Skin Barrier Enhancing Alternative Preservation Strategy of O/W Emulsions by Water Activity Reduction with Natural Multifunctional Ingredients

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Abstract: Water activity (a_w) as an important parameter for self-preservation can help to control microbial growth in cosmetic formulations. However, high amounts of water-binding substances are required to lower the a_w enough to affect microbial growth. Since consequences for the skin barrier have been poorly studied so far, we investigated the effect of a_w-lowering agents on both the antimicrobial properties of o/w emulsions and skin physiological parameters. A combination of selected natural humectants (Sodium lactate, Propanediol, Erythritol, Betaine and Sodium PCA) with a total concentration of 28 wt% in an o/w emulsion was able to reduce its a_w from 0.980 ± 0.003 to 0.865 ± 0.005. The challenge test results of the a_w-lowered emulsion showed a convincing microbial count reduction in potentially pathogenic microorganisms. The addition of as little as 0.5% of the antimicrobial multifunctionals Glyceryl Caprylate and Magnolia Officinalis Bark Extract further enhanced the antimicrobial effect, resulting in adequate antimicrobial protection. Moreover, twice-daily application of the a_w-lowered emulsion for a period of four weeks led to a skin barrier-enhancing effect: TEWL significantly decreased, and SC hydration significantly increased. Thus, we present an opportunity to replace conventional preservatives with a natural alternative preservation strategy that has been shown to offer benefits for the skin.

Keywords: water activity; self-preservation; hurdle technology; formulation; skin barrier; humectants; multifunctional ingredients; antimicrobial

1. Introduction

Microbial stability is one of the most important requirements for a cosmetic product, as uncontrolled microbial growth can pose serious consequences for consumers’ health. Most cosmetic formulations contain enough water and nutrients to provide a breeding ground for microorganisms, such as bacteria, yeasts and molds. Spoilage microorganisms can lead to changes in color, odor or consistency of a formulation, but they can also be pathogenic and cause hazardous infections, such as corneal ulcers [1] or septicemia [2].

In order to ensure the quality of a product and its safety for consumers, chemical preservatives are added to personal care products. However, there is an increasing number of studies with reports on common preservatives causing skin irritation or allergic reactions. For example, formaldehyde-releasing imidazolidinyl urea has been suggested to be responsible for skin reactions in individuals with sensitive skin [3–5]. Several studies have further found that the commonly used isothiazolinone can cause allergic reactions [6]. Moreover, in 2004, a study from Darbre et al. [7] discovered that methylparaben is present in human breast cancer tumors. Although no causal connection between parabens and the development of breast cancer could be established, the study attracted considerable attention. As a result, the safety of preservatives is being questioned by consumers, leading to product rejection and a demand for new solutions. For this reason, cosmetic manufacturers
are increasingly looking for alternative preservation strategies to circumvent use of the criticized classical preservatives.

Interest in the development of self-preserving cosmetics has risen over the past few years [8,9]. Self-preserving products are based on the principles of “hurdle technology”, which have been applied in the food industry since the 1970s to control microbial growth in food, but can also be transferred to the cosmetic industry [8,10]. This concept combines various factors, such as a low redox potential, an acidic or basic pH or low water activity, each of which act as hurdles to the survival of microorganisms.

This work focuses on water activity ($a_w$) as a hurdle for microbial growth. Unlike the water content of a formulation, which indicates the total amount of water including the molecularly bound water, $a_w$ is a measure of “free”, unbound water in a product. Restricting the availability of unbound water leads to deterioration of the initial conditions for microbial growth. Consequently, lowering the $a_w$ with water-binding agents can inhibit microbial growth and improve the microbial stability of a cosmetic product [11].

As in food products with a high water content, high quantities of water-binding substances are required to reduce the $a_w$ of skincare emulsions to such an extent that microorganisms are affected. For example, Pushpalatha et al. [12] developed a self-preserving herbal oral care formulation that required at least 40% glycerol in combination with other polyols to sufficiently reduce the $a_w$ and make the formulation microbiologically stable. However, in a leave-on formulation, such a high glycerol concentration creates an unpleasant, sticky feeling on the skin. Furthermore, the consequences of correspondingly high concentrations of hygroscopic ingredients for the skin barrier have not yet been studied in detail.

