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Optimization of the Composition of a Cosmetic Formulation Containing *Tremella fuciformis* Extract (Fungi)

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Abstract: According to recent trends, people are more interested in cosmetic products based on natural raw materials, known to be safe for humans, including extracts obtained from selected plants, rich in active ingredients, such as proteins, vitamins, micro- and macro-elements, phospholipids, antioxidants, and natural preservatives. An example of such raw materials is *Tremella fuciformis* extract, which is a natural alternative to hyaluronic acid. It retains water deep in the skin cells and stimulates the skin to fight free radicals, which are responsible for the premature aging of the skin. The aim of this study was to optimize the composition of cosmetic formulations based on natural substitutes for the synthetic substances, as well as their characterization, which included the determination of the physicochemical and skin care properties. Formulations containing *Tremella fuciformis* extract had the effect of reducing TEWL by 12.4%, compared to a formulation that did not contain this active ingredient, and allowed adequate hydration of the epidermis, which was confirmed by apparatus methods. Additionally, dermatological tests were also conducted for the formulations obtained, which showed that no erythema or swelling/irritation was observed in any of the test volunteers 48 and 72 h after the application of the product.

Keywords: natural cosmetics; hyaluronic acid; *Tremella fuciformis* Extract

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1. Introduction

For thousands of years, plants and the active substances they contain have been important natural resources, used in various industries such as the food industry, construction, clothing, and in the production of pharmaceuticals and cosmetics. Recently, thanks to modern analytical methods, it has become possible to isolate biologically active compounds of plant origin from plants, which have so far been rarely used due to the impossibility of their isolation [1]. Today, many cosmetic products containing natural substitutes for the synthetic compounds are available. However, do they work in the same way and exhibit the same physicochemical and care properties? Is the principle of their operation the same? Or is it just a marketing ploy? This study focuses on the optimization of the composition of cosmetic formulations based on *Tremella fuciformis* Extract, a natural substitute for the synthetic substance hyaluronic acid, and the determination of their physicochemical and care properties.

Tremella fuciformis (order *Tremellaces*; family *Tremellacea*; common names such as snow mushroom, snow ear, silver ear mushroom, white jelly mushroom, and white auricularia) is counted among edible mushrooms, widely cultivated and valued, among other things, as a medicinal ingredient in traditional Chinese medicine. Although it was first found in Brazil, it has developed as one of the most popular cultivated mushroom species in China and other Asian countries. The fruiting bodies of this mushroom, clustered in a series of flat-lobed or wavy leaves, are gelatinous with a white or light-yellow color.

In recent decades, many researchers have reported that the polysaccharides in these fungi exhibit anticancer, immunomodulatory, antioxidant, anti-ageing, brain memory

repair, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties. The main compounds found in the extract of *Tremella fuciformis* are xylose, mannose and glucuronic acid linked by an α-1,3-glycosidic bond to side chains that consist of galactose, arabinose, and small amounts of fucose. In addition, an analysis of the fresh weight of the fruiting body of these fungi showed that it contains 6.7–10.0% protein, 65.0–71.2% carbohydrates, 0.6–12.8% fats, 2.4–2.7% crude fiber, 4.0–5.4% inorganic salt, 15.2–18.7% water, and a small amount of class B vitamin [2–15].

Furthermore, polysaccharides found in the fruiting body of these fungi have been shown to exhibit antioxidant activity by neutralizing free radicals from hydroxyl and superoxide, and they show anti-ageing activity by increasing resistance to oxidative stress [16,17]. In addition, these compounds are thought to be promoters of wound healing, as shown in studies conducted on isolated pig skin [18].

A study by Chen and colleagues has shown that the extract is perfectly safe for ingestion and topical application. When used in skin care formulations, it has a similar or even better effect than hyaluronic acid on binding to water in the skin, which influences skin hydration by forming an occlusive layer on the skin surface [19].

2. Materials and Methods

2.1. Preparation of the Emulsion Base

In order to prepare the emulsion base, which was used to compare the care and physicochemical properties of the base alone and the emulsion with *Tremella fuciformis* Extract added, a formulation, whose composition is given in Table 1, was used.

Table 1. The formulation used to prepare the emulsion base, the function of the raw materials used, and INCI.

Raw Material	INCI	[wt.%]	Function
Citric acid	Citric Acid	for pH regulation	pH adjuster
Sodium gluconate	Sodium Gluconate	0.2	Chelator
Xanthan gum	Xanthan Gum	1.0	Rheology modifier
Euxyl K712	Sodium Benzoate, Potassium Sorbate, Aqua	1.0	Preservative
Tocopherol	Tocopherol, Helianthus Annuus Seed Oil	1.0	Antioxidant
MGS	Methyl Glucose Sesquistearate	2.0	Emulsifier
Glycerin	Glycerin	7.0	Humectant
Refined grape seed oil	Vitis Vinifera (Grape) Seed Oil	25.0	Emollient
Water	Aqua	to 100.0	Solvent

Procedure for preparing the base emulsion:

1. All the raw materials needed according to the recipe were weighed (Table 1);
2. The aqueous phase was prepared: water was poured into a beaker on a magnetic stirrer and upon continuous stirring at about 500 rpm/min sodium gluconate was added;
3. Glycerin was mixed with xanthan gum (known as a blend);
4. The blend was added to the beaker with water and stirred for about 2 min until a homogeneous, transparent mixture was obtained;
5. The oil phase (emollient and emulsifier) was placed in a separate beaker;
6. The beaker with water and the beaker with the oil phase were heated to a temperature of about 75–80 °C;
7. A preservative was added to the aqueous phase;
8. The melted oil phase was added to the aqueous phase;
9. The combined phases were stirred on a magnetic stirrer for 2 min (700–800 rpm/min);

10. The emulsion was homogenized for 2 min at 9500 rpm and stirred by hand with a glass dipstick to 20–25 °C;
11. Tocopherol was added and pH was adjusted to 5.0–5.5.

After the aforementioned emulsion was obtained, its stability was checked by means of a centrifuge test. It was carried out by weighing 2 g of the emulsion into an Eppendorf-type tube and placing it on the rotor of a rotary centrifuge and setting the parameters:

- Rotor speed range: 5.5 rpm;
- Centrifugation time: 2 min.

