The skin microbiota and stressful circumstances

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Abstract

The skin microbiota is described in countless articles [1] as a powerful ally of the skin. Losing or damaging this beneficial microbiota is detrimental to the health of the skin. The skin microbiota is especially damaged when exposed to short and rapid changes, such as to excess UV light. A strategy to support the skin microbiota is to use a prebiotic such as inulin, which acts as a power food that provides the necessary energy to cope with drastic changes. This study quantifies both the damage and recovery of the skin microbiota by UV-exposure, the use of ethanol, preservatives, surfactants, and glycolic acid. The protective and recovering benefits of different types of inulin in leave-on and rinse-off applications are measured using 2 techniques. The number of microorganisms was counted by cultivation on agar-TTC nutrient. The skin microbiota was also profiled through detailed interpretation of the sequencing-based 16S or IST profiling experiment.

UV light killed the skin microbiota in less than 20 minutes by 99.99%. A timid recovery started only after 4 hours. However, with inulin, already 80% of the skin microbiota recovered after 4 hours. Without the use of inulin, the skin was substantially invaded by microorganisms from the environment. Similar results were observed when the skin was challenged with other drastic changes.
A facial wash was tested on an acne prone skin. The surfactant increased the acne-pathogens, while inulin reduced them by 50%. Therefore, inulin is an interesting support for the skin microbiota and for the skin itself.

Keywords: (skin microbiota; UV-protection; inulin; acne).

Introduction The most successful strategy in skin care is to take care about the integrity of the skin. The use of mild ingredients, such as mild surfactants and emulsifiers avoids damage

to the precious epidermis. Thanks to the addition of hydrating and protecting ingredients, such as oils and hydrating agents, the skin is reinforced. Recent insights demonstrate that taking care of the skin integrity cannot be achieved without protecting the skin microbiota. How to preserve the integrity of the skin microbiota is still poorly understood, let alone how to maintain it.

The skin microbiota is essential to the skin for ~~some~~ many reasons:

1) Keeping the skin clean and radiant by removing waste proteins (keratinocytes) and oxidized skin lipids, sebum and sweat [2].
2) Production of an endless collection of anti-microbial peptides and acids that keep pathogens (including viruses) away [3].
3) Members of the skin microbiota communicate with the skin's immune cells increasing the resistance of our skin. The skin microbiota educates our immune system [4].
4) Antioxidants from the first categories such as SOD and RoxP are produced by the protective microbiota [5]. These antioxidants do prevent the formation of free radicals and protect the skin from premature aging, damage, and inflammation.
5) A strain of S. epidermidis produces 6-N hydroxyaminopurine. This substance reduces and prevents the formation of cancer cells [6].
6) Many skin microbes produce molecules such as glycerine and proteins that keep moisture on the skin.

To present it simply, but clearly: the skin microbiota operates as the perfect hydrating and protecting day cream. To perform all these protective actions, the skin microbiota needs a lot of energy. It finds a part of the necessary energy from the waste (desquamated corneocytes, sebum) secreted by the skin. However, this food is far from enough. Therefore, the skin-waste is upcycled towards metabolites such as amino-acids, lactic acid by members of the skin microbiota such as *S. epidermidis* and *Cutibacteria*. These metabolites feed other members of the skin microbiota society (*Micrococci, Brevibacteria*,…). These members on their turn produce again different metabolites that support other members. In other words, the skin microbiota receives the necessary power from all the members working together and supporting each other with food. All skin-microbes are united through a food web (figure 1)

