological quality and safety of cosmetic products.

1. General Relevance of a System Application

The application of quality management systems is seen as an indispensable tool to reliably reach quality targets. Quality must be understood as precondition for product safety: Certain product characteristics (acceptance criteria for quality) are essential for obtaining safe products. Accordingly, the system application is indispensable for reaching the safety targets in the same sense. The system application therefore guarantees that the consumer can constantly and reliably be provided with products and services of the defined quality and the necessary safety.

To reach these targets, a number of objectives have to be fulfilled when applying quality management systems, which are compiled here in only a few critical elements – "key objectives" (**Tab. 1**). Through the system installation and its subsequent application in the sense of these "key objectives", failures and risks are avoided, and an improvement is possible in time. Working in the system generally requires and facilitates activities, which are done with a holistic view, in contrast to working with single and punctual activities and results. This leads to an improved reliability of reaching quality and safety targets.

This is of special importance for the microbiological working area, since the microbiological quality and safety of cosmetics depend upon various factors which are located in different processes. Therefore, as many findings as possible must be gained from the system in order to control processes and procedures and to make valid decisions, thus safeguarding reaching the targets.

Some of the "key objectives", such as "defining processes and procedures" and "documentation", are well known in the daily working practice. Others are only sporadically employed, as experience shows. This is often caused by a limited knowledge of system principles or the result of wrong cost considerations. To obtain relevant information from the system requires sufficient adequate "control checks and tests" to be located in the processes. This must be based upon a

good knowledge of the processes and of the possibilities of test systems. "Changes/improvements" are part of all working processes – this is also the case in the product life cycle of cosmetic products. Very often, however, the fact that such activities may also have an impact on other procedures not directly connected is overlooked. The system approach makes it possible to anticipate the effect of further reaching implications and to initiate additional measures if necessary. Finally, it should be pointed out that "organisational structure and personnel qualifications" must be defined in such a way that personnel be sufficiently qualified for the specific microbiological tasks and that an adequate communication of new findings and risks can take place, in order to detect failures in time, to make scientifically correct decisions and employ corrections resp. corrective actions.

The "risk management" is of basic relevance and an integral part of all quality management systems. Due to the legal requirement of a final safety report with the safety assessment, the risk management plays an important role for cosmetic products – this includes the microbiological area as well. Microbiological risks, however, can only reliably be avoided, if risk management is regularly applied during development and production in a systematic and holistic approach. This covers then the definition of processes, procedures and microbiological targets, the localization and performance of control checks and tests, and the evaluation of findings and results. Likewise, risk management is particularly necessary in the event of any changes and improvements.

2. GMP and MQM

The EC cosmetics regulation [1] demands the use of the GMP as a quality management system for the manufacturing process – the ISO 22716 [2] has to be seen as part of the EC cosmetics regulation due to the publication in the official journal of the EC [3]. The GMP guidelines in the ISO norm contain, of course, all general specifications and measurements (see "key objectives" in 1.), which are needed in order to achieve the appropriate quality. Accordingly, most of the microbiological

Objective	Target	Explanations, Examples
Define Processes / Procedures	• To avoid failures, • Products with reproducibly consistent character	• Processes, procedures, measures, test-methods
Set Targets	• Product character, • Each single target in conformity with overall-target	• Target values for quality and safety, • Product-/material-specifications • Target-results for tests/control-checks
Perform Controls	• Make sure, that all targets are reached • Targets of all checks and tests to be in conformity with overall-target	• Control-checks, measurements, reviews and complaint-handling. • Control-extent • Suitable test-methods • Approval steps • Suitability and implementation of the system
Establish Documentation	• Requirements and their fulfilment documented • Traceability	Requirement-/performance documents • Processes/procedures/materials • Results • Requirements of organisation and personnel
Adequate Changes and Improvements	• Continuous improvement/ optimization • Changes respecting overall-system • Change management	• Changes due to operational, cost and optimization reasons • Improvements due to detected risks: counter measures / follow up-actions
Build up Organisation and Provide Qualified Personnel	• Suitably qualified personnel for performance of procedures and decisions • Defined duties and authorization • Communication	• Qualification demands • Duties and authorization • Training activities • Organisational structure
Apply Risk-Management	• To avoid failures • To detect and evaluate risks • Define effective counter measures / follow up-actions	• Holistic view in all processes/procedures • To be applied for decisions on all levels • Selection of materials/methods • Evaluation of results and approval • Recall-management • Safety assessment

Tab. 1 "Key-Objectives" for Working in Quality Management-Systems

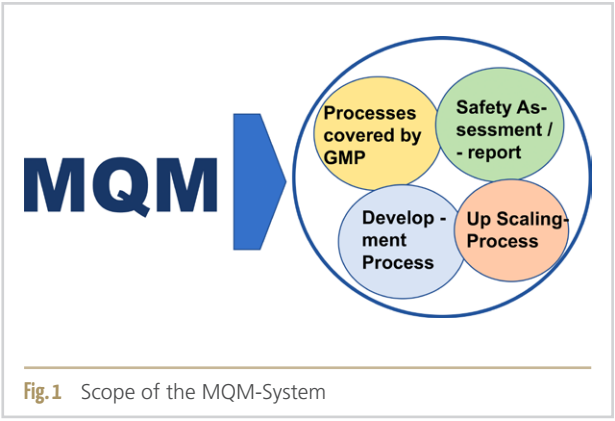

Fig. 1 Scope of the MQM-System

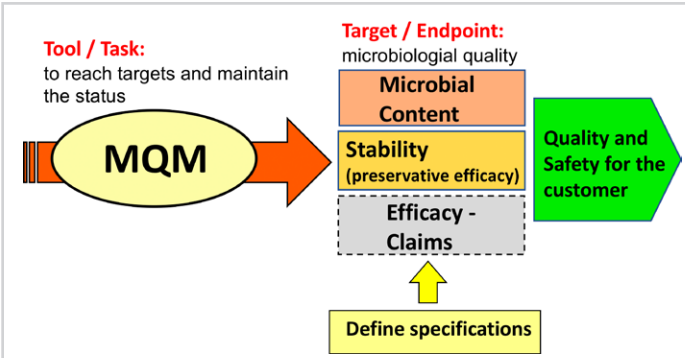

Fig. 2 Assignment of Tasks for the MQM

tasks connected with the microbiological quality and safety of cosmetic products are covered by the GMP system. However, not all processes relevant for microbiology are in the scope of GMP (ISO 22716), and therefore, this system alone cannot be considered sufficient for the microbiological objective.

What we need is a quality management system that compiles all the microbiological aspects required for the development, manufacture and marketing of cosmetic products. It is important to include the development process which is missing in the GMP (ISO 22716) [4]. However, the up scaling-process also plays an important role when striving for a reliable microbiological product safety [5]. Finally, system requirements result from the microbiological safety assessment which must be fixed in an adequate way [6]. All these requirements lead to the MQM-system (microbiological quality management) (**Fig. 1**). The MQM, which ensures the helpful compilation of all microbiological activities in the cosmetic area, has been claimed already many years ago. The understanding of the MQM's relevance as QMS, however, specially developed as a direct consequence from requirements subsequently legislated for product safety in the EC cosmetics regulation (1223/2009) [7, 8].