2.2. Optimization of the Addition of Tremella fungi Extract to the Emulsion Medium

To incorporate *Tremella fungi* Extract (Evonik) into the emulsion substrate at a weight concentration of 0.1 wt.%, the following procedure was applied:

1. A weighed amount of the active substance was added to the emulsion substrate;
2. The weighed amount of the active substance was mixed with the same amount of water and then added to the emulsion substrate;
3. The weighed amount of the active substance was mixed with the same amount of glycerin and added to the emulsion substrate;
4. The weighed amount of the active substance was mixed with the same amount of ethanol and added to the emulsion substrate;
5. The weighed amount of the active substance with a 200 times greater amount of water was mixed and stirred on a magnetic stirrer for about 15 min. at 500 rpm/min, and then the mixture was added to the emulsion substrate;
6. The weighed amount of the active substance was mixed with a 300 times greater amount of water, stirred on a magnetic stirrer for about 30 min. at 500 rpm/min, and then added to the emulsion substrate;
7. The amount of the active substance weighed in the preparation stage of the emulsion substrate was added to water, stirred on a magnetic stirrer at about 500 rpm/min for about 30 min, then sodium gluconate was added, the mixture was blended, and the rest of the procedure for the preparation of the emulsion substrate was followed;
8. The weighed amount of the active ingredient was added to the emulsion substrate obtained at the preparation stage—sodium gluconate. Then, the active ingredients were added and stirred on a magnetic stirrer at about 500 rpm/min for about 30 min, and then the blend was added and the rest of procedure of the emulsion substrate preparation was followed.

Procedure for Obtaining a Formulation Containing *Tremella fuciformis* Extract

We proceeded as described in Section 2.1, taking into account the addition of the active ingredient at the emulsion medium preparation stage by adding sodium gluconate and *Tremella fuciformis* Extract to water.

2.3. Microbiological Purity Tests

To assess the microbiological quality and safety of the obtained preparations, preliminary tests were performed to detect bacteria, mold, and yeast. For this purpose, Schulke Mikrocount Duo qualitative microbiological tests for bacteria, mold, and yeast were used. In the tests, a plate covered on both sides with the sample to be tested using a clean, disinfected instrument was immersed in a container. On the yellow side of the plate, the infection with bacteria was observed, while on the pink side we observed the infection with mold and yeast. After 24–48 h of incubation at 25–30 °C, bacteria could be detected, while mold and yeast could be detected after 3–5 days.

After preliminary tests, microbiological purity tests were performed according to the ISO standards [20–25]. They allow for the detection of microorganisms that could cause undesirable effects, such as an allergy after using the preparation. Basic microbiological tests for cosmetics include those for:

- Total number of microorganisms (mesophilic bacteria, as well as yeasts and molds);
- Presence of *Pseudomonas aeruginosa*;
- Presence of *Staphylococcus aureus*;
- Presence of *Candida albicans*;
- Presence of *Escherichia coli*.

The tests were performed in accordance with the procedures described in the relevant standards, which are shown in Table 2 along with the requirements that should be met in order to consider a cosmetic preparation safe.

Table 2. Tested characteristics (presence of microorganisms), test methods, and requirements.

Tested Characteristic	Research Method	Reference	Requirements
Total number of aerobic mesophilic microorganisms	ISO 21149:2017/Amd 1:2022	[20]	$\leq 1 \times 10^3$ aerobic microorganisms in 1 mL
Total number of yeasts and molds	ISO 16212:2017/Amd 1:2022	[21]	$\leq 1 \times 10^3$ yeasts and molds in 1 mL
Presence of *Candida albicans*	ISO 18416:2015/Amd 1:2022	[22]	Absence in 1 mL
Presence of *Staphylococcus aureus*	ISO 22718:2015/Amd 1:2022	[23]	Absence in 1 mL
Presence of *Pseudomonas aeruginosa*	ISO 22717:2015/Amd 1:2022	[24]	Absence in 1 mL
Presence of *Escherichia coli*	ISO 21150:2015/Amd 1:2022	[25]	Absence in 1 mL

Checking the microbiological purity of cosmetic products involves growing bacterial and fungal strains (Table 3) on a suitable agar medium, then introducing each microorganism into a separate sample of the cosmetic preparation and incubating the inoculated samples for an appropriate time according to the test method. A prerequisite for a properly conducted procedure is to neutralize the effect of the preservative, which at the same time will not have an inhibitory effect on bacteria and fungi. Therefore, it is necessary to demonstrate the effectiveness of the neutralizer used before each test. During microbiological testing, Letheen Broth modified neutralizer was used at a dilution of 1/10, and its effectiveness was confirmed during the validation of the method.

Table 3. Microbial strains tested.

The Strain Tested
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922
Candida albicans ATCC 10231
Aspergillus brasiliensis ATCC 16404

2.4. Dermatological Tests

To evaluate the irritant/allergenic properties of the two selected emulsions, a semi-open ordinary contact test was conducted. The purpose of the test was to evaluate the irritant/allergenic properties of the product in a healthy adult volunteer using the single-application patch test method. Table 4 shows the characteristics of the testing panel along with the site of skin application.

Table 4. Characterization of the testing panels and site of application of the product.

Volunteers	Mean Age ± SD	Place of Application
Woman	51 ± 16	Back
Woman	46 ± 17	Shoulder
Man	32 ± 0	Shoulder

Patch testing according to Jadassohn-Bloch (modified by Rudzki) was conducted under the supervision of a dermatologist. The allergenic and irritant properties of the formulation were evaluated on a group of 20 healthy volunteers with a negative history of allergic responses, aged 18–69 years, where 95% of all volunteers were women and 5% were men (Table 4). The test was conducted using Finn Chamber patches with filter papers, onto which the test product was applied, and then the whole thing was glued to the volunteer's skin (arm or inter-blade area). The samples were removed after 48 h. The first reading was taken 15 min after the patch was removed, and then after 72 h. The reading was performed according to the generally accepted scale in dermatological testing and then evaluated by a dermatologist. A positive reaction (erythema) confirms the allergenic properties of the product, while a negative result (no erythema) confirms the absence of allergenic properties.

The evaluation of the results was carried out in accordance with the recommendations of the International Contact Dermatitis Research Group (ICDRG) and included the score scale shown in Table 5.

Table 5. Dermatological outcome rating scale.