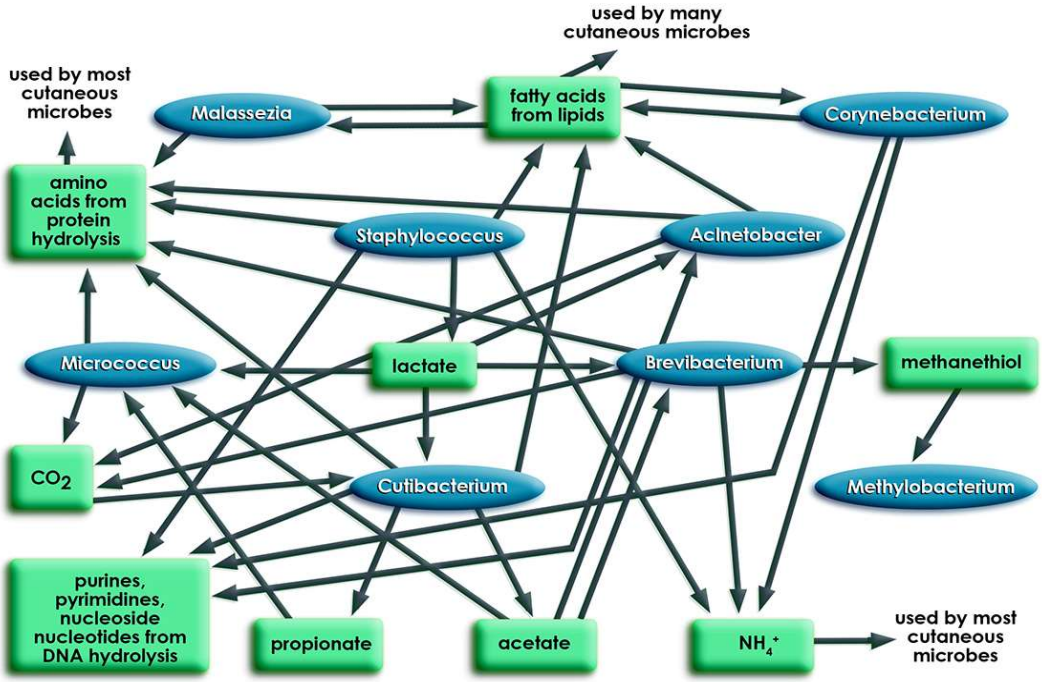

Figure 1. The Food web of the skin microbiota.

Sudden violent environmental changes change/disturb/damage the skin microbiota. This leads to a temporary interruption of the beneficial and protective properties that the skin microbiota offers the skin. The efficient upcycling and recycling of food is interrupted~~. The~~ so the skin microbiota cannot perform anymore as before. An immediate result is that the skin is left alone and unprotected. If these interruptions occur frequently and/or last for a longer period, the skin might become more sensitive, dries out, becomes irritated and on the long-term ages faster. What is more, since the skin lost its powerful protector, infections might occur. Skin problems such as acne [7], atopic dermatitis [8], psoriasis [9] are associated with a changed (unbalanced) skin microbiota where the presence of one micro-organism became dominant. The most common sudden violent changes that disturbs the cooperation of all the members of the skin microbiota, are: UV-light, disinfectants, preservatives, sudden pH changes, and surfactants.

Observing the skin microbiota is a challenge as it consists of more than 1000 different species (bacteria, fungi, viruses, and mites) that are constantly adapting and changing. The skin microbiota can be divided in 2 parts:

1) The resident skin microbiota, which represent the largest abundance in a healthy skin. They can grow on the skin and are attached to the skin surface through adhesins. The main members are Staphylococci, Cutibacteria, Micrococci, Corynebacteria, Brevibacteria, Malassezia. They support each other through the food web. All known beneficial properties are attributed to this group [10].
2) The transient skin microbiota is the group of microbes that cannot grow on the skin, they are not attached to the skin and no known benefits have been attributed to this group. Usually these are microbes coming from the environment and accidentally occur on the skin.

A promising strategy to support the skin microbiota under all circumstances is cosmetic grade inulin. Inulin is a prebiotic, which means that it can support the growth of resident and protective skin micro-organisms, but a possible pathogen cannot be supported by a prebiotic. Inulin is a natural polysaccharide of fructose monomers. It is extracted from Chicory root, Agave or Sun root. Inulin ~~supports~~ directly supports 3 members of the protective microbiota: *Micrococci, Corynebacteria and Brevibacteria*. It means that they can metabolize inulin. Through the food web, these metabolites support the other members. In this way inulin indirectly supports the entire protective microbiota.

Materials and methods: To study the influence of disturbing circumstances for the skin microbiota and to discover the supporting effect of inulin, the following test protocol was followed:

1) All tests have been performed in-vivo. Since the efficiency of the skin microbiota comes mainly from the mutual cooperation and interaction between all the members, it is difficult to simulate this complex microbe-society under in-vitro conditions. In-vitro simulation can also not simulate the skin-skin microbiota interaction.
2) One hour prior to the start of the test, each person was treated with the same simple O/W-lotion, containing no preservative and no perfume. This lotion had as a purpose to reset the environment for each individual skin microbiota. In this way all skin microbiotas of the test persons started in the same way. The formulation of the lotion is: Water - qs, C12-15 Alkyl benzoate - 10%, Sodium acrylate - 0.3%.