The MQM-system as enlarged quality management system for the microbiological working area covers, of course, the GMP guidelines as an integral part. By means of the MQM-system, however, the system-requirements are additionally transferred into processes which are not in the scope of the ISO 22716 norm, but which are essential for the microbiological quality and safety. In this way, the MQM-system becomes an essential tool for reaching the microbiological requirements given in the EC-cosmetic regulation. This regulation contains in Annex 1 [1] microbiological quality criteria which exclude microbiological product risks and must therefore be understood as safety requirements. Microbiological product claims (e.g. antimicrobial efficacy [9]) also present quality criteria, and sufficient proof of the effect must be given in the PIF (product information file) (Art. 11 of the cosmetics regulation [1]) – however, such criteria are not part of the safety assessment as mentioned in Annex 1 of the cosmetics regulation. The cosmetics producer is responsible for reliably fulfilling the legal requirements. This can only be reached by a system approach, which is given with the installation and maintenance of the MQM-system (**Fig. 2**). Thus, the MQM system for the microbiological field also represents the appropriate weight-of-evidence approach required for the safety statements (Art. 10, (1) b of the Cosmetics Ordinance [1]).

As already mentioned, every quality management system has the objective of eliminating product risks. Although this target is not explicitly mentioned in the norm 22716, it should be understood as an integral part of the system. This is all the more true as GMP is part of the EC Cosmetics Regulation, and sufficient product quality and safety are the core ideas of the legislation. Safety and risk are reciprocal forms of statements on the same content aspect, and thus risk management is an integral part of microbiological tasks for achieving microbiological product objectives [7, 8, 10]. Corresponding requirements must therefore be transferred to the MQM as an extended system.

3. Examples: System-impacts on Microbiological Settings and Conclusions

The following text shall describe in some examples why a system application is of special importance for the microbiological working area. The MQM system approach makes sure that the microbiological quality data are well substantiated and allow for a valid quality and safety statement (weight-of-evidence approach). The examples indicate the relevance of a well-functioning communication between the microbiology and various organisational units for the necessary exchange of information. Furthermore, the system makes sure that essential procedures and measures resulting from expert decisions are reliably implemented. All this, of course, calls for an adequate qualification of the personnel (on all hierarchical levels) for the execution of microbiological duties, for expert evaluation and decisions.

3.1 Testing for Preservative Efficacy

Preservation of cosmetic products and its efficacy testing are an important part of microbiological quality and safety. Even though the definition of a suitable preservation for the given formulation is done during the development process,

the production method may have a relevant impact on the maintenance of the efficacy. Contamination risks during manufacture, aspects of raw material quality and hygiene-management have to be observed as well [5]. This leads to the demand for preservative efficacy testing not only during the development process, but also for products resulting from the manufacturing process. Therefore, the up-scaling process, which leads to the method for the industrial production, plays a central role for the resulting microbiological product safety. Consequently, formula aspects, production method and production environment (e.g. contaminants from practical situation) must be taken into account for the preservative efficacy test (PET) [7, 11, 12]. The system application ensures that necessary information is exchanged between all organizational areas involved. The same requirements are necessary if the manufacture or parts of this process are transferred to new sites which are usually connected with certain changes in production conditions. Accordingly, such activities will usually require new efficacy testing. In all such cases, it is essential that product samples are provided and used for the tests, which can clearly be connected with the relevant procedures (sampling procedures, documentation).
There is no legal obligation regarding the test method and evaluation for the PET. Therefore, the producer must provide evidence for the market-fitness of their products. This can usually be done through market surveillance with a careful complaints management. All these possible influences on the test results must be taken into account during microbiological safety assessment, since only a valid PET result can lead to an effective exclusion of risks.

3.2 Manufacturing Process

Since a number of microbiological risks are located in the manufacturing process, various microbiological tasks have to be defined for this process. All parts and procedures of this process are covered by the GMP-system, which therefore contains microbiological tasks such as control checks and approval for raw materials and product, the hygiene system with necessary measures and controls and also the complaints management. Manufacturing aspects significantly contribute to market a microbiologically safe cosmetic product. The importance of the system is underlined through the demand for a defined production method in the PIF (product information file) [1] – the production method is, of course, part of GMP requirements – and for a GMP-conform manufacture.
The safety report with the safety assessment should be established based on "relevant information" [1]. For the microbiological quality and safety statement, the production method is definitely "relevant information", since this method influences the product risks through its impact on hygiene conditions and well as on the product stability (preservation) (see 3.1 and [5, 6, 7, 12]). Changes in the manufacturing process – especially in the production method – therefore always require a microbiological risk assessment. This might lead to adaptation of control and hygiene measures, but also a new PET and a new safety assessment might be necessary. All such activities need a communication of changes, which should be fixed in the system procedures. System procedures must also be installed to make sure that resulting counter measures/follow-up actions are reliably implemented.

3.3 Microbiological Safety Assessment

The microbiological safety assessment is part of the safety assessment which has to be performed before the product is launched into the market [1]. Defined microbiological quality criteria must be fulfilled to guarantee an appropriate microbiological product safety – especially limit values for the microbial content of raw materials and product and the microbiological stability (preservative efficacy). The adequate criteria must be defined for each product after a respective risk management [13, 14]. It goes without saying that processes and specifications for development, production and marketing must then be defined to ensure that the requirements are met and that the consumer regularly receives a safe product that meets the quality promise.
After a regular risk exclusion has to be observed for the determination of suitable process flows, measures and tests/controls and for approval steps, a final risk assessment is carried out by the safety assessment. The assessor has to analyse and to evaluate if the microbiological product characteristics fulfil safety requirements. For this purpose, specifications and data/results have to be checked, processes and procedures must be adequately fixed and test/control data must show the necessary results [6, 15]. The assessor must therefore view the relevant documents and data from the relevant processes and document the results of his or her assessment. Due to the importance of changes and improvements for microbiological quality and product safety (see above), the microbiological safety assessment must ensure that in such cases appropriate handling (change management) takes place and thus risks are avoided.
For a microbiological safety assessment, the application of an MQM system is important in two respects:
Firstly, the system is used to define the safety assessment procedures. These procedures cover, for instance, the selection of a suitable and qualified assessor (internal or external) and the authorisation to inspect documents and data. They should also define the obligation to take effective measures if failures are detected: The producer must initiate and perform a root cause analysis in case of detected contaminations, and correction and corrective actions must be taken. Such new findings also require a new safety assessment to be carried out.
Secondly, the quality management system contributes to reliably reaching microbiological quality and safety targets. For instance, limit values for the microbial content can only be effective if respective controls are carried out. The PET results are only valid if an appropriate method is used and if relevant product samples are tested (see 3.1). The MQM system substantiates the data for the microbiological safety assessment and the implementation of the microbiological product

safety. Therefore, it is recommended to check for the implementation of an MQM-system in the microbiological safety assessment [6, 15].

4. System Implementation

The cosmetics manufacturer is responsible for achieving the microbiological quality and safety objectives of their products. Therefore, he must make sure that appropriate quality management systems (GMP, MQM) are installed and implemented. The successful implementation of quality management systems can only be achieved if the corporate management backs this project and understands it as part of the company's quality policy. If the company does not possess respective qualifications to build up and install the MQM-system, it should subcontract an external partner.

It is essential to staff all positions of microbiological working areas (defining targets, performing tests/control checks, selection of test methods, evaluation of findings/results) with adequately qualified personnel. A particularly high qualification level and expert experience are required for risk management decisions and for the final microbiological safety assessment. Whenever external partners are appointed, this must clearly be taken into account when building up the system, because only then the advantage of the system application can positively affect reaching the microbiological targets. Generally, the system responsibility remains with the principal [16].