Erythema	**Point Classification**
No erythematous reaction	0
Slight erythematous reaction	0.5
Erythematous reaction and/or papules	1
Erythematous reaction and/or papules and/or vesicles	2
Erythematous reaction and/or papules and/or vesicles	3
Erythematous reaction, visible ulceration/ulceration and/or papules and/or vesicles and/or blisters	4
Swelling/Infiltration	**Point Classification**
No swelling	0
Very slight swelling (almost invisible)	1
Light swelling	2
Moderate swelling (about 1 mm above the skin surface)	3
Severe swelling (extended even beyond the application area)	4

Interpretation of results: The average irritation index (x_a) is calculated based on the skin reaction readings of 25 volunteers. The product is then classified according to the following scale (Table 6).

Table 6. Interpretation of the results of the dermatological examination.

Average Irritation Rate (x_a)	**Product Classification**
$x_a < 0.5$	Non-irritating
$0.5 \leq x_a < 2.0$	Slightly irritating
$2.0 \leq x_a < 5.0$	Moderately irritating
$5.0 \leq x_a$	Strongly irritating

Where x_a—average irritation index.

2.5. Stability Tests with Turbiscan Apparatus

To check the stability of the obtained emulsions, analysis was performed with the Formulation Turbiscan Lab Expert apparatus, which allows the detection of instabilities such as sedimentation, creaminess, flocculation, and coalescence.

The device uses static multiple-light scattering to detect particle migration and size changes. The head moves up and down the entire sample while working with two de-

tectors: transmission (T) and backscattering (BS). The signal coming from the detectors is related to the size and concentration of the particles, and their changes are related to the destabilization of the sample.

The procedure for checking sample stability consisted of:

- Preparing the samples—placing them in a special vial, in such a way that there are no air bubbles in it;
- Performing a zero measurement (T0) on the Turbiscan Lab Expert apparatus;
- Incubation of the sample in an oven at 40 °C for 4 weeks;
- Repeating the measurement after 7 days (T1), 14 days (T2), 21 days (T3) and 28 days (T4) of incubation.

2.6. Apparatus Studies

In order to confirm the functional properties of the two selected emulsions, we conducted instrumental tests of the level of skin hydration (Corneometer) and the level of transepidermal water loss from the epidermis (Tewameter).

The studies were carried out using a special measuring apparatus (Courage + Khazaka electronic GmbB) and were performed on the skin on the inner side of the forearm. After the initial measurement, 0.003 mg of the product was applied to each field.

Corneometer®−CM 825 (Courage + Khazaka electronic GmbH, Köln, Germany)—testing the level of skin hydration using the capacitance method and a single mode. The depth of penetration ranged from 10 μm to 20 μm in the stratum corneum. Measurements were taken before, 1 h, 3 h, and 5 h after application. Each numerical result obtained is the arithmetic average of a minimum of five unit measurements.

Tewameter®−TM HEX (Courage + Khazaka electronic GmbH, Köln, Germany)—Testing the intrinsic transepidermal water loss level of the epidermis, the measurement was performed before, 1 h, 3 h, and 5 h after application.

These measurements were made on a group of five volunteers, aged 29–38 years, where 100% were women. The mean age of the volunteers was 33; SD = 4.6.

2.7. Physicochemical Properties of Cosmetic Preparations

2.7.1. Organoleptic Evaluation

Organoleptic evaluation was performed to determine the perception of the resulting emulsions. The test was carried out by applying a portion of the preparation (about 2 g) to the outer part of the hand, and gently spreading it. Subjective evaluation of the finished cosmetic preparations concerned the following:

- Color;
- Smell;
- Consistency;
- Whiteness;
- Ease of spreading;
- Absorption;
- Leaving a sticky layer;
- Additional observations.

2.7.2. Viscosity

To test the viscosity of the obtained emulsions, a Brookfield-type Rheotec RC02 rotational viscometer was used, which is a viscosity meter with adjustable shear torque that measures viscosity resistance on a rotating disk immersed in the test medium. The test was carried out at 20.2–20.4 °C, using an R5 spindle at different speeds depending on the emulsion under test. The test of each sample was repeated three times.

2.7.3. Density

To determine the mass of the substance per unit volume of the emulsion under test density, a 100 mL pycnometer was used. The test consisted of freezing an empty

pycnometer, then filling it with the test preparation and weighing it again. The difference in the weight of the pycnometer filled with the test sample and the empty pycnometer divided by the volume of the pycnometer (100 mL) allowed for the determination of the density of the test preparation in [g/mL].

2.8. Comparison of the Absorption Properties of Tremella fungi Extract and Hyaluronic Acid

To compare the absorption properties of hyaluronic acid (Chemat) and *Tremella fungi* Extract (Evonik), which is its natural mimetic, 0.5 g of each substance was weighed in two separate crucibles, which were then placed in a glass desiccator along with a beaker of water. After 1, 2, 3, 4, 5, and 6 h, both crucibles with active substances were weighed, and the weight of the absorbed water was compared.

3. Results

3.1. Emulsion Base

The method used to prepare the emulsion substrate resulted in a stable emulsion.

3.2. Optimization of the Addition of Tremella fungi Extract to Emulsion Medium

The optimization of the parameters of the method for obtaining a cosmetic formulation with the addition of *Tremella fungi* Extract allowed us to draw the following conclusions:

- Direct addition of the active ingredient to the emulsion substrate results in a very thick and stringy emulsion, and large lumps are visible, which are not felt during spreading (Figure 1a);
- Addition of an active ingredient mixed with water to the emulsion substrate in the amount equal to the amount of the sub-active ingredient results in an emulsion with smaller lumps that are not perceptible during spreading (Figure 1b);
- Addition of the active ingredient mixed with glycerin in an amount corresponding to that of the active ingredient and addition of the mixture to the emulsion substrate results in a very thick, dragging consistency with visible medium-sized lumps that are not perceptible during spreading (Figure 1c);
- Addition of the active ingredient mixed with ethanol in an amount equivalent to that of the active ingredient and addition of the mixture to the emulsion base results in a thick emulsion with visible small lumps that are not perceptible during spreading (Figure 1d);
- Mixing of the active ingredient with a 200 times greater amount of water and stirring on a magnetic stirrer for about 15 min at 500 rpm, and addition of the mixture to the emulsion substrate, results in a dense emulsion with very small lumps, undetectable during spreading (Figure 1e);
- Mixing of the active substance with a 300 times greater amount of water, stirring on a magnetic stirrer for about 30 min. at 500 rpm, and addition of the mixture to the emulsion substrate leads to a dense emulsion with single lumps, undetectable during distribution (Figure 1f);
- Addition of the active ingredient at the emulsion substrate preparation stage: adding it to water, stirring the mixture on a magnetic stirrer at about 500 rpm for about 30 min, adding sodium gluconate, stirring, and continuing according to the emulsion substrate preparation procedure, results in a thick emulsion with individual very small lumps visible, not detectable during spreading (Figure 1g);
- Addition of the active ingredient at the emulsion substrate preparation stage: adding sodium gluconate to water followed by the active ingredient and stirring on a magnetic stirrer at about 500 rpm for about 30 min, adding a blender and continuing to follow the emulsion substrate preparation procedure, yielded a thick, smooth emulsion with no visible lumps (Figure 1h).