3) To identify the skin microbiota, a sample was taken by ~~means of~~ swapping firmly a DNA/RNA-Shield swap (Zymo Research))over a skin surface of approximately 6 cm^2 for 1 min. Since all test persons where healthy (except for the test on acne-prone skin) this procedure reveals the initially healthy, balanced skin microbiome.
4) To quantify the skin microbiota, an Agar-TTC slide was pressed firmly for 1 min over a skin surface of 10 cm^2.
5) Then, the skin microbiota was challenged with a sudden disturbance (UV, Ethanol, preservative mix, glycolic acid or surfactant)
6) After X hours the skin microbiota was sampled again for identification and quantification. The sampling was performed on an area close to the initial sampling
7) The skin microbiota was profiled through detailed interpretation of the sequencing-based 16S or IST profiling experiment. Short sequence reads were generated using the Illumina MiSeq platform.
8) The quantification was obtained through incubating the Agar-TTC slide for 48 hours at 30°C (+/- 1°C)
9) The change in abundance and the amount of each microorganism was compared to its original state.
10) The above procedure was followed for the placebo (product without inulin) and for the same formulation with inulin. All formulations were kept as simple as possible to minimize interference of other ingredients.
11) The inulin types used in this study were: short chain inulin (preBIULIN® FOS) for surfactant and alcohol formulations and long chain inulin (preBIULIN® AGA) for leave on application.
12) The test SPF30 formulation used in the UV challenged skin microbiota was as follows:

Ingredient	**Placebo**	**Inulin**
Aqua		
preBIULIN® AGA (Inulin)	-	2%
Glycerin	3%	3%
Preservative	0,8%	0,8%

SunZno SA (Zinc Oxide, Stearic Acid)	19.25%	19.25%
Neocare P3R (Polyglyceryl-3 (Poly)ricinoleate)	3%	3%
Miglyol 812 (Caprylic/Capric Triglyceride)	5.5%	5.5%
Cetiol V (Decyl Oleate)	6%	6%
Gosulin IL MB (Isoamyl Laurate)	9%	9%

13) The ethanol lotion used in the ethanol challenged skin microbiota was as follows:

Ingredient	**Placebo**	**Inulin**
Aqua	60%	59%
preBIULIN® FOS (Inulin, Fructose)	-	1%
Ethanol	40%	40%

14) The cream used in the preservative challenged skin microbiota was as follows:

Ingredient	**Placebo**	**Inulin**
Aqua	74.95%	72.95%
Xanthan Gum (Inulin, Fructose)	0.2%	0.2%
Hyaluronic acid (1,4-1,6 MDa) (Sodium Hyaluronate)	0.1%	0.1%
Glycerin	3%	3%
Glyceryl Stearate	2,5%	2,5%
PEG-100 Stearate	2,5%	2,5%
Lanette O (Cetearyl alcohol)	2%	2%
Squalane	3%	3%

Macadamia Nut Oil	3%	3%
Apricot Kernel Oil	3%	3%
Cetearyl Ethylhexanoate	2%	2%
Cetearyl Isononanoate	2%	2%
Diazolidinyl Urea	0.45%	0.45%
Phenoxyethanol	1%	1%
Methyl Paraben	0.2%	0.2%
Propyl Paraben	0.1%	0.1%
preBIULIN® AGA	-	2%

15) The products used in the glycolic acid challenged skin microbiota was as follows:

Ingredient	
Aqua	89.5%
Glycolic Acid	8.5%
preBIULIN® C90 (Inulin, Cellulose gum, Xanthan gum, Fructose, Cellulose)	2%

Cream		
Ingredient	**Placebo**	**Inulin**
Aqua	78.9%	77.4%
preBIULIN® AGA (Inulin, Fructose)	-	1.5%
Xanthan Gum	0.2%	0.2%
Glyceryl Stearate	2,5%	2,5%
PEG-100 Stearate	2,5%	2,5%
Lanette O (Cetearyl alcohol)	2%	2%
Miglyol 812 (Caprylic/Capric Triglyceride)	10%	10%

16) Test product for the acne prone skin test:

Ingredient	**Placebo**	**Inulin**
Aqua	90%	89%
preBIULIN® FOS (Inulin, Fructose)	-	1%
Sodium Cocoyl Glutamate	10%	10%

Results:

The skin challenged by UV-light

The skin of the forearms was exposed to the sun during the months June – August at 11 am. The exposure time was 20 min. After sun exposure the test subjects went out of the sun for the rest of the duration of the test (4h). 5 minutes prior to the sun exposure the SPF30 cream (Placebo) was used on the left forearm arm, while the right forearm was treated was treated with the SPF30 cream enriched with long chain inulin (SPF30-Inulin)

The skin microbiota was quantified before sun exposure, after 20 minutes of sun exposure, then again 2 and 4 hours later. The initial number of bacteria was 10^4 CFU/cm2 [10]. The results are shown in figure 2.