The organisational structure has to ascertain a reasonable placement of the microbiological working units, and these units must be provided with duties and authorization as appropriate. If different microbiological units belong to different organisational areas, an appropriate communication/cooperation should be fixed in order to enable a holistic evaluation for all microbiological decisions. Since close cooperation is also required with other organizational areas such as development, production, procurement, quality, and product safety, all relevant communication issues with these areas must also be covered in the system.

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Basic Requirements for Microbiological Testing of Cosmetics

B. Fellenberg, B. Heinken, D. Melchior, J. Nussbaum

Introduction

Microbiological issues play a central role in the quality assurance of cosmetics. This requires appropriate microbiological expertise and know-how. In addition to microbiological training, this microbiological expertise can also be acquired through professional experience, literature, further training and seminars. Basic microbiological knowledge is most helpful.
On the homepage of the DGK (Deutsche Gesellschaft für Kosmetik) you can find two new documents from the group "Microbiology and Industrial Hygiene", which provide valuable assistance regarding the microbiological safety and harmlessness of your products (https://web.dgk-ev.de/publikationen-fg2/).
The basic requirements for microbiological testing are explained in one of the documents in the form of answers to individual questions.
If you have more questions, please do not hesitate to send them to *Mrs. Joelle Nussbaum* (joelle.nussbaum@bav-institut.de). The topics will be discussed an answered by the working group of the DGK.

1. Why Do Basic Requirements for the Performance of Microbiological Tests of Cosmetics have to be Fulfilled?

In short: Consumer protection in Europe has become a high priority over the last decades. Insufficient methodological certainty in the performance of microbiological tests may lead to false negative or false positive results. This can endanger the safety of the consumer and lead to significant financial damage for the company.

Long: In addition to the known regulations, the cosmetics market is also subject to continuous monitoring by authorities and consumer protection organizations. Microbiological contamination of cosmetic products not detected in time (e.g. by false negative results) and a subsequent uncontrolled proliferation of microorganisms in a product can lead to consumer hazards, complaints, costly recall measures as well as listing of the company and the product in the European Safety Gate, former called RAPEX system (https://ec.europa.eu/consumers/consumers_safety/safety_products/rapex/alerts/?event=main.search&lng=de). In particular, the risk of critical user groups such as small children or sick people requires special protection and attention. False positive results can lead to delays in the internal product release and have an impact on production planning, storage capacity and delivery times.

2. Is it Necessary to Test my Product from a Microbiological Point of View?

In short: As a rule, microbiological testing of every cosmetic product is necessary to ensure the quality and safety of the product as well as consumer protection.

Long: *When does a product have to be tested?*
The person launching a product on the market is responsible for ensuring that the specified limit values for microbial counts and the absence of specified microorganisms are observed in order to ensure product quality and thus reduce the risk for the consumer. For this purpose, a microbiological risk assessment shall be carried out, specifically for each product with the aim of determining whether and to what extent a cosmetic product is to be tested.
This risk assessment considers a wide range of factors such as product composition, application and frequency of use, type of packaging, etc.
The majority of cosmetic products offers ideal conditions for the growth of microorganisms due to their composition. The manufacturer must keep the microbiological risk under control by suitable selection of a preservation system, taking into account further parameters (e.g. packaging) and defining control tests (in process controls, end product controls).

3. What is a Low-risk Product?

In short: ISO 29621 describes the properties of products which, if present or fulfilled, usually do not require (or only to a significantly reduced extent) microbiological tests such as, for example, preservative efficacy testing of the product. These products are called low-risk products.

Long: The parameters for classifying cosmetics as low-risk products are, for example:

- pH value < 3 or > 10
- ethanol or other alcohols > 20%
- water activity < 0.75

Examples for low-risk products: Hair straightening agents, body oils, lipsticks, hair dyes, antiperspirants, bleaching agents, products with organic solvents, alkaline compounds, etc.
It must be ensured that the products fulfill at least one of the above characteristics, nevertheless some products have to be microbiologically tested due to other factors (as a result of hygienic problems at the workplace, critical raw materials of vegetable/mineral origin).

4. What Needs to be Considered during Microbiological Sampling?

In short: When taking a sample, it must be ensured that it is representative for the entire batch. Sampling and subsequent storage must not alter the sample and thus influence the result.

Long: *What are suitable sampling vessels?*
For the microbiological analysis of end products, the samples are analyzed directly from the primary packaging. Sterile disposable vessels are ideal for sampling raw materials, semi-finished products and water. Due to the risk of contamination with foreign bodies, the use of glass vessels during sampling should be avoided.

What needs to be considered during sampling?
The sample must be representative for the entire batch.
When water is sampled, it must be taken into account that the sampling also reflects the daily use operations: if water is used without a preliminary drain in production, sampling also takes place without a preliminary drain (sampling taps must always be prepared accordingly). In addition, the sampling points in the system must be arranged in such a way that they allow a statement to be made about the status of the overall system (early recognition of developing problems).
As a rule, sterile disposable material or disinfected sampling equipment should be used to transfer the sample. The sampling personnel shall be trained in aseptic handling.
Finally, a negative example: Do not immerse the sampling vessel directly into the product, even with gloved hands.

What must be observed when storing and transporting samples?
As a rule, raw material and product samples do not require separate storage and transport conditions.
Water samples should be stored and transported at 2–8°C. The testing should be accomplished within 24 hours. Detailed information on this can be found in the Drinking Water Ordinance TrinkwV 2001 (https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/3_Downloads/E/Englische_Dateien/Drinking_Water_Ordinance.pdf).

5. How Often Do I have to Test my Product?

In short: In case the product is not low-risk (see point 2), each batch must be released on the basis of the results of the microbiological test.

Long: In order to release a batch in accordance with the GMP standard (ISO 22716), microbiological results must be available for this batch.
For example, it is almost impossible to transfer older results to an unchecked batch under certain circumstances. Attention must be paid ensuring constant microbiological and hygienic production conditions and consistent raw material quality.
In the cosmetic environment, there are often fluctuations in the process, especially with:

- Water systems, storage tanks, pipes
- different batches of raw materials, intermediate stored raw materials
- many employee-dependent individual work steps (during production or cleaning/disinfection)

Therefore, a microbiological final inspection of each batch of "not low-risk cosmetic" is essential. The testing of a semi-finished product does not replace the testing of the finished product. Between the manufacturing plant and the filling nozzles, some process steps still have the probability to influence the microbiological quality of the product.

What's a batch and how is a batch defined?
A batch is usually defined from a production point of view. It is the mixing of a defined quantity of water with defined quantities of raw materials in a vessel. If the same recipe is produced/mixed subsequently (water and raw materials are prepared and added again), it is defined as a new batch. Some companies define a batch using coding procedures. When establishing a sampling plan, the microbiological aspects shall always be considered. In the case of a large batch (e.g. several thousand individual containers or filling over more than one working shift), sampling at the beginning, in the middle and at the end of filling is recommended. If filling takes place over several shifts and batches, this must also be considered. At least one sample per shift should be tested. Downtimes during filling are to be considered separately.
In the case of a continuous production process, appropriate sampling intervals shall be defined.

6. Which Method Should I Use for the Microbiological Examination?

In short: For the microbiological testing of cosmetics, only test methods shall be used which showed a positive suitability test and thus provided reliable and reproducible results.