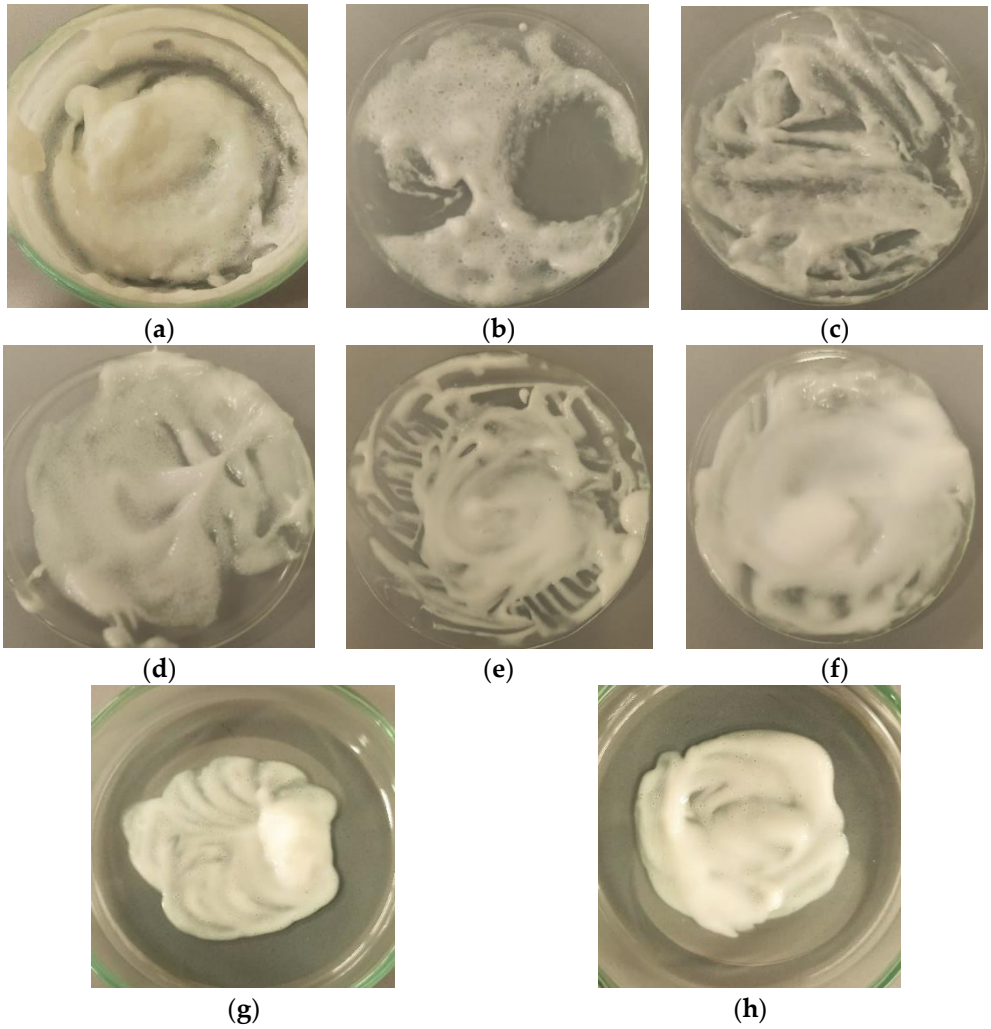
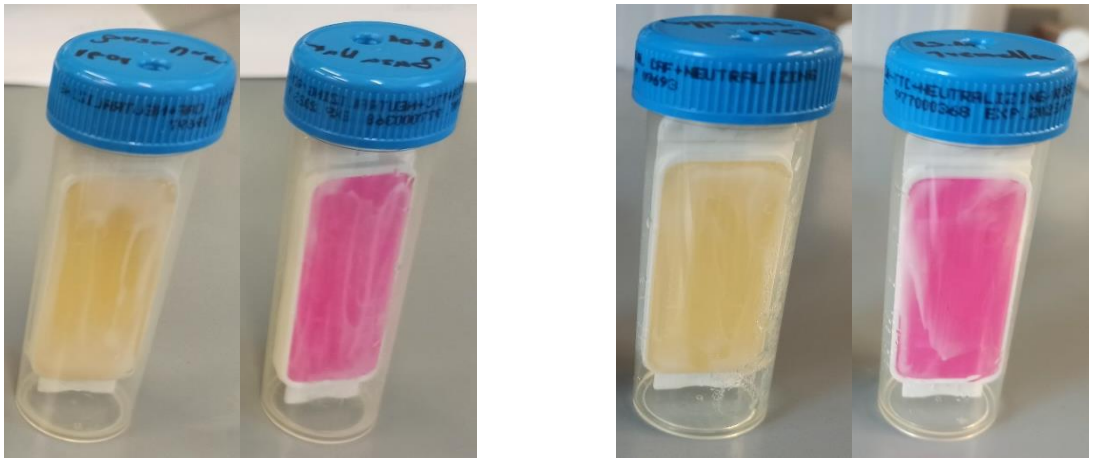


Figure 1. Emulsions obtained after adding *Tremella fungi* Extract: (**a**) directly in an emulsion; (**b**) after mixing with water in the amount corresponding to that of the active substance; (**c**) after mixing with glycerin in the amount corresponding to that of the active substance; (**d**) after mixing with ethanol in the amount corresponding to that of the active substance; (**e**) after mixing with 200 times greater amount of water; (**f**) after mixing with 300 times greater amount of water; (**g**) after adding active substance during emulsion preparation—after sodium gluconate; (**h**) after adding active substance during emulsion preparation—before sodium gluconate.