To rule out a detrimental effect, we used natural humectants to lower the $a_w$ of o/w emulsions, in accordance with their maximum use concentrations, as recommended by the Cosmetic Ingredient Review (CIR) safety assessments [13–16]. Thereby, we focused on using endogenous components of the Natural Moisturizing Factor (NMF) or natural, harmless alternatives that are known to protect the skin from dehydration. We then investigated both the antimicrobial properties of the $a_w$-lowered emulsion by challenge tests and its influence on the skin barrier by a randomized forearm-controlled study. In addition, we studied how the linkage of a low $a_w$ and antimicrobial additives affects the antimicrobial efficacy and whether the number of antimicrobial agents required can be reduced. This should provide an opportunity for a natural alternative preservation strategy without conventional preservatives that is skin-friendly and may even offer benefits for the skin due to the use of multifunctional ingredients.

2. Materials and Methods

2.1. Raw Materials

The antimicrobial agents Cosphaderm® GMCY (INCI: Glyceryl Caprylate) and Cosphaderm® Magnolia Extract 98 (INCI: Magnolia Officinalis Bark Extract), as well as the ingredients Cosphaderm® Feel (INCI: Triheptanoin), Cosphaderm® X 34 (INCI: Xanthan Gum) and the solute Cosphaderm® Propanediol natural (INCI: Propanediol), were provided by Cosphatec GmbH (Hamburg, Germany). The remaining solutes used were ERYLITE® (INCI: Erythritol), which was supplied by Jungbunzlauer Suisse AG (Basel, Switzerland), as well as Sodium Lactate, Sodium PCA and Natural Betaine (INCI: Betaine), which were supplied by alexmo cosmetics GmbH (Stuhr, Germany). Cetyl Alcohol was also supplied by alexmo cosmetics GmbH. Shea Butter (INCI: Butyrosperum Parkii Butter) and MCT oil (INCI: Caprylic/Capric Triglyceride) were provided by Gustav Hees GmbH (Leonberg, Germany), and Imwitor® 372 P (INCI: Glyceryl Stearate Citrate) and Softisan® 154 (INCI: Hydrogenated Palm Oil) were provided by IOI Oleo GmbH (Hamburg, Germany). Lactic Acid and Sodium Hydroxide were supplied by Carl Roth GmbH + Co. KG (Karlsruhe, Germany). All raw materials were used without further purification. For preparation of the formulations, demineralized water, filtered by an ion exchanger, was used.
2.2. Formulations

The five formulations studied were o/w emulsions containing varying amounts of an a\textsubscript{w}-reducing solute combination (Table 1) and antimicrobial agents (Table 2), expressed in weight percent, with the ratio between lipophilic and hydrophilic phases remaining unchanged (Table 3). The emulsions contained either 28%, 20% or 0% solutes. To the emulsions with 28% solutes or 0% solutes, 0.5% of a combination of the antimicrobial agents Glyceryl Caprylate (GMCY) and Magnolia Officinalis Bark Extract (ME) was added, respectively.

Table 1. Composition of the a\textsubscript{w}-reducing solute combinations.

| Solute | INCI                | Composition of Combination [%] |
|--------|---------------------|--------------------------------|
|        |                     | 20%                            |
|        | Propanediol natural | Propanediol                     | 5.7                            |
|        | Sodium Lactate      | Sodium Lactate                  | 5.7                            |
|        | ERYLITE\textsuperscript{3} | Erythritol                     | 3.6                            |
|        | Natural Betaine     | Betaine                        | 3.6                            |
|        | Sodium PCA          | Sodium PCA                      | 1.4                            |
|        |                     | 28%                            |
|        |                     | 8.0                            |
|        |                     | 8.0                            |
|        |                     | 5.0                            |
|        |                     | 5.0                            |
|        |                     | 2.0                            |

Table 2. Composition and use concentration of the antimicrobial agents.

| Antimicrobial Substances | INCI                | Ratio       | Use Concentration [%] |
|-------------------------|---------------------|-------------|-----------------------|
| Cosphaderm\textsuperscript{®} GMCY | Glyceryl Caprylate, Magnolia Officinalis Bark Extract | 60:40       | 0.5                   |
| (GMCY)/Cosphaderm\textsuperscript{®} Magnolia Extract 98 (ME) |                       |             |                       |

Table 3. Basic composition of the studied o/w emulsions.