Long: Which test methods are to be used for the microbiological testing of cosmetics and raw materials and what needs to be considered?
In the testing of cosmetics, the corresponding ISO standards have become state of the art. This does not mean that other methods may not be used (e.g. European Pharmacopoeia methods). It is important that the suitability of the methods used has been demonstrated.
The following provides an overview of the test methods required to comply with the microbiological limits mentioned in ISO 17516:

Quantitative:

- ISO 21149: Aerobic mesophilic bacteria
- ISO 16212: Yeasts and moulds

Qualitative:

- ISO 18415: Specified and non-specified microorganisms in 1g
- ISO 18416: Detection of *Candida albicans* in 1g
- ISO 21150: Detection of *Escherichia coli* in 1g
- ISO 22717: Detection of *Pseudomonas aeruginosa* in 1g
- ISO 22718: Detection of *Staphylococcus aureus* in 1g

The general framework is provided by ISO 19838 (Guide to cosmetic products for the application of ISO standards in the field of microbiology of cosmetic products).
Normal work steps of a microbiological test can be:

- Weighing of the product: usually a quantity of at least 1g of a product is weighed in, in order to achieve reliable results and comply with the detection limit
- Dilution and neutralization: the initial weight is mixed with a dilution solution in a ratio of 1:10 (the diluent contains neutralizing agents such as lecithin, tween, etc.). This step is essential to neutralize possible antimicrobial substances in the product (and to prevent false negative results)
- Sample preparation (quantitative): a defined (corresponding to the product dilution) subset of the diluted sample is applied directly to culture media (agar plates), and these cultures are incubated for a defined time
- Sample preparation (qualitative): the diluted sample (at least 1g product has to be tested) is incubated (enrichment).

Evaluation: As microorganisms grow, they are counted (quantitative method) and the number of CFU/g sample is calculated taking into account the dilution (CFU = colony-forming unit). The qualitative test methods indicate the result as positive or negative in 1g product.

What is a suitability test?
A suitability test shows that the method is suitable for detecting the microorganisms in the product. For this purpose, the product is contaminated with a certain number of microorganisms and the method used must be able to recover this number (minus a microbiological measurement uncertainty) (recovery at least 50%).

Can I also use test methods other than those described above?
In general, the use of other test methods is also possible, a corresponding suitability of the method (see above) must be shown. So-called "rapid methods" are used as an alternative to the classical culture-based tests described above. These methods have advantages and disadvantages depending on the question and the field of application. In individual cases, these methods can be used, e.g. for testing raw materials. But this has always to be validated and examined in detail.

What is the Preservation Efficacy Test (Challenge test)?
The challenge test is a test procedure to confirm the adequate preservation of a product. For so-called low-risk products, this test is generally not required (see point 3). Most tests are carried out in accordance with ISO 11930 or alternatively the European Pharmacopoeia 5.1.3 method is used.
These test procedures include the following steps:

- cultivation of the specified microorganisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus brasiliensis*)
- contamination of the product with each of the microorganisms separately
- storage of the contaminated product over a period of 4 weeks
- determination of the germ count in the inoculated product at different points in time
- determination of germ reduction kinetics

7. May I Carry Out the Tests Internally? If so, what Requirements Need to be Considered?

In short: To operate your own microbiological laboratory, the requirements of the Biostoff VO (German Bio Substance Regulation) and the Infektionsschutzgesetz (German Infection Protection Act) must be complied with. The ISO 21148 standard as well as various standards (BRC, IFS HPC, etc.) also place requirements on personnel, premises and equipment. Testing is preferably carried out in laboratories offering appropriate accredited procedures (ISO 17025).

Long: *What is important when operating a microbiological laboratory?*
For the operation of an own microbiological laboratory, the requirements of the Biostoff VO (BiostoffVerordnung, German Bio Substance Regulation) and the Infektionsschutzgesetz (IfSG, German Infection Protection Act) have to be met.

- Working with pathogens or for targeted examination for certain microorganisms (exclusion of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* in 1g according to ISO 17516 or identification of microorganisms or inoculation or maintenance of a strain collection for challenge test), the laboratory requires a permit according to §44IfSG.

- §45 IfSG applies to all other microbiological activities. There is an obligation to notify according to §49IfSG.
- The authorities of the respective federal states often offer forms on the Internet for notification/permission.
- The laboratory requires a risk assessment according to §4 Biostoff VO for the activity with microorganisms.

Further information on the establishment and operation of microbiological laboratories in accordance with the "Infektionsschutzgesetz" can be found in the "Laboratory Guide" (IFSG_Leitfaden 2018) published by the Regierungspräsidium (regional council of) Tübingen.
Further information on the operation of a microbiological laboratory is published by the Berufsgenossenschaft Rohstoffe und chemische Industrie (Trade Association Raw Materials and Chemical Industry).
The protection level of the microbiological laboratory depends on the risk group of the microorganisms used. The requirements for premises and equipment are described in the following bulletins of the BG Chemie (Berufsgenossenschaft Rohstoffe und chemische Industrie):

- Work instruction B 011: Safe working on microbiological safety cabinets (Leaflet B011)
- Work instruction B 002: Laboratories, equipment and organizational measures (Leaflet B002)

8. What has to be Considered when Subcontracting Microbiological Analysis for Sample Release?

In short: If external laboratories are subcontracted for microbial analysis, the laboratory shall show sufficient technical expertise and experience in the handling of cosmetic products. It is recommended to select an ISO 17025 accredited laboratory.

Long: Planning subcontracting means a precise definition depending on the production context and bearing in mind the expected requirements (e.g. purity tests according to ISO 17516, challenge test according to ISO 11930). A clear and simple report format of the results should be ensured, which is understandable for all employees and not only for microbiologists. This is achieved by specifying internal and external specifications and specification limits, which are then marked as "fulfilled" or "not fulfilled" in the report.
A professional support for the interpretation of positive results as well as for microbiological and hygienic questions is very important.
In the case of a positive result, it is recommended that further analysis steps, such as a repetition of the examination, as well as identification of the detected microorganisms is defined in advance.
Thus, the laboratory has to guarantee that all important information is available for the final batch release in the right time.

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Ethik'Biote Daily Care – Soft & Powdery Touch | EU07533

Phase	Ingredients	INCI	Supplier	Function	% w/w
A	Water	Aqua			Qs 100
	FLUIDIFEEL™ EASY	Lauryl Glucoside and Myristyl Glucoside and Polyglyceryl-6 Laurate	SEPPIC	Emulsifier	3.00
	SOLAGUM™ AX	*Acacia Senegal* Gum and Xanthan Gum	SEPPIC	Natural Thickening Polymers	0.80
B	**EMOGREEN™ L15**	C15-19 Alkane	SEPPIC	Emollient	10.00
	APRICOT OIL	*Prunus Armeniaca* (Apricot) Kernel Oil	Bertin		5.00
C	**EQUIBIOME™**	Propylene Glycol and *Arctium Lappa* Root Extract	SEPPIC	Active Ingredient, Moisturiser	1.00
	GEOGARD 221	Benzyl Alcohol and Dehydroacetic Acid	LONZA		0.80
	Tocopherol	Tocopherol			0.05
	Apricot perfume	Fragrance	Expressions Parfumées		0.20

Procedure:
1. Introduce phase A into the tank and add the SOLAGUM ™ AX. Homogenize to avoid lumps.
2. Weigh phase B separately then add it to phase A with stirring to emulsify (12 m/s, 4 minutes).
3. Add phase C and homogenize.

