

aurafirm P

DATA PACK



PREBIOTIC ACTIVE THAT PROTECTS AND MAINTAINS A HEALTHY MICROBIOME

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aurafirm P

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INGREDIENT PROFILE

aurafirm P is a prebiotic active that protects and maintains a healthy microbiome. The key characteristics of this active ingredient include:

- Prebiotic effect due to the presence of oat beta-glucans and starch
- Essential amino acids which are important for the metabolic activity of the epidermis
- Organic acids such as lactic acid, which have the ability to hydrate the skin and improve the stratum corneum barrier function



*Product image for illustration purposes only, actual product may vary

INTRODUCTION

Oat Cosmetics' **aurafirm** ingredients are a family of active ingredients created by the fermentation of Oat COM, our advanced colloidal oatmeal, using proprietary cultures of *Lactobacillus*.

FERMENTATION

aurafirm P is made by the fermentation of 25% of Oat COM (our advanced colloidal oatmeal). Oat COM is fed to the *Lactobacillus* strain which undergoes a patented fermentation process where Oat COM is completely converted to biomass and organic acids. Fermentation breaks down the cell wall structures of the oat, leading to the release or synthesis of bioavailable molecules as various antioxidant compounds and amino acids. These antioxidant compounds can act as free radical terminators, metal chelators, singlet oxygen quenchers or hydrogen donors to radicals. The fermentation results in:

- **aurafirm P** (5-10 % of Oat COM), a filtered paste
- **aurafirm N