3.3. Microbiological Tests

Preliminary qualitative microbiological tests performed using Schulke Mikrocount Duo for bacteria, mold and yeast showed no sign of microorganisms in the emulsion medium or the emulsion with *Tremella fuciformis* Extract (Figure 2).



(**a**) (**b**)

Figure 2. Schulke Mikrocount Duo microbiological tests were performed for: (**a**) emulsion medium; (**b**) emulsion with *Tremella fuciformis* Extract.

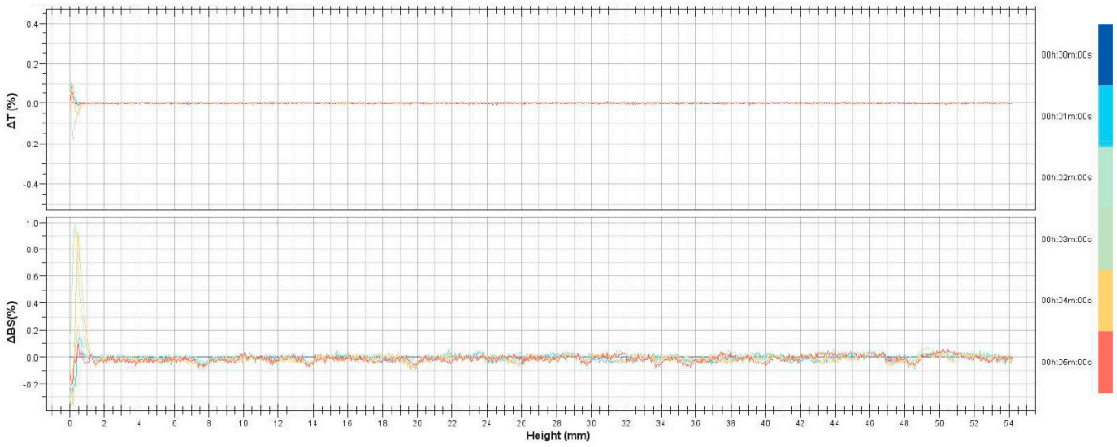
The results of the microbiological tests conducted according to the relevant ISO standards showed compliance with the accepted standards for samples of both preparations (Table 2).

3.4. Dermatological Tests

Dermatological tests showed that none of the test probands experienced erythema or swelling/irritation at 48 and 72 h after application (Table S1 in Supplementary Materials) for samples of both formulations.

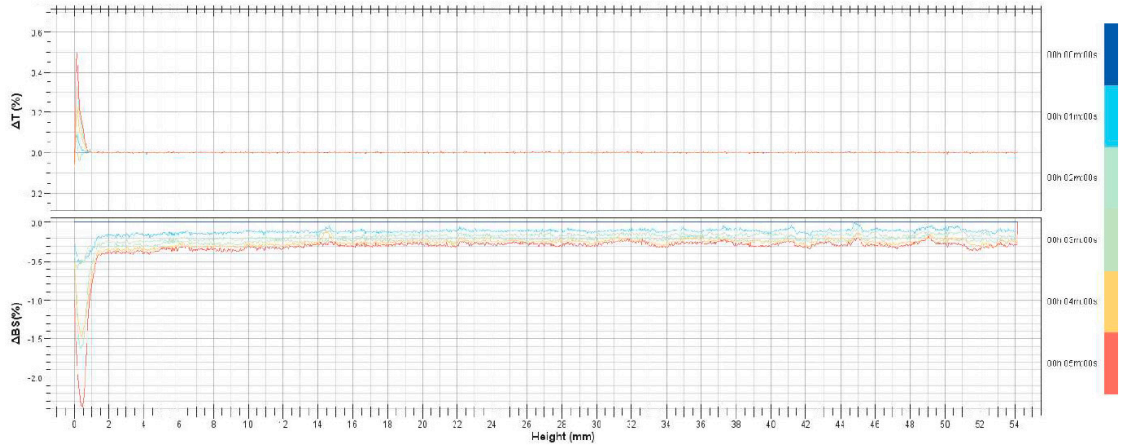
3.5. Stability Tests—Turbiscan

Results of the stability tests performed with the Turbiscan Lab Expert instrument are presented in the form of delta transmittance and delta reflectance plots across the base sample (Figure 3) and the emulsion sample with *Tremella fungi* Extract (Figure 4) at T0 and after 4 weeks of incubation (T4).



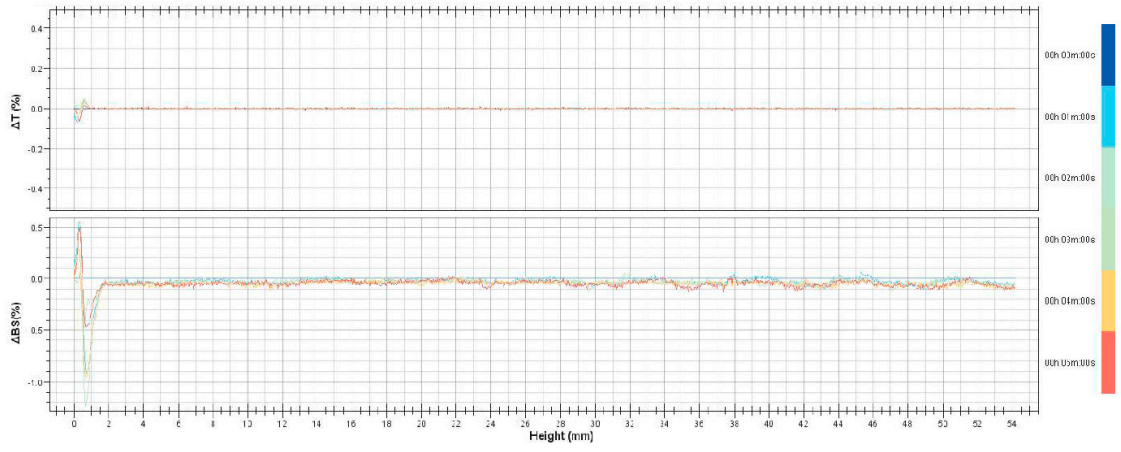
(**a**)