Properties:
• Appearance: White liquid • pH-value: 5.5 • Viscosity 1M at RT: 3 300 mPa.s Brookfield S2S6
• Viscosity 1M at 45°C: 2 800 mPa.s Brookfield S2S6 • Viscosity recovery at RT (after 1M at 45°C): 3 200 mPa.s Brookfield S2S6
• Stability: Stable 1M at RT, 45°C and thermal cycles -5 à 40°C; Stable to centrifugation (3 000 rpm, 20 minutes)

Baby Natural Micellar Water | 67/CTBBY1841/00

Phase	Material Name	EU INCI	Supplier	% Material
A	Aqua/Water	AQUA	–	85.25
	Glycerin (657319)	GLYCERIN	–	4.00
	Hydrolite® 5 green (996442)	PENTYLENE GLYCOL	Symrise	0.90
	Sodium Benzoate	SODIUM BENZOATE	BASF	0.45
B	Miranol® C2M CONC NP	DISODIUM COCOAMPHODIACETATE	Solvay	3.00
C	**Allplant Essence Organic Camomile (311617)**	CHAMOMILLA RECUTITA (MATRICARIA) FLOWER/LEAF/STEM WATER	Symrise	1.00
	DragoCalm® (674463)	AQUA GLYCERIN AVENA SATIVA KERNEL EXTRACT	Symrise	5.00
D	Citric Acid 50% Sol.	AQUA CITRIC ACID	–	0.40
				100.00

Operating Instructions:
Blend phase A. Add phase B under gentle agitation and blend until homogeneous. Add phase C and blend until homogeneous. Adjust pH to 5 with phase D.
For questions concerning product safety and dangerous substance classification please ask for the Safety Data Sheets of the used Symrise products.

Specifications
• pH Value: 5 • Viscosity: N/A

POWERFUL LOOK De-puffing and Vitalizing Eye Gel

F17009.03

vytrus biotech

Phase	INCI	Trade Name	% (w/w)
A	WATER (AQUA)		83.02
	CAFFEINE		2.00
	DISODIUM EDTA		0.10
B	GLYCERIN		5.00
	PROPANEDIOL	Cosphaderm® Propanediol Natural	3.00
	HYDROXYETHYLCELLULOSE		0.50
C	ACRYLATES/C10-30 ALKYL ACRYLATE CROSSPOLYMER		0.40
D	PRESERVATIVE		1.00
	PARFUM (FRAGRANCE)		0.10
	CAPRYLYL/CAPRYL GLUCOSIDE, WATER (AQUA), SODIUM COCOYL GLUTAMATE, GLYCERYL CAPRYLATE, CITRIC ACID, POLYGLYCERYL-6 OLEATE, SODIUM SURFACTIN	Symbio® solv clear	0.50
E	SODIUM HYDROXYDE		0.08
	WATER (AQUA)		0.30
F	GLYCERIN, SARCOCAPNOS CRASSIFOLIA CALLUS LYSATE, CITRIC ACID	**SARCOSLIM RE-SHAPE** PRCF	3.00
	OLEA EUROPAEA (OLIVE) CALLUS CULTURE LYSATE, GLYCERIN, CITRIC ACID, CYAMOPSIS TETRAGONOLOBA GUM, XANTHAN GUM, TOCOPHEROL	**OLEA VITAE** PLF	1.00

Manufacturing process:

1. Add the caffeine to the water and heat to 40°C whilst stirring. Add the other components of phase A and keep stirring until totally dissolved.
2. Pre-disperse the hydrohyethylcellulose and add it to phase A avoiding the incorporation of air bubbles.
3. Disperse C in phase A+B.
4. Pre-dissolve phase D and add it to the phase A+B+C.
5. Allow cooling down to 40°C. Add consecutively the components of phases E and F and homogenize 10 min at 12000 rpm.
6. Check the pH and adjust it to 5.5–6.5 if necessary.

Properties:

- Appearance: white gel
- Viscosity: 6000–12000 cP (FUNGILAB Alpha series, PB spindle, 12 rpm, 20°C)
- pH: 5.50–6.50

DISCLAIMER: The information contained herein is meant to demonstrate how our products can be used. The given data, including claims and procedures, are suggestions without any guarantee, aimed at supporting customers' development. Any product manufactured corresponding to the present recipe is used at own risk and may require additional testing prior to marketing in order to comply with local regulations.

"War for Talents"

Interview with Marcus Mausberg, Managing Director, mausberg consulting

Mr. Mausberg, please tell us a bit about your career.
I've been working in the cosmetics and detergent industry since 1989. It all began with an apprenticeship as a chemical laboratory assistant at Henkel. I had already begun working in product development and analytics for cosmetics and detergents during my apprenticeship. After eight years in the laboratory, I switched to the consulting-based sale of raw materials and active ingredients. Then, a good ten years later, in 2007, I founded *neochem,* a distribution company focusing on personal care and HI&I raw materials and active ingredients. In 2011, we also acquired *domal Wasch und Reinigungsmittel GmbH* in Stadtilm and because of the sheer scale of the group's activities, service companies had to be set up to handle the diverse range of activities in IT, marketing, accounting and regulatory affairs management.


Marcus Mausberg

To what extent did you come into contact with the "War for Talent" during these nearly thirty years of your career?
In 2007, I founded a classic market follower in an existing and well-functioning market, the commodities trading market. Not only were the competitors established and, in many respects, superior to the "one-man show" *neochem,* but they also had corresponding reputations with potential applicants. That became my personal "War for Talent". Even back then, it was my challenge to attract good and motivated – even passionate – employees to *neochem*. I, and later my team, managed to do that over and over again. Even today, we "neochemlers" are still a strong community.

So you won your "War for Talent"?
I would definitely have to say no. Although the "battles" for talent were mostly won at *neochem,* the war was lost to an equal extent at *domal.* I had to experience first-hand and very dramatically that you can't win a "war" with the wrong warriors. The path that this took ultimately culminated in the insolvency of *domal,* which for me was one of the most formative experiences of my career.

How exactly do you define the term "War for Talent"?
The expression "War for Talent" basically describes the familiar principle of the market economy, namely the relationship between supply and demand. The expression was first discussed in 1997 by a well-known management consultancy firm. This refers to the increasing struggle for talent, i.e. qualified junior staff with development potential. The fact is that there is an oversupply of manpower for simple tasks that can be performed with simple training. At the same time, the opposite is the case for professions requiring specialist knowledge and expertise gained through professional experience: The demand for these skilled workers clearly exceeds the supply.

How do you identify this "War for Talent" in our industry?
In addition to the perceived factors of "duration of the search for qualified employees" and "average salary of the final selection", factors which my clients have repeatedly identified, statistics can also be used for this purpose. For example, the development of the birth rate in relation to the growth in the industry plays a decisive role here. Whereas in the 1960s just under 18 children were born per year per 1,000 inhabitants, between 1985 and 1994 there were only around 11 births per year per 1,000 inhabitants. The number of college graduates and skilled workers who have entered the labor market in the last ten years is correspondingly lower. At the same time, however, the market for detergents, cleaning agents and cosmetics has grown continuously and will continue to grow. According to IKW, total sales in the detergents, cleaning agents and cosmetics sector in 1990 amounted to approx. 8.4 billion euros. Twenty years later – in 2010 – it was already almost 16.8 billion euros and since then, the market has further grown close to 18.6 billion euros. Currently, this industry directly employs nearly 50,000 people. If you compare the birth rate and growth, you can sense that the "War for Talent" is not only in full swing, but – to stick to the context of "war" – is already claiming its first "victims".