| Phase | Ingredient                     | INCI            | Quantity [%] |
|-------|--------------------------------|-----------------|--------------|
| A     | Demineralized Water            | Aqua            | ad. 100%     |
|       | Solutes \textsuperscript{1}    |                 | varying      |
|       | Antimicrobial agents \textsuperscript{2} | X 34            | 0.50         |
|       |                                | Xanthan Gum     |              |
|       |                                |                 |              |
| B     | MCT Oil                        | Capryl/Capric Triglyceride | 5.00         |
|       | Softisan 154                   | Hydrogenated Palm Oil | 1.00         |
|       | Cetyl Alcohol                  | Cetyl Alcohol   | 2.00         |
|       | Shea Butter                    | Butyrosperum Parkii Butter | 5.00       |
| C \textsuperscript{3} | Solute \textsuperscript{4}   | -               | varying      |
|       | Demineralized Water            | Aqua            |              |

\textsuperscript{1} Propanediol, Sodium Lactate, Erythritol and Sodium PCA (Table 1); \textsuperscript{2} Glyceryl Caprylate/Magnolia Officinalis Bark Extract (Table 2); \textsuperscript{3} Not applicable for formulations without solutes; \textsuperscript{4} Betaine was added to cooled emulsion due to its heat sensitivity.

The formulations were prepared according to the following protocol: The components of phase A and phase B were combined separately. For phase A, Xanthan Gum was dissolved in demineralized water under stirring at room temperature, and solutes as well as antimicrobial ingredients were added if necessary. Both phases were heated to 70 °C. Phase B was then added to phase A under stirring at 1000 rpm. The slightly cooled emulsion was homogenized with the T 25 digital ULTRA-TURRAX\textsuperscript{®} (IKA\textsuperscript{®}-Werke GmbH & CO. KG, Staufen, Germany) at 9000 rpm for two minutes. Phase C was incorporated into the stirring emulsion at room temperature. The pH value was adjusted to 5.50 ± 0.05 with Lactic Acid (90%) or Sodium Hydroxide (30%) one day after preparation.

2.3. Water Activity Measurement

Water activities were measured with a Waterlab (Stereoglass\textsuperscript{®} S.r.l., Perugia, Italy) dew point water activity meter from I&L Biosystems with an accuracy of ±0.003 a\textsubscript{w} (at 25 °C). An
internal temperature control within the sample chamber ensured that measurements were performed and standardized at 25 ± 1 °C. Sample containers were filled up to the mark with the formulation to be analyzed. The “N3 mode” was set as the measurement program. In this mode, the measuring process with measurement intervals of about one minute is completed once the deviation of three subsequent data points is less than 0.003 a_w.

 Calibration was carried out with calibration standards consisting of saturated salt solutions.

2.4. Challenge Test

The antimicrobial efficacy of the preservative system was evaluated by challenge tests according to the specifications of the European Pharmacopoeia (Ph. Eur.) 5.1.3 by an external laboratory (J.S. Hamilton Poland Sp. z o.o., Gdynia, Poland). The following germs were tested: Escherichia coli (E. coli) ATCC 8739, Pseudomonas aeruginosa (P. aeruginosa) ATCC 9027, Staphylococcus aureus (S. aureus) ATCC 6538, Candida albicans (C. albicans) ATCC 10231 and Aspergillus brasiliensis (A. brasiliensis) ATCC 16404. Tryptic soy agar was used as culture medium for the bacteria, Sabouraud dextrose agar for C. albicans and potato dextrose agar for A. brasiliensis.

An amount of 20 g of each formulation was inoculated with 0.2 mL of the calibrated inoculum (1.0 × 10^5–1.0 × 10^6 colony forming units (cfu)/g) and incubated at room temperature for 28 days. The colony forming units of 1 g of each sample were determined by counting after 2, 7, 14 and 28 days.

The assessment of the antimicrobial protection of the formulations was performed using the evaluation criteria of ISO 11930 [17], which is specifically designed for cosmetic products (Table 4). The evaluation criteria refer to the results of the log reduction, calculated by forming the decadic logarithm from the quotient of the number of cfu at the beginning of the test and at the respective time points. The test is considered passed if an A or B criterion is achieved. Achieving an A criterion is recommended, as it expresses that a cosmetic product meets the requirements for protection against microbial contamination. The B criterion is also sufficient to pass the test but indicates that a formulation is less effectively protected than a formulation with an A criterion. If necessary, additional parameters (e.g., adapting the packaging) must then be considered.