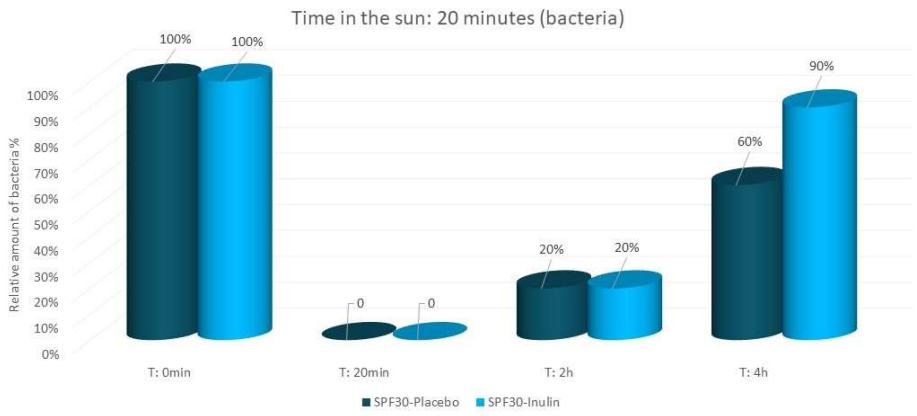

Figure 2. The evolution of viability of the skin microbiota Before/during/after UV-exposure.

The skin microbiota was identified before sun exposure and 4 hours after the exposure. ~~later~~. The results on the main resident microbiota (*Cutibacteria, S. epidermidis, Micrococci and Corynebacteria*) are shown in figure 3.

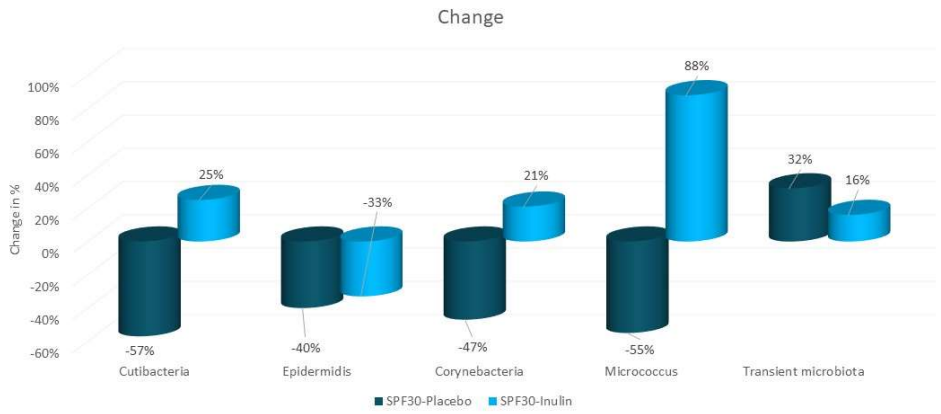

Figure 3. The impact of UV on the protective species of the skin microbiota and the transient microbiota.

The skin challenged with 40% ethanol

The skin of the hands was treated with a 40%-ethanol lotion. The left hand was treated with the ethanol lotion (Ethanol-Placebo), while the right hand was treated with ethanol lotion enriched with short chain inulin (Ethanol-Inulin).

The skin microbiota was identified before ethanol exposure, 1 minute and 4 hours after ethanol exposure. The microorganisms whose abundance changed by more than 30% were considered to be significantly disturbed. The evolution of the recovery of the skin microbiota is shown in figure 4.