What does this look like in concrete terms?
One typical symptom is that positions remain vacant much longer than they were a year or two ago. Today salaries are much higher, sometimes to an even unrealistic extent. But these are only the effects, taking a look at the underlying mechanism proves far more interesting.

Which would be ...?
Let's go back to the relationship between supply and demand: If demand is high and supply is low, the one who wins will be the one who is perceived as more attractive under the same economic conditions. Soft factors such as the presentation of the company are increasingly important: What is the company's image like? Does the company have a strong brand? How strong is the company's innovative force? To what extent are employees supported in their work-life balance? Are there incentives or bonuses? These and similar questions are being asked more and more often when addressing my talent. We are experiencing a massive change of generations. I myself come from the "live-to-work" generation, who defined themselves through their career and professional development.

And that's different today?
Today, we are increasingly dealing with the "work-to-live" generation, which focuses much more on "work-life balance" and understands professional advancement and the income generated more as a tool for achieving its own goals and desires. The focus has increasingly shifted to the person as such and not the job. However, this does not mean that this generation is doing a worse job than the previous one; the job merely is no longer the center focus as such.

What matters for today's talent?
A sense of inner solidarity and identification. Today's talent want to identify more than ever with the company's values. They want to move forward just as much as the company itself. This talent will be able to put things into motion in this respect! For many, pride in the company and in their work is also an essential element of the meaning of life. If one's own work is perceived as meaningful and sustainable, then their demands on the work-life balance are met. A former employee described a job change with the words "same shit – different flies". In this vein: when changing jobs, they must have the opportunity to free themselves from the "same shit". The "different flies", for example a higher salary, are often still the motivation to change. But the desire for identification and solidarity is becoming more and more important.

What can companies do to survive in the "War for Talent"?
It is important to invest in employer branding. This ultimately pays off in above- average corporate success. It is essential to credibly present your company as an attractive employer. This marketing measure achieves two things: on the one hand, better, more suitable talent will be interested in the open position. On the other hand, your own qualified, experienced and productive talent will identify more closely with your own actions and those of the company and will thus be more loyal to the company for a longer period of time. Equally important is modernizing recruiting as such or replacing it completely with contemporary "talent scouting".

What do you mean by recruiting?
The term recruiting has its origins in the military. This refers to the classic recruitment of personnel: a personnel department creates a job advertisement, which is distributed in the media, interested individuals apply, job interviews are held, and at the end an employee is hired. At least that's how it went for me in 1989, when I started at Henkel and my parents proudly told me that their son was now "at Henkel" and would stay there until the end of his working life.

What has to change?
The fundamental task will remain the same: the task is to find talent and to hire them. But the way this is done is changing. Today, more than half of all applications reach companies via digital channels. Applications are increasingly being generated by means of a wide variety of channels: from the company's own career portal, the employment agency, job exchanges or networks or directly via networkers – i.e. HR consultants.

Which approach would you recommend to companies?
"Talent Scouting" – that is: a departure from outdated structures and exaggerated formalities – towards short and direct communication channels and the creation of a corporate culture with employees who fit together well not only because of their experience and qualifications. The first step must be a detailed analysis of the position and the creation of a realistic talent profile. For the subsequent process, this means simplifying paths, shortening waiting times and processes and making them more direct. This saves time and money for everyone involved and prevents surprises and disappointments that can arise from unfulfilled expectations on both sides and usually lead to the new employee's early departure.

What are the advantages of talent scouting?
The relationship and communication between employer and potential employees must be right. Companies must actively approach applicants and candidates on the external job market. Professional "talent scouting" ensures that the applicant also fits in well with the company as a person in the long term – especially if communication is done right in advance

and both sides have a good feeling. As a talent scout, I have a different mindset than a classic recruiter and I communicate differently with talent. "Talent scouting" is the professional identification and acquisition of individual hand-picked talent that fit the company exactly.

Is communication everything?
No, but it is an essential differentiator between classic recruiting and talent scouting. After the aforementioned analysis of the position to be filled and the creation of the talent profile, the task is to find the right talent and assess them by means of interviews and a personality test. In my career, I have interviewed hundreds of candidates for various positions and salary categories. At peak times, I had up to 160 employees at a total of four locations. In these years and also during my time in the various start-ups, I have repeatedly experienced how much a company's prospects of reaching its targets live or die depending on the corresponding players involved. While as an entrepreneur, I often had to make do with only a satisfactory solution, as a consultant I can now put my finger on the wound and work on optimizing the result.

What factors characterize successful "talent scouting"?
Curiosity and creativity, the desire and strength to communicate. I am convinced that curiosity is the driving force behind creativity. Today's "state of the art" can quickly become tomorrow's left-overs. Only those who are curious will always learn about new developments and come up with new ideas. This is where creativity comes into play. For me as a talent scout, the ability to try out new things and develop my own ideas is an absolute must. With my imagination and my wealth of ideas, I can support my clients in winning their "War for Talent". I need to be creative from the moment I address talent to the moment I judge who is the best talent for the job. Curiosity in this context primarily concerns my curiosity about new people and the personalities that make these individuals tick. Which, in turn, help me succeed in other aspects.

Which are ...?
The desire to communicate and the strength of communication – the desire to use every available means of communication. Today, people expect communication at eye level. The days of the "distinguished consultant in a double-breasted suit" are long gone. Media such as Instagram and WhatsApp are also moving into the world of talent scouting. The infamous Generation Y knows about their coveted role in the "War for Talent". Fast, honest feedback is expected throughout the scouting process. Communication is characterized by a quick exchange of arguments on both sides of the table. The classic role distribution of the interviewer and interviewee, even the classic question and answer interview, is becoming more and more obsolete. As a talent scout, I am the extended arm of my client, at the same time also the "Carte Blanche", since I proceed much more directly in the entire scouting process.

So what is your conclusion?
The "War for Talent" represents a major challenge for companies. But there are various measures that help to attract promising talent. The first is to create a positive working environment and sense of identity within the industry. Workplace design also plays an important role. The choice of employer depends on whether there is a creative working atmosphere with meeting zones and an open dialog. Or are managers sitting behind closed doors instead, summoning their staff as subordinates rather than service providers to company meetings? This second set of measures has a significant impact on employee loyalty and retention. Work-life balance measures ensure that employees remain motivated and healthy. I even have clients who co-finance the pedelec their employees use to commute to work: For instance, employers contribute to the CO_2 balance and at the same time to the fitness and satisfaction of their employees. The weekly fruit and vegetable basket or further training courses as well as courses in health and fitness are already part of everyday life in smaller companies.

How does *mausberg consulting* support companies in finding suitable talent?
We have been working in talent scouting and management consulting since 2015. We develop programs for employer branding, support clients and talent during the entire scouting process and coach employees in the areas of sales and marketing. Our focus is on talent scouting for medium-sized enterprises and group companies. Since the middle of this year, we have also been active in these areas across all industries. For example, we recently helped a very established advertising and communications agency fill a strategic position in business development. This is a milestone for us because we had to rely on our processes in an industry in which we do not yet have the degree of networking as is the case in the detergents and cleaning agent industry and the cosmetics industry.

Thank you very much for the interesting interview!
Thank you!

www.mausberg-consulting.com

The Claims Support Seminar of proDERM Academy

Hamburg/Germany, December 2–4, 2019.