Table 4. Criteria according to ISO 11930 for assessing the microbial stability of a cosmetic product [17].

| Microorganisms | Criteria | Day 7 | Day 14 | Day 28 |
|----------------|----------|-------|--------|--------|
| **Bacteria**   | A        | ≥3    | ≥3 + NI| ≥3 + NI|
|                | B        | -     | ≥3     | ≥3 + NI|
| **C. albicans**| A        | ≥1    | ≥1 + NI| ≥1 + NI|
|                | B        | -     | ≥1     | ≥1 + NI|
| **A. brasiliensis** | A | -     | ≥0    | ≥1 + NI|
|                | B        | -     | ≥0     | ≥0 + NI|

NI: no increase compared to the previous count.

Results of the challenge tests were related to the minimum a_w values given in the literature, which are required for the growth of the tested pathogenic microorganisms (Table 5).

Table 5. Minimum a_w values for the growth of pathogenic germs in cosmetics [18].

| Microorganisms | Minimum a_w |
|----------------|-------------|
| P. aeruginosa  | 0.97        |
| E. coli        | 0.95        |
| S. aureus      | 0.86        |
| C. albicans    | 0.87        |
| A. brasiliensis| 0.77        |
2.5. Study Design

To investigate the effect of an o/w emulsion with a low $a_w$ on physiological skin parameters, twelve healthy volunteers (three men and nine females, aged between 18 and 50) were enrolled in a randomized forearm-controlled study after they were fully informed of the study protocol and gave informed consent. Exclusion criteria included active skin diseases or excessive hair growth at the volar forearms, allergies to cosmetic ingredients, sunbathing during the study period, participation in another cosmetic study as well as pregnancy or breastfeeding.

The study was performed under standardized conditions (22 ± 2 °C room temperature, 50 ± 10% relative humidity) at the premises of Cosphatec GmbH (Hamburg, Germany) in September 2021. Subjects were informed not to apply topical products at the test site for at least twelve hours prior to the measurements and to avoid water contact at the test site for at least six hours prior to the measurements.

After an acclimatization period of 20 min, the skin parameters were measured 10 cm above the distal wrist crease on both volar forearms to obtain baseline values. Subsequently, subjects were instructed to apply 0.2 g of the test emulsion twice daily to the randomly selected volar forearm for a period of four weeks. After four weeks, skin parameters were measured again and compared with the baseline values.

2.6. Evaluation of Skin Parameters

Skin barrier function was assessed by measuring transepidermal water loss (TEWL) using a Tewameter® TM Hex (Courage & Khazaka Electronic GmbH, Cologne, Germany), expressed in g/m²/h. For each test site, one measurement was taken per assessment time for approximately 20 seconds at intervals of one second until the deviation between the individual readings was less than 0.03 g/m²/h.

SC hydration was determined by measuring the electrical capacitance of the skin surface with a Corneometer® CM 825 (Courage & Khazaka Electronic GmbH, Köln, Germany), expressed in arbitrary units (a.u.). Three measurements were performed slightly offset next to each other for each test site per assessment time, and the resulting mean value was calculated.

2.7. Statistical Analysis

Statistical analyses were performed with IBM® SPSS® Statistics 27 (IBM Corporation, Armonk, New York, NY, USA). The Shapiro–Wilk test was used to confirm normal distribution of the data. Significant differences between the means at the study begin (baseline) and after four weeks were determined using a paired t-test. The significance level was set at 5% ($\alpha = 0.05$). Thus, a $p$-value of $\leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Impact of Solute Combinations on $a_w$ of o/w Emulsions

A combination of common humectants with water-binding properties (Sodium Lactate, Propanediol, Erythritol, Betaine and Sodium PCA) was added to the emulsion at a concentration of 20% and 28% (solute ratio remaining unchanged), and the resulting $a_w$ was measured. The specific compositions of the solute combinations are listed in Table 1.

The results of the $a_w$ measurements performed on the emulsions containing 20% or 28% solutes, respectively, compared to the reference without solutes are presented in Figure 1.