Figure 3. *Cont.*

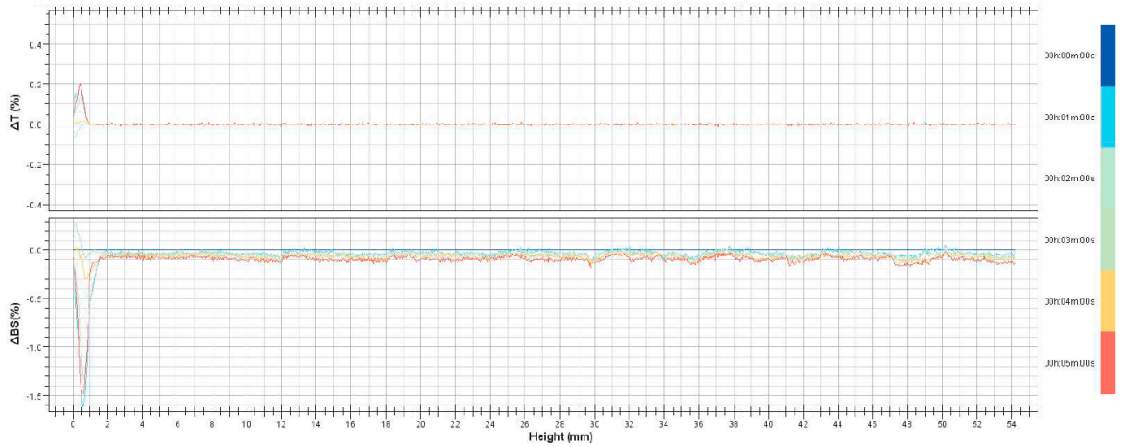


(**b**)

Figure 3. Transmission and backscattering profiles of the emulsion substrate: (**a**) immediately after preparation (T0); (**b**) after 4 weeks of incubation at 40 °C (T4).



(**a**)



(**b**)

Figure 4. Transmission and backscattering profiles of emulsions with *Tremella fuciformis* Extract: (**a**) immediately after preparation (T0); (**b**) after 4 weeks of incubation at 40 °C (T4).

Additionally, the stability of the emulsions after 4 weeks of incubation at 40 °C was checked using the Turbiscan Stability Index (TSI), which provides information on the overall destabilization of the samples (Figure 5) [26–28].

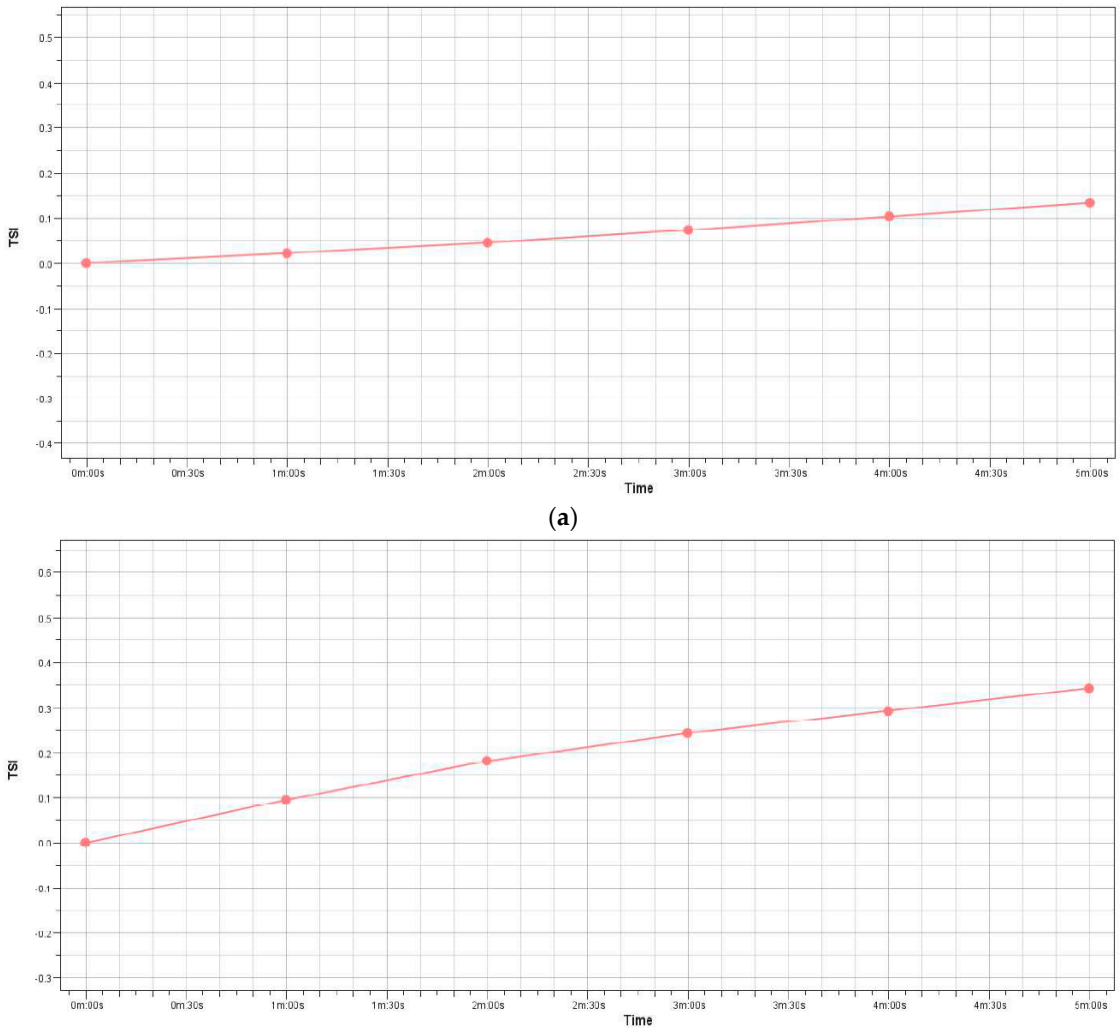


Figure 5. TSI stability index of the obtained emulsions as a function of time: (**a**) emulsion substrate; (**b**) emulsion with *Tremella fuciformis* Extract.

3.6. Apparatus Studies

3.6.1. Skin Hydration Level Test—Corneometer® −CM 825

The skin hydration test conducted for both test samples showed an increase in epidermal hydration 5 h after the application of the cosmetic formulations compared to the initial value in all volunteers participating in the study (Figure 6).