Realities & Practicalities

Following the huge 2018 success of this ever popular seminar sponsored by the proDERM Academy, the 2019 edition held here in Hamburg early December had high expectations, and neither speakers, delegates nor organisers disappointed. Once again, the proDERM Academy Claims Workshop successfully brought together a wide faculty of experts from diverse backgrounds covering all aspects of the claims development process. As with previous years, and following a warm welcome from host ***Professor Klaus Peter Wilhelm***, Day 1 opened with a practical session at the proDERM Clinical Research Centre. Demonstrations and practical knowledge were headed up by ***Stephan Bielfeldt*** and his team on how to generate evidence for claims substantiation.

Claims & The Consumer

Clearly energised, the following 2 Days comprised lectures and discussions on bringing claims to fruition from regulation to effective implementation. Day 2 opened with a general keynote presentation from ***Theresa Callaghan*** (Callaghan Consulting International, Germany) setting the scene for following lectures. Theresa discussed the essentials of a claim, and described a common sense approach when developing a claim in terms of concepts, product users, expectations of claims, what is to be measured and the weight of evidence required before a claim can be finalised.

This led into a cautionary yet slightly humorous presentation by ***Erik Schipper*** (i3 Innovate, Sweden) who delved more deeply into the importance of the consumer when creating claims and gave practical examples of the consequences of getting it wrong. His message was clear – be very aware of consumer insights when developing claims and the importance of how they fit together. Erik's presentation was a cautionary tale to drive home the message – focus, and clearly understand 3 key issues – what consumers say they do, what consumers actually do and why consumers do what they do, and importantly should never be biased toward a marketing whim!

Old & New Challenges

Consumer trials for claims development presented by ***Shazia Ginai*** (Neuro-insight, UK). Shazia showed that the world really is awash with cosmetic claims and as such the bombardment of them by advertising companies give rise to many consumer perceptions which are mis-read by developers if they are not careful. Consumer research is very powerful as it showcases the realistic perspectives and they go hand-in-hand with the technical support provided by laboratory and CRO testing. Consumer language and avoidance of bias and "forcing" in questioning are paramount in consumer studies. ***Harald van der Hoeven*** (CLR Berlin, Germany) provided an outstanding presentation on the importance of the raw material supplier in the claims development process and used the development of actives for the microbiome as an on-trend example. If we do not understand the microbiome how can we make claims? The biggest debate surrounded the concern of experts that the industry knows too little of the impact of influencing the skin's microbiome, and the possible problems that are likely to ensue.

Where to Get the Data

Firstly, ***Stephan Bielfeldt*** (proDERM, Germany) opened the session with a concise overview of how information to develop a claim can be provided by clinical setting. Clinical studies can really help bolster and help create strong claims for cosmetics and are one of the best approaches to obtain credible and relevant information for claims and a wide variety of methods and tools are available.

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Sylvia Zebrowski (proDERM, Germany), provided us with a guide to watch out for when using statistics in the development of claims, and make sure you use the correct test for your data. Too many studies use the wrong statistical analysis test resulting in invalid data. This is important when presenting data for advertising purposes and for when challenges of claims end up in court, or challenges by ASA's for example.

Building Claims

Delegates were provided with a selection of data covering consumer assessments, market share and technical study performance data for a new product from which they had to create a number of solid claims. Working in teams, were

encouraged to interpret the data and compile strong and provocative claims. The results were lively, intense, creative and competitive with some very robust challenges and defences! The take-home message here was clear: "does the consumer understand what you are getting at, and is it actually relevant to them." An added humorous note too, always be careful where you place your commas, asterisks, and Asterix!

The 3 R's – Rules, Regulations & Reviews

We were pleased to welcome ***Emma Meredith*** (Director General of the CTPA, UK) who provided us with clarity on the new claims guidelines published in July 2019. Given that each country in the EU has made its won decision on these guidelines, Emma provided some persuasive arguments on why it is important to ensure that any 'free-from' claim for example complies with the 6 claims criteria. Many do not. In addition, Emma also presented the Brexit scenario. In principle the UK will follow suit with the EU legislation when it comes to claims requirements and compliance.

Meera Cush (Delphic, UK) then explained claims compliance from the perspective of the Responsible Person (RP). Whilst major brands and multinational companies have their own internal RP function(s), SME's (small & medium enterprises) contract this service to either independent companies or even their contract manufacturer. The responsible person has a tough task meeting obligations in relation to claims since legislation is not black and white; the role requires a level of practicality and flexibility; and it is important to keep in mind overarching goals and consequences of non-compliance. Issues with Brexit also arose in line with Emma's presentations.

Making Impact: Seals, Endorsements, TV Advertising

Klaus-Peter Wilhelm (proDERM, Germany) took us through using seals and endorsements as part of the overall "claims" package, and provided a number of interesting examples on how these can work to improve sales and product credibility. It is important to take into account that seals and endorsements must also comply within the regulations with responsibility falling to the manufacturer and seal provider, especially when challenged either legally or by a competitor. The strength and credibility of a seal will always depend on the integrity and reputation of the seal provider!

Chris Gummer (Cider Solutions, UK) then brought us full circle and the event was closed out with a lively informative presentation on how to prepare claims support evidence for advertising in the UK. With some excellent examples and challenges thrown in, Chris outlined the tough and detailed requirements of UK advertising and Clearcast already gives guidance on the requirements for claim support and advertising in other EU countries.

Judging from the feedback the course delivered as part of proDERM Academy programmes, will become essential for anyone (especially marketing, R&D and legal) involved in claim substantiation or claims regulation in the cosmetics industry. We look forward to welcoming anyone involved in making claims to our next Claims Workshop.

A review by Theresa Callaghan

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in-cosmetics Global 2020 Announces Stellar Line-up for 30th Year

Barcelona/Spain, March 31 – April 2, 2020.

Celebrating 30 years of innovation, the industry's biggest cosmetics and personal care event announces exceptional programme of seminars and exhibitors for 2020.

Returning to Barcelona from 31 March – 2 April 2020, in-cosmetics Global is set to provide an unrivalled platform for R&D specialists and formulators at the forefront of the burgeoning cosmetics and personal care industry.

Celebrating its 30th anniversary and sixth year in the Spanish city, the leading event for personal care ingredients will bring together suppliers with leading finished product manufacturers, to network, learn about future trends and discover new products, ingredients and services.

With the value of the global cosmetic products market expected to reach USD 805.62 billion by 2023 [1], in-cosmetics Global will offer insights into the key trends driving this ongoing market growth. The show will welcome leading experts from Euromonitor, Mintel, GlobalData and BEAUTYSTREAMS, delivering the latest intelligence and market analysis.

Top Tier Exhibitors from Across the Globe

in-cosmetics Global continues to attract the industry's biggest global names in beauty and personal care. 100% of the top 20 suppliers, including BASF, Evonik and Croda, are expected in 2020. The next edition will also welcome over 50 Spanish suppliers who will cover everything from suncare to decoratives, including the likes of Industrial Quimica Lasem S.A.U (IQL), Neftis Laboratorios SL and Thor Especialidades S.A.

More than 20 new exhibitors are confirmed including the likes of Forestwise, leading manufacturers of sustainable and high-quality herbal extracts and natural carotenoids from the forests of Indonesia. These companies make up just some of the 800 suppliers expected to exhibit at the 2020 event.

Keeping up to Date with the Latest Market Trends

The popular Marketing Trends Theatre returns with a wide range of sessions, offering insight into the latest trends, facts, figures, market analysis and research data.