A total of 20% of the solutes were able to reduce their $a_w$ by 6.4% from 0.980 ± 0.003 (reference formulation without solutes) to 0.917 ± 0.006. Thus, a value was reached that impairs the growth of the gram-negative bacteria $P$. aeruginosa ($a_w = 0.970$) and $E$. coli ($a_w = 0.950$) according to the critical values in Table 5. With 28% solutes, an even greater $a_w$ reduction of 11.7% to 0.865 ± 0.005 was achieved. Following the minimum $a_w$ values that allow microorganisms to grow (Table 1), the higher solute concentration additionally affects the proliferation of $C$. albicans (minimum $a_w = 0.870$) and $S$. aureus (minimum $a_w = 0.860$).
Adequate protection against *A. brasiliensis* was not expected because the critical $a_w$ (0.770) is lower than the measured $a_w$ of the formulation. Nevertheless, with 28% of the solute combination, the $a_w$ was lowered to such an extent that it should be unfavorable for most potentially pathogenic germs in cosmetics.

![Figure 1. Mean water activities of o/w emulsions with 20% or 28% of the solute combination (colored bars) compared to the reference o/w emulsion without solutes (grey bars) at 25 °C with standard deviation error bars ($n = 2$) and percent reduction indicated.](image)

3.2. Effect of $a_w$ on Microbial Stability of o/w Emulsions

In order to verify the assumption that the growth of certain microorganisms is restricted at an $a_w$ of 0.865 and 0.917, challenge tests were carried out on the o/w emulsions containing either 20% or 28% of the solute combination (Table 1) and on the o/w emulsion without solutes (reference).

While all of the tested microorganisms, with the exception of *A. brasiliensis*, were able to grow in the formulation without solutes (Figure 2a), a significant antimicrobial effect was observed in the formulations with a lowered $a_w$ (Figure 2a,b). With 20% solutes ($a_w = 0.917$), *E. coli*, *P. aeruginosa* and *C. albicans* were maximally reduced after 14 days at the latest (Figure 2b). The reductions in *S. aureus* and *A. brasiliensis* were less pronounced, which was expected considering their critical $a_w$ values reported in the literature (Table 5). Alternatively, the growth of *C. albicans* was inhibited, although the reported minimum $a_w$ for growth is lower (0.870).

The antimicrobial activity was even more marked in the formulation containing 28% solutes ($a_w = 0.865$) (Figure 2c). A reduction in *A. brasiliensis* was achieved despite the low critical $a_w$ value that is below the formulations’ $a_w$. The log reduction in *S. aureus* met criterion B of ISO 11930, while the log reductions in the other microorganisms even fulfilled criterion A, indicating that the formulation was successfully protected against microbial spoilage solely by the $a_w$-lowering solutes.

To analyze if $a_w$ reduction can be used in combination with antimicrobials in order to reduce their required application concentration, challenge tests on emulsions with different $a_w$ values and a sub-sufficient amount of the antimicrobial multifunctionals GMCY and ME were performed. The $a_w$ values of these formulations were slightly higher compared to the formulations with the same number of solutes but without GMCY/ME.

The challenge test results of the formulation without solutes ($a_w = 0.988$) showed that the addition of 0.5% of the antimicrobial multifunctionals GMCY and ME reduced microbial counts—especially *E. coli* (Figure 3a). As expected for the chosen sub-sufficient application concentration, the microbial count reduction in *P. aeruginosa*, in particular, was not enough to pass the test without lowering the $a_w$. 

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Figure 2. Challenge test results of the o/w emulsion (a) reference without solutes ($a_w = 0.978$), (b) with 20% solutes ($a_w = 0.913$) and (c) with 28% solutes ($a_w = 0.868$), indicating the colony-forming units (cfu/g) for the germs E. coli, P. aeruginosa, S. aureus, C. albicans and A. brasiliensis after 2, 7, 14 and 28 days.

Figure 3. Challenge test results of the o/w emulsion with 0.5% Glyceryl Caprylate/Magnolia Officinalis Bark Extract (a) without solutes ($a_w = 0.988$) and (b) with 28% solutes ($a_w = 0.876$), indicating the colony-forming units (cfu/g) for the germs E. coli, P. aeruginosa, S. aureus, C. albicans and A. brasiliensis after 2, 7, 14 and 28 days.
In comparison, the formulation with reduced $a_w$ (0.876) clearly showed a much better performance against microbial growth (Figure 3b). Here, the addition of 0.5% GMCY and ME led to faster maximum reductions in *E. coli*, *P. aeruginosa* and *C. albicans*. Furthermore, *S. aureus*, which had the lowest microbial count reduction in the formulation without antimicrobial additives, was maximally reduced on day 7 of 28. Since the log reductions in all tested microorganisms met criterion A (ISO 11930), the test was passed, indicating that the o/w emulsion was effectively protected against microbial spoilage.