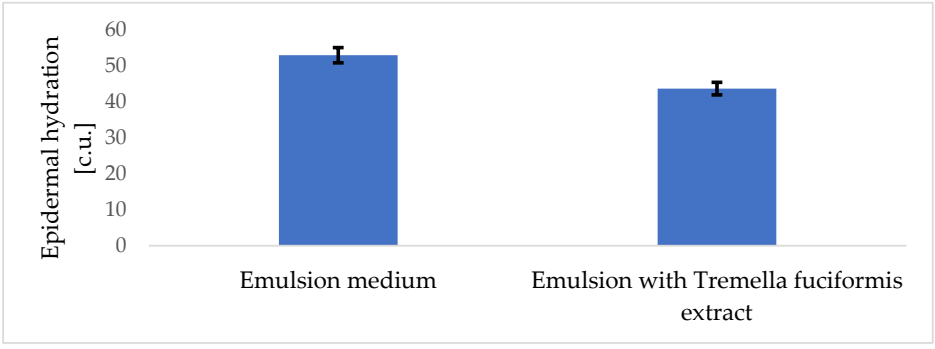


Figure 6. Results of apparatus—Corneometer® −CM 825—average epidermal hydration level—comparison between emulsion medium and emulsion with *Tremella fuciformis* Extract.

3.6.2. TEWL Level Testing—Tewameter®—TM HEX

An examination of the level of transepidermal water loss from the epidermis for both test samples showed a decrease in the level of transepidermal water loss from the epidermis 5 h after the application of the cosmetic formulations compared to the initial value (Figure 7).

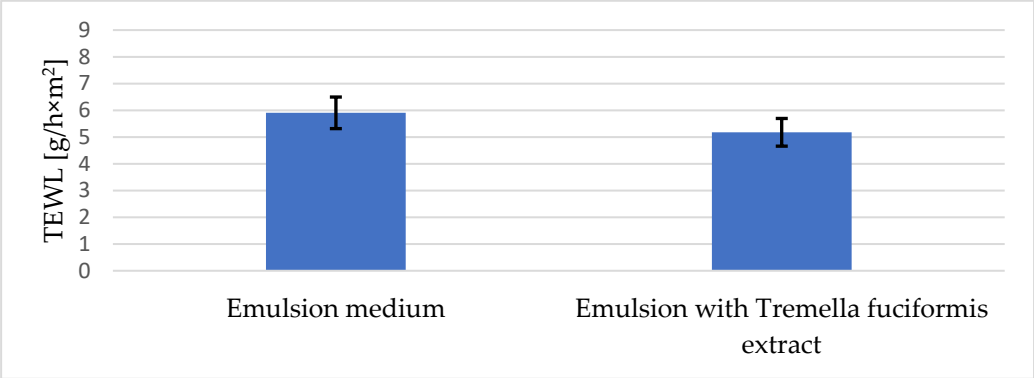


Figure 7. Results of the apparatus Tewameter®—TM HEX: average TEWL level—comparison between emulsion medium and emulsion with *Tremella fuciformis* Extract.

3.7. Physicochemical Properties

3.7.1. Organoleptic Evaluation

The organoleptic characteristics of the prepared preparations obtained are shown in Table 7. Both preparations are white in color and odorless, do not leave a sticky film on the skin, and are quickly absorbed. The emulsion substrate turns slightly white when spreading and has a thicker consistency.

Table 7. Organoleptic test results.

Trait under Study	Emulsion Substrate	Emulsion with *Tremella fuciformis* Extract
Color	White	White
Odor	None	None
Consistency	Medium dense	Dense
Whitening	Slightly	None
Spreading	Very smooth	Very smooth
Leaving a better layer	None	None
Absorption	Fast	Fast
Other observations	Light texture, but rich under the finger	Light consistency

3.7.2. Viscosity and Density

Viscosity testing of both preparations was carried out with an R5 spindle and RPM = 200. The averaged viscosity results and tested densities are shown in Table 8.

Table 8. Viscosity and density of the tested preparations.

Type of Emulsion	Density [g/mL]	Viscosity [mPas]
Emulsion substrate	0.983	1537
Emulsion with *Tremella fungi* Extract	0.990	1607

3.8. Comparison of the Absorption Properties of Tremella fuciformis Extract and Hyaluronic Acid

A water absorption study showed that both hyaluronic acid and *Tremella fuciformis* Extract were highly hygroscopic. Water absorption by hyaluronic acid increases for up to 3 h then decreases. *Tremella fuciformis* Extract, on the other hand, shows an increasing trend throughout the entire test period, that is, for 5 h. Detailed results are shown in Figure 8.

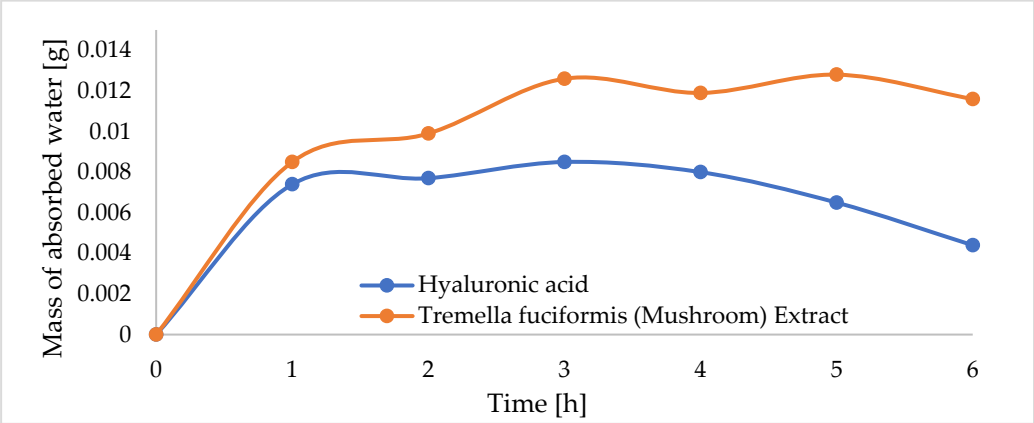


Figure 8. Time dependence of the mass of water absorbed by the tested active substances.

4. Discussion

The best method of preparing emulsions with *Tremella fuciformis* Extract is to add the active ingredient at the emulsion preparation stage. It has been shown that the addition of sodium gluconate before the addition of the active substance causes an incomplete dissolution of the extract, resulting in the appearance of individual lumps in the finished preparation. Changing the order in which these substances are added to water allows the active ingredient to dissolve completely and produce a smooth emulsion.