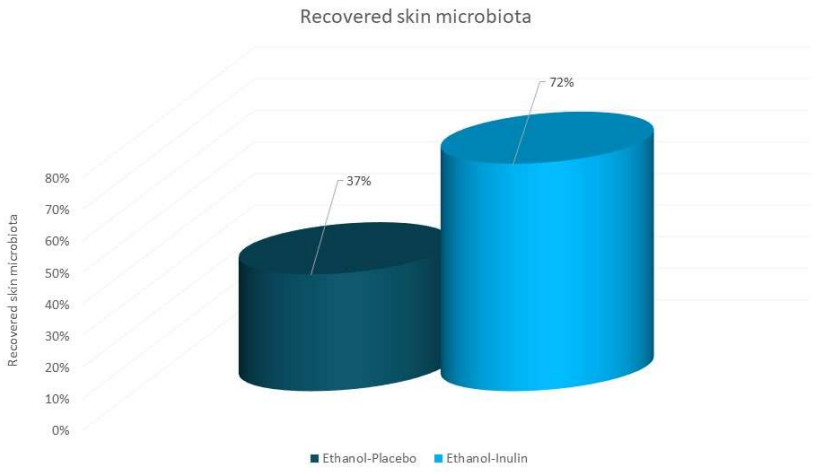

Figure 4. The Recovery of the skin microbiota 4 hours after ethanol disturbance.

The skin challenged with preservative

The skin of the forearms was treated with a cream containing a high number of preservatives. The left forearm was treated with the cream (Pres-Placebo), while the right forearm was treated with the cream enriched with long chain inulin (Pres-Inulin). The skin microbiota was quantified before applying the cream, then 1 and 4 hours after application. The effect of such a cream on the skin microbiota is demonstrated in figure 5.

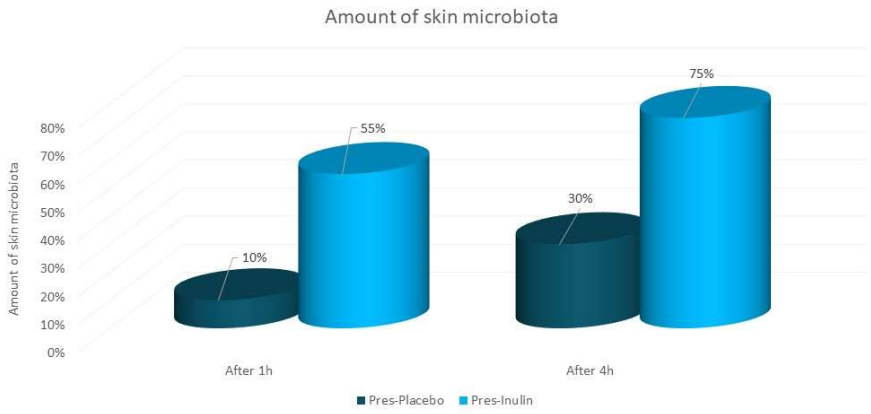

Figure 5. Recovery of the viability of the skin microbiota after using a preservative mixture.

The skin challenged by glycolic acid

The skin of the forearm was treated with a 8.5% glycolic acid gel. The gel was kept for 20 minutes and then removed with cotton patches. The left forearm was afterwards treated with a cream (Cream-placebo), while the right forearm was treated with the cream enriched with long chain inulin (Cream-Inulin). The skin microbiota was quantified before the glycolic acid treatment, 20 minutes, 2 hours and 4 hours later. The impact of such a treatment is demonstrated in figure 6.

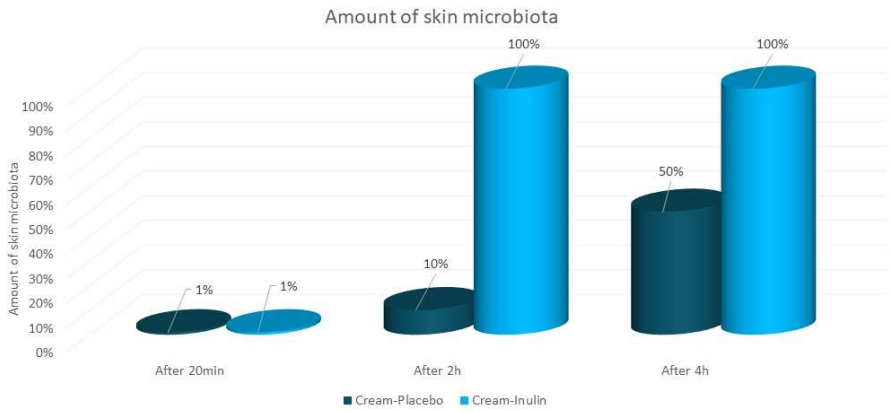

Figure 6. The viability of the skin microbiota of a glycolic acid treatment.