We further examined whether synergistic effects were achieved by combining low $a_w$ with antimicrobial ingredients, which would imply that a reduction in the concentration of both antimicrobial additives and $a_w$-lowering humectants is possible. Therefore, the log reductions in *S. aureus*—the microorganism with the lowest microbial count reduction—in the o/w emulsions with only 28% solutes, only 0.5% GMCY/ME or both in combination were compared with the theoretical calculated additive effect (Figure 4). In case of synergy, the log reduction in *S. aureus* in the emulsion with the combined system would exceed the additive effect. The maximum achievable log reduction in *S. aureus* was 4.88, which was already reached on day 14 for the additive effect. Thus, the time period in which synergistic effects could be detected was limited up to day 7. For the other tested microorganisms, the period was even shorter, hence only the plot of the *S. aureus* log reductions is presented.

![Figure 4](image-url)

**Figure 4.** Plot of the log reductions in *S. aureus* in the o/w emulsion containing either 28% solutes, 0.5% Glycerol Caprylate/Magnolia Officinalis Bark Extract or both systems in combination as well as the calculated additive effect, which results from the addition of the individual systems. The maximum achievable log reduction was 4.88, therefore, the additive effect (if it exceeded this value) was set to 4.88.

Figure 4 shows that the antimicrobial effect of the combined system ($a_w$-lowering solutes and the antimicrobial agents GMCY/ME) was considerably better than that of the individual systems. The maximum log reduction in *S. aureus* in the emulsion with the combined system was reached notably faster. In addition, on days 2 and 7, the log reductions exceeded the calculated additive effect, indicating a synergistic effect. Thus, the $a_w$ reduction allows for lowering of the concentration of the antimicrobials GMCY/ME necessary to meet the safety standards of cosmetic formulations even more than if the effect were merely additive.

### 3.3. Effect of an $a_w$-Reduced o/w Emulsion on Skin Physiological Parameters

The effect of a leave-on formulation containing hygroscopic solutes on the physiological skin parameters TEWL and SC hydration was investigated in a study involving twelve volunteers aged 28 ± 5. The test formulation used was the o/w emulsion ($a_w = 0.867$),
containing 28% of the solute combination (Table 1) and 0.5% GMCY/ME, which passed the challenge test with an A criterion.

The results of the measurements of both parameters show a skin barrier enhancing effect of the \( a_w \)-lowered emulsion (Figure 5). The mean values of the TEWL at the untreated forearm (control) remained stable and showed no significant differences between the measurement time points (Figure 5a). After four weeks of twice-daily application of the \( a_w \)-reduced formulation, the TEWL decreased statistically significantly from \( 6.65 \pm 0.974 \, \text{g/h/m}^2 \) to \( 5.98 \pm 1.18 \, \text{g/h/m}^2 \) (\( p < 0.05 \))—a decrease of 10.1%. A detrimental effect to the skin barrier due to application of the \( a_w \)-reduced formulation could therefore be excluded.

![Figure 5. Mean values (n = 12) of (a) transepidermal water loss and (b) stratum corneum hydration on forearms as a function of measurement time points (baseline and after 4 weeks) with error bars indicating standard deviation and * symbols indicating statistically significant results (\( p < 0.05 \)).](image)

In agreement with the results of the TEWL measurements, the SC hydration results showed a positive effect of the \( a_w \)-reduced formulation on the skin barrier (Figure 5b). After four weeks, the SC hydration on the control forearm remained at baseline levels, while the SC hydration on the treated forearm increased statistically significantly by 15.6% from \( 31.9 \pm 6.87 \, \text{a.u.} \) to \( 37.8 \pm 3.04 \, \text{a.u.} \) (\( p < 0.01 \)). Thus, an improvement in skin condition was observed after four weeks of application.

4. Discussion

Water activity serves as an important parameter for controlling microbial growth in foods [10]. In the cosmetics industry, despite the increasing interest in self-preserving products, there have been relatively few published approaches to influence the growth of microorganisms through \( a_w \) reduction in personal care formulations. However, these have shown that improvement in microbial stability can be achieved [12,18].

Our results clearly confirm that the importance of \( a_w \) with regard to the antimicrobial properties of cosmetic formulations can be easily transferred from the food to the cosmetics industry. Both \( a_w \)-lowered emulsions (\( a_w = 0.913 \) and 0.868) without additional antimicrobial agents have shown a convincing antimicrobial effect.

In general, a reduction in water activity may be accomplished by using water-binding substances, such as polyols, amino acids, protein hydrolysates and salts [8]. In this study, we focused on substances that lower the \( a_w \) efficiently and, at the same time, can be used as humectants in cosmetic formulations. A total of 28% of the solute combination consisting of Propanediol, Sodium Lactate, Erythritol, Betaine and Sodium PCA were able to lower the \( a_w \) by 11.7% to 0.865 \pm 0.005. This value is unfavorable for most of the relevant potential pathogenic microorganisms in pharmaceutical or personal care formulations according to the critical \( a_w \) values given in the literature [18,19].
The challenge test results of the o/w emulsion with an \( a_w \) of 0.868 confirmed excellent efficacy of the formulation against gram-negative bacteria, yeasts and even molds. Only the microbial count reduction in *S. aureus* was less pronounced, but it still resulted in a criterion B (ISO 19930).

In a study performed by Kerdudo et al. [18], the antibacterial efficacy of a cream with an \( a_w \) of 0.898 ± 0.009 improved in comparison to the reference cream with an \( a_w \) of 0.961 ± 0.001, and the log reductions in the bacteria *P. aeruginosa*, *E. coli* and *S. aureus* fulfilled criterion B according to the European Pharmacopoeia challenge test method [20]. However, no sufficient protection against yeasts and molds was obtained.

Based on the critical \( a_w \) values reported in literature [18,19] and the results of Kerdudo et al. [18], no protection against *A. brasiliensis*, but better efficacy against *S. aureus*, were expected. Since the counts of *A. brasiliensis* were unexpectedly reduced in the control formulation with a high \( a_w \), it is conceivable that the emulsion provided an unfavorable environment for the mold. The growth conditions were probably further restricted by \( a_w \) reduction, leading to a significant microbial count reduction in *A. brasiliensis*. In contrast, the less pronounced microbial count reduction in *S. aureus* might be due to the type of \( a_w \)-lowering agents used. It is known that the type of solute has an impact on how well a microorganism can handle a low \( a_w \) [21]. Numerous studies have shown that *S. aureus* accumulates increased levels of compatible solutes in the intracellular space in environments with low \( a_w \) [22–24]. It is assumed that the accumulation of compatible solutes lowers the intracellular osmotic potential and thus prevents the outflow of water from the cell [25]. In a study by Miller et al. [24], betaine and proline were identified as the most effective osmoprotective substances. Since the studied o/w emulsion with an \( a_w \) of 0.868 contained betaine as a solute, it would be conceivable that *S. aureus* accumulated betaine as an osmoprotective substance and was thus less affected by \( a_w \) reduction despite reaching the critical \( a_w \) of 0.86. This could also be a reason why Kerdudo et al. achieved a better bacterial count reduction in *S. aureus* with natural glycerol as \( a_w \)-lowering agent, although the \( a_w \) of the formulation they studied was higher (0.898). Ultimately, the results confirm that the critical \( a_w \) values do not represent sharp limits and can be shifted, i.e., depending on the temperature, pH, composition and other factors of the formulation, as reported by Kabara et al. [19]. Against this background, it could be advantageous in the water activity reduction to use multiple \( a_w \)-lowering solutes to attenuate as many microorganisms as possible.