Both preliminary qualitative microbiological tests and quantitative analysis showed that both preparations meet the conditions for cosmetics and are safe for use by consumers.

The results of the dermatological tests, in consistence with the assumptions made, indicate that the irritation index (x_a), calculated from the sum of the scores for erythema and swelling/irritation is equal to 0, for both formulations throughout the study period. It can, therefore, be concluded that both formulations do not irritate or sensitize the skin and meet the requirements of the skin compatibility test.

Stability tests performed using the Turbiscan Lab Expert instrument allowed for a comparison of transmission and backscattering profiles. The transmission profiles for both substances after preparation (T0) and after 4 weeks of incubation at 40 °C (T4) are zero. An analysis of the backscattering profiles showed that no migration phenomena, such as sedimentation or creaminess, occurred in any sample. Uneven signals appearing along the entire length of the emulsion substrate samples may be related to the presence of air bubbles in the test sample (Figures 3a and 4a). The anomalies present in the emulsion sample with the active ingredient after 4 weeks of incubation indicate a slight change in particle size, i.e., flocculation.

An analysis of the TSI index, which was measured throughout the sample volume, reveals the overall stability of the tested formulations. The smaller the value, the more stable the sample. For the emulsion substrate, the TSI index increases mildly and does not exceed the value of 0.15 (Figure 5a). On the contrary, for the emulsion sample with the active ingredient, the TSI index increases more dramatically and does not exceed the value of 0.35 (Figure 5b), indicating the lower stability of this emulsion compared to the emulsion substrate. These results correlate with the changes seen in the backscatter profile of both samples. Nevertheless, both emulsions can be considered very stable, as the TSI value does not exceed 0.5 [29].

The tests of the skin hydration level of the emulsion base showed (Figure 6):

- One hour after application, an increase in skin hydration from 51% to 72%, by 58% on average, compared to the initial value;
- After 3 h from the application, a further increase in skin hydration from 63% to 70%, by an average of 65% compared to the initial value;
- Five hours after application, a further increase in skin hydration from 70% to 80%, on average 75% compared to the initial value.

For the emulsion containing the active substance, the measurements with the apparatuses showed:

- One hour after application, an increase in skin hydration from 48% to 83%, by 72% on average compared to the initial value;
- Three hours after application, a further increase in skin hydration from 78% to 87%, by an average of 82% compared to the initial value;
- Five hours after application, a sustained increase in hydration from 57% to 75%, on average 65% compared to the initial value.

The average epidermal hydration level for the emulsion is 52.81 g/h·m^2 ± 2.09, while for the emulsion containing *Tremella fuciformis* Extract it was 43.56 g/h·m^2 ± 1.69.

These values are shown in Table 9.

Table 9. Percentage increase in epidermal hydration compared to initial value and percentage reduction in transepidermal water loss from the epidermis compared to baseline.

Volunteers	Emulsion Base—Change [%]			Emulsion with *Tremella fuciformis* Extract—Change [%]		
	T1	**T2**	**T3**	**T1**	**T2**	**T3**
			Epidermal hydration			
X_a	**58**	65	75	72	82	65
			TEWL			
X_a	**−32**	−27	−27	−26	−26	−28

Where X_a—average value.

Studies of transepidermal water loss from the epidermis after application of the emulsion substrate showed (Figure 7):

- One hour after application, a reduction in TEWL from 27% to 38%, by an average of 32% compared to the initial value;
- At 3 h after application, a sustained reduction in TEWL from 21% to 37%, by an average of 27% compared to the initial value;
- Five hours after application, a sustained reduction in TEWL from 19% to 37%, by an average of 27% compared to the initial value.

For the emulsion containing the active substance, the measurements with the apparatuses showed:

- One hour after application, a reduction in TEWL from 20% to 34%, on average, compared to the initial value;
- At 3 h after application, a reduction in TEWL from 16% to 34%, by an average of 26% compared to the initial value;
- Five hours after application, a sustained reduction in TEWL of 14% to 35%, an average of 28% compared to the initial value.

The average TEWL value for the emulsion is 5.91 g/h·m^2 ± 0.56 while for the emulsion containing *Tremella fuciformis* extract 5.18 g/h·m^2 ± 0.49.

These values are shown in Table 9.

Furthermore, after analyzing changes in epidermal hydration levels and coupled changes in TEWL levels (Figures 6 and 7), we observed the ability of prepared formulations containing the *Tremella fungi* Extract to reduce the value of the TEWL parameter by 12.4% compared to emulsions not containing the extract. The increase in hydration content can be

explained by the action of the extract used, which retains or binds water or draws it from deeper layers, as well as due to the occlusive properties of the lipids contained in the cream, which have a protective effect to prevent excessive water loss (Figure 7). Moreover, both formulations increase the hydrolipid barrier of the skin and protect the skin from external agents. Furthermore, both the emulsion substrate and the formulation containing *Tremella fungi* Extract cause intensive and long-lasting moisturization effects on the skin. Similar properties in preparations containing *Tremella fungi* Extract were observed, among others, by Ho and colleagues [15] and Lan [30].

The higher viscosity and density of the emulsion with the active ingredient than those of the emulsion substrate, confirms the absorption capacity of the *Tremella fungi* Extract, which absorbs water from the environment by increasing the weight of the formulation. In addition, the polysaccharides contained in it form a network in water, modifying (increasing) the viscosity of the final product [31].

Tremella fungi Extract, compared to the emulsion medium, absorbed water to a greater extent within 5 h, and the level of absorption did not decrease over time as it did with hyaluronic acid (Figure 8). This confirms an earlier study from 2012, which showed that cosmetics with 0.05 wt.% polysaccharides from *Tremella fuciformis* held moisture better than those with 0.02 wt.% hyaluronic acid [32].

5. Conclusions

In conclusion, during the study, we observed better perceptual properties and skin care effects (epidermal hydration and TEWL) of the emulsion with the addition of *Tremella fuciformis* Extract compared to the medium without the active substance. Microbiological and dermatological tests were carried out according to European Union regulations. These studies did not show any adverse effects of the formulations on human skin. Preliminary apparatus tests confirm the possibility of the commercial use of emulsions with this active substance, as the TEWL level for formulations containing *Tremella fuciformis* Extract decreased by 12.4% compared to formulations without this active substance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics10030082/s1, Table S1: Dermatological test results in 48 and 72 h after application.

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