The acne prone skin challenged by surfactant

The acne prone skin was washed with a surfactant solution. The left part of the face was washed with the surfactant solution (Surf-Placebo), while the right side was washed with the surfactant solution enriched with short chain inulin (Surf-Inulin). The product was left ~~1 minute~~ on the skin for 1 minute. The skin microbiota was quantified before the test and 2 hours after washing. The results are demonstrated in figure 7. The evolution of C. acnes and S. epidermidis, the presumed pathogens in case of acne, was followed up in detail.

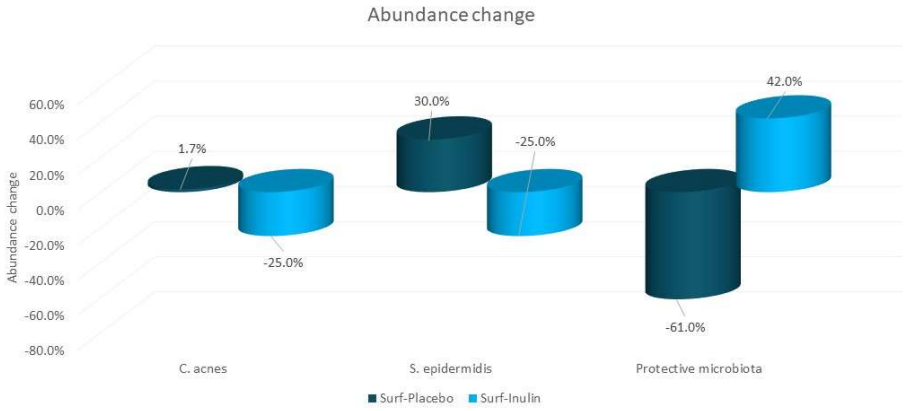

Figure 7. The impact of a cleanser on acne-pathogens and the protective microbiota.

Discussion.

In literature the disturbing factors for the skin microbiota are described generally, but in this study for the first time the impact of the most common disturbing factors are quantified. UV exposure has the most destructive impact, as the complete skin microbiota on the surface of the skin is killed after 20 minutes. Even after 4 hours the skin microbiota did not recover. The protective micro-organisms such as *Cutibacteria, S. epidermidis, Micrococci and Corynebacteria* were significantly reduced. It means that the skin is lacking the necessary protection, such as protection against the invasion of environmental microbes~~,~~ or destructive free radicals. Even 4 hours after sun exposure the skin is not properly protected. This might be an additional reason why the human skin is so vulnerable under UV light. It lost its main protective layer.

The disturbance of antimicrobial ingredients such as ethanol and preservatives were also particular invasive. Ethanol is particular destructive in the first seconds of applications, due to the body heat the ethanol evaporates fast and the skin microbiota is released from this nuisance. However, preservatives, although less destructive, ~~they~~ stay on the skin and can complete their destructive action on the skin microbiota. In this particular study the number of preservatives was excessively high to challenge the skin microbiota significantly. In

commercial products, the dosage of preservatives is much lower. This particular mix of preservatives reduced the amount of skin microbiota with 90% in just 1 hour. Such a reduced skin microbiota cannot protect the skin efficiently anymore.

Glycolic acid was invasive for the skin. The skin microbiota was seriously reduced (from 10^4 CFU/cm^2 towards 10^2 CFU/cm^2) immediately after application. This is a reduction ~~of~~ by 99%. After 2 hours the skin microbiota started a significant, but incomplete recovery. This gigantic attack by glycolic acid can be explained by the fact that the survival of microorganism depends on the activity of their enzymes. These enzymes are necessary to metabolize food such as proteins lipids and polysaccharides. The performance of the enzymes depends on the pH. Below pH 4.5 the enzymes necessary to survive are inhibited. Glycolic acid moreover dissolves the corneocytes, which is the main food source for the skin microbiota. The microorganisms are left with inactivated enzymes and almost no food to survive.