Highlights include; ***Gabrielle Beckwith***, Senior Analyst – Beauty and Fashion at Euromonitor, who will offer a tour of the world's beauty, from A-beauty to K-beauty and ***Michael Nolte***, Creative Director at BEAUTYSTREAMS, who will define consumer archetypes and how their lifestyles and buying habits will impact the beauty industry in 2021 and above: lifestyle shifts impacting product development and storytelling. While

Andrew McDougall, Associate Director – Beauty & Personal Care at Mintel, will question what the panorama of humanity means for the beauty industry.

Stylus' senior beauty editor ***Lisa Payne*** will reveal five beauty consumer tribes that brands need to maintain relevance and further their businesses in the ever-changing beauty landscape. In branding beyond green: a how-to guide for designing responsible beauty brands, Partner and Creative Director of Free The Birds, ***Nick Vaus***, will look at what responsibility means in beauty – tackling some of the common myths around sustainable ingredients and packaging.

The Marketing Trends Theatre will also host two roundtables – the first, focusing on Indie brand development, and the second debating the growing trend for CBD in beauty with representatives from Prohibition Partners, HO KARAN, and OTO.

[1] https://www.Reuters.com/brandfeatures/venture-capital/article?id=30351

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Time to get Technical

The comprehensive and free-to-attend educational programme will feature 80 technical seminars, where R&D professionals can familiarise themselves with key personal care ingredients and hear leading suppliers share hints, tips and in-depth solutions knowledge.
A session on taking care of our protective skin barrier and why we love ceramides will be delivered by Evonik's Global Head of Product Development Innovation Management Active Ingredients, ***Jennifer Schild***. Discussing formulating high-performance haircare products in the clean beauty trend, ***Caroline Mabille***, Global Business Development Manager Hair Care at Solvay Novecare, will showcase a selection of Solvay ingredients – such as eco-friendly conditioning Jaguar® and cruelty-free & gluten-free strengthening protein, Mackproplus® Rice C – that help formulators in the design of efficacious products in the clean beauty trend.
Principal Scientist at Ashland, ***Roger McCullen***, will talk about embracing hair's physiochemical properties to leverage its natural texture. He will present exclusive data to demonstrate the differences in the lipid characteristics of various hair types, using advanced imaging technologies to explain the properties of the surface and internal structure.

Shiny and New for 2020

Reflecting the rapid growth of beauty technology, the 2020 event will see the launch of a new Beauty Tech Zone at in-cosmetics Global, focusing on eight key product segments and functions including personalised purchase, ongoing support and sales strategies. Through a series of dedicated talks and exhibitor showcases, the Beauty Tech Zone will be a networking hub that brings together relevant buyers, investors, marketers and retailers.
Additionally, a new #PitchPerfect competition will give three exhibitors the opportunity to present concepts to a mixed panel of judges and win the first-ever 'Best Technology 2020' award for their innovative concept, solution or ready-made product.

Find Inspiration in the Formulation Lab

The Formulation Lab returns to give R&D professionals and those with a laboratory background an unrivalled opportunity to soak up formulation advice and practical techniques from the likes of Evonik, Sensient, Azelis and Ashland in a purpose-built lab. This year, in-cosmetics Global will be partnering with leading accredited online Organic Cosmetic Science School, Formula Botanica, who will be hosting formulation training sessions open to all R&D and non-R&D professionals.
Other Formulation Lab sessions will see ***Carol Corvez***, Global Formulation Senior Manager at Lucas Meyer Cosmetics, demonstrate how to formulate a sprayable emulsion in a sustainable way using Lysofix™ – a unique pure lysophospholipid O/W emulsifier for flexible formulations. Sharing the latest innovations in natural and mild cleansing, ***Claudia Brunn***, Manager Global Product Development Surfactants & Oleochemistry at BASF Personal Care and Nutrition GmbH, will present Texapon® SFA (INCI Disodium 2-Sulfolaurate) – a new natural anionic surfactant that enables the creation of ultra-mild, stable cleansing formulations with a special foam sensation.

Limitless Opportunities for Learning

Taking place on 30th March, the in-cosmetics Global pre-show Regulatory Conference also offers regulatory and compliance professionals an opportunity to advance their understanding of the world's biggest markets and their key regulatory modules. Covering everything from microplastics to halal certification, the paid-for conference helps professionals to stay compliant within differing international personal care frameworks. Spaces are limited, so attendees are encouraged to book their places and take advantage of the early-bird rate, available until 14 February 2020.
Elsewhere, personal care professionals can discover and discuss the most prevalent industry issues at one of five half-day Workshops. These paid-for lessons help attendees identify and plan for future business opportunities as well as navigate potential challenges surrounding topics including skin microbiome to preservation challenges.

in-cosmetics Guides the Way

in-cosmetics Global has been curated to allow visitors to make the very most of their time at the show. A number of specialist tours and trails will help attendees achieve this, including the Indie Trail, following its successful launch at the 2019 event.
Guided by formulation expert, ***Rouah Al-Wakeel***, R&D professionals are exclusively invited on a series of R&D tours to be introduced to selected exhibitors. The 2020 tours will focus on: protection, haircare innovation and naturals.

An Interactive Zone for Everyone

The vibrant Innovation Zone showcases, in one interactive space, the most innovative personal care ingredients designed to enable the next wave of formulations for personal care products. Products will be launching at the show or in the six months prior, providing R&D professionals unique exposure to innovation and a competitive edge.
The zone will also host Formulation Displays, where a number of exhibitors will offer the chance to test formulations. Event partners Mintel will also be holding Live Demonstrations, hosted by its Beauty & Personal Care team, who will present the latest products available for the hottest marketing trends. The Sensory Bar will offer stimulating, direct interaction with products including fragrances for haircare and transforming textures for moisturisers. For those looking for new creations for the next wave of colour cosmetics, the Make-Up Bar will present new formulations covering everything from pigments to technologies.

Offering yet another interactive opportunity, the Sustainability Corner will feature the latest advancements and accomplishments in sustainable personal care, including raw materials and products that meet certain environmental and social standards.
The Fragrance Zone will bring together prestigious fragrance houses, producers and distributors. Attendees can gain expert advice, learn about regional differences and source the latest aromatic components for personal care formulations from leading fragrance suppliers, including Luzi AG and CPL Aromas. For the latest developments in high-end lab equipment, the Lab Zone will bring together leading companies to share their knowledge of emerging trends and innovations. In addition, the Testing & Regulations Zone will inspire solutions to ensure the safety, stability and shelf-life of personal care products.
Highlighting contrasting global developments, country-specific, Pavilions will bring together niche suppliers from France, the UK, South Korea, Brazil, Spain, South Africa, Colombia, Peru and Tunisia.
Roziani Zulkifli, Exhibition Director of in-cosmetics Global, commented: "We're incredibly proud to be celebrating 30 years of in-cosmetics Global. During this time we have championed innovation and played host to some of the most influential product launches in the industry. Last year, personalisation and the rise of digital were highlighted as the next trends and the establishment of these drivers in 2019 is reflected with the launch of the new Beauty Tech Zone in 2020. We're excited to see what innovation will be revealed and what will be pinpointed as the next market drivers. As one of the most cost-effective platforms for R&D professionals, in-cosmetics Global is not to be missed."
For more information and to receive the latest updates, register to attend via the website:

www.in-cosmetics.com/register

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