Based on hurdle technology, the combination of specific parameters that act inhibitory on microorganisms can further enhance the antimicrobial effect [10]. Berthele et al. [26] pointed out that a cosmetic product with an \( a_w \) higher than 0.8 can be adequately protected without any incorporated listed preservatives, if the right combination of other hurdles, namely pH and ethanol concentration, is maintained. Nevertheless, further studies with changes to the experimental design (i.e., less ethanol, a less acidic or basic pH) have been proposed to better meet industry needs. In our study, we investigated how the linkage of antimicrobial multifunctional ingredients and low \( a_w \) affects antimicrobial efficacy. The results of the challenge tests showed that an addition of as little as 0.5% of the multifunctional ingredients GMCY and ME to the \( a_w \)-lowered emulsion considerably reduced the *S. aureus* counts and improved efficacy against *P. aeruginosa*, *E. coli* and *C. albicans*. Accordingly, the challenge test was passed with an A criterion, while the formulation with the normal \( a_w \) failed the test. Furthermore, a synergistic effect was confirmed. While the microorganisms were subjected to osmotic stress by the low \( a_w \), GMCY and ME have been suggested to have cell membrane disrupting properties [27–29]. In addition, a part of the antimicrobial effect may have been contributed by Sodium Lactate, which was included in the solute combination. Since the pH of the formulation was 5.5, a minor part of the sodium lactate is present in the form of undissociated Lactic Acid, which is supposed to damage cells by disrupting the proton gradient [30]. Consequently, the various stress factors disrupting osmotic homeostasis, cell membrane integrity and proton homeostasis have resulted in the achievement of a synergistic effect against *S. aureus*. This is consistent with a publication
by Leistner [10], according to which synergistic effects can be achieved if different hurdles simultaneously affect the cell by independent mechanisms or target independent cellular structures. Further studies of the $a_w$-lowering solutes in combination with other membrane disruptive antimicrobial systems would be useful to elucidate the relationship.

Ultimately, by reducing the water activity to 0.876, in combination with small amounts of the multifunctional substances GMCY and ME, a formulation was developed that fulfilled the microbial safety requirements of a cosmetic product. Thus, the formulation could be tested in a study on volunteers to further analyze the influence of the $a_w$-reducing solutes on the skin barrier.

TEWL and SC hydration are common parameters for assessing skin barrier function. A high TEWL indicates a disrupted skin barrier function and has been observed in skin diseases, such as atopic dermatitis [31]. Knowledge about the effect of high amounts of humectants on the skin barrier is limited. Fluhr et al. [32] reported that undiluted glycerol could potentially lead to a dehydration of the skin upon osmotic action. Such a detrimental effect could be ruled out for the developed $a_w$-lowered formulation containing 28% humectants. Instead, a skin barrier enhancing effect was obtained: After twice-daily treatment with the $a_w$-reduced emulsion for four weeks, the TEWL significantly decreased by 10.1%, and the SC hydration significantly increased by 15.6%. Hence, the skin was lastingly hydrated due to the regular application of the $a_w$-lowered emulsion. We confirmed that humectants promote skin hydration even at the low water activity of the applied formulation, as reported by Alber et al. [33]. As $a_w$-lowering solutes, Alber et al. used glycerol or urea at a concentration of 20%. However, in personal care formulations, these humectants are usually used at lower concentrations since glycerol affects sensory properties and urea has a keratolytic effect [34]. This could be the reason why, to our knowledge, there are no cosmetics on the market that are based on, or advertised as, formulations that are highly reduced in water activity. To create a more practical and customer-oriented approach for the personal care sector, our $a_w$-lowering solute combination was carefully composed regarding ingredient concentrations. It further contained Sodium Lactate and Sodium PCA as substances of the NMF, which are usually insufficiently present in dry skin [35]. Since NMF components are known to protect the skin from drying when applied on the SC, they might have contributed to the skin barrier-enhancing effect.

Thus, we have successfully linked water activity with microbial stabilization as well as skin barrier stabilization in one interlocking approach.

5. Conclusions

In this work, we have, for the first time, connected water activity reduction as a method to improve microbial stability of a formulation with a positive effect on skin barrier function. The strategy of using a combination of attractive natural humectants, such as Propanediol or components of the NMF, as $a_w$-lowering agents provides a skin barrier-enhancing formulation that simultaneously exhibits an improved microbial stability. Therefore, only minimal additional effort is required to achieve adequate antimicrobial efficacy, as ensured by a small amount of antimicrobial multifunctional ingredients, e.g., Glyceryl Caprylate and Magnolia Officinalis Bark Extract.

Thus, an alternative, natural and modern preservation strategy with a positive effect on the skin barrier has been developed, offering the possibility to replace conventional preservatives. Moreover, the strategy is not limited as the applied $a_w$-lowering solute combination can be modified by other small polar molecules and combined with further antimicrobial multifunctional agents.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. Ethical review and approval by the Ethics committee were waived for this study because it was not a clinical trial conducted in the interest of medical research. Instead, it was a claim study for a cosmetic formulation with non-invasive measurements that do not injure the skin. Furthermore, the cosmetic product tested was safe to use, as confirmed by a challenge test. In addition, the guidelines of Good Clinical Practice (GCP) were taken into account in the study conduction. The study investigator holds a GCP certificate and is familiar with study procedures.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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