The disturbing effect of a mild surfactant on an acne prone skin was quite convincing. In rinse off conditions the surfactant favored the possible pathogens *S. epidermidis* and did not affect the C. acnes. Both micro-organisms are protective in a healthy skin, but in an acne prone skin they are associated with the disease. The worst part is that the surfactant reduced drastically the other remaining protective resident micro-organisms. This group was formed by other *Cutibacteria*, other *Staphylococci* (except *S. aureus*), *Microccoci, Corynebacteria, Brevibacteria* and *Acinetobacter.* These protective micro-organisms are the only chance to overcome the dominancy of the pathogens. They are the only resistance. However, when the pathogens are favored and the only chance for recovery is reduced, the presence of acne is even more secured. Washing an acne prone skin with a surfactant is most likely not the appropriate strategy.

The addition of inulin was able to support the skin microbiome under all disturbing circumstances. Inulin seems to be mainly efficient in speeding up the recovery of the skin microbiota, which means that the skin is faster fully protected in the presence of inulin. Inulin was not able to protect the skin microbiome from the destructive UV light, but inulin had a significant recovery effect on the main members (*Cutibacteria, Microccoci* and *Corynebacteria*) of the protective resident skin microbiota after 4 hours. The recovery of *S. epidermidis* was not significant. When the skin microbiota is destroyed by ethanol, inulin

cannot reduce this damage. This can be seen as positive, as it means that inulin does not interfere with the desired disinfecting effect. However, after 4 hours 72% of the skin microbiota was able to recover thanks to the presence of inulin. Without inulin the skin microbiota recovered only by 37%. Such a skin microbiota fails to support the skin.

Inulin turns out to be extremely efficient in keeping the skin microbiota operational in the presence of a high number of preservatives. The damage caused by the preservatives after 1 hour was significantly reduced thanks to the presence of inulin. After 4 hours the skin microbiota was fully recovered with inulin. Without inulin still 70% of the skin microbiota was not recovered.

Although the damaged caused by glycolic acid was gigantic and invasive, the recovery with inulin was even more spectacular. In this case a full recovery was observed after only 2 hours.

Presumably the most promising and interesting discovery was the use of inulin in a rinse off facial wash on the acne prone skin. In this test, which was performed under rinse off conditions, inulin was able to reduce the abundance of the possible pathogens in favor of the protective resident microbiota. As inulin has no anti-microbiocidal properties, inulin must have supported the growth of the protective microbes. When their abundance increased, proportionally the abundance of the pathogens decreased. It was not possible to determine whether the number of pathogens had decreased in this test.

Conclusion.

The sudden harmful situations for the skin microbiota described in literature do indeed decimate and disturb the skin microbiota. UV-light, ethanol and glycolic acid are the most destructive. Preservatives are less destructive, while surfactants seem to have a more disturbance effect on the abundance of the individual species. In all cases inulin offers ~~in all case~~ a significant recovery of the skin microbiota within 2 to 4 hours, which presumably results indirectly into a more efficient protection and recovery of the skin. Therefore, inulin is a valuable ingredient in protecting the skin and making all types of cosmetic products such as peeling, preserved emulsions, washes, etc.… much more skin and skin microbiota friendly.

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Conflict of Interest Statement. NONE.

References.

1. Chen Y, Fischbach M (2018) Skin microbiota-host interactions. Nature 553 (7689: 427-436
2. Moskovicz V, Gross A, Mizrahi B (2020) Extrinsic factors shaping the skin microbiome. Microorganisms 8 (7): 1023
3. Rademacher F, Glaser R (2021) Antimicrobial peptides and proteins: interaction with the skin microbiota. Experimental Dermatology 30 (22)
4. Grice A, Segre J (2003) The skin microbiome. Nat Rev Microbiol 9 (4): 244-253.
5. Stodkilde K et al (2022) Structure of the Cutibacterium acnes-specific protein RoxP and insights into its antioxidant activity. Front Cell Infect Microbiol 12: 803004
6. Nakatsuji T et al (2018) A commensal strain of sStaphylococcus epidermidis protects against skin neoplasia. Sci. Adv. 4:eaao4502
7. Lee Y, Byun E, Kim H (2019) Potential Role of the microbiome in acne: a comprehensive review. 8(7): 987
8. Gong J et al (2006) skin colonization by Staphylococcus aureus in patients with eczema and atopic dermatitis and relevant combined topical theray: a double-blind multicentre randomized controlled trial. Br J dermatol. 155 (4): 680-7
9. Wang W, Jin H (2018) skin microbiome: an actor in the pathogenesis of psoriasis. Chin Med J 131 (1): 95-98
10. Selwyn S (1980) Microbiology and ecology of human skin. Practitioner 224:1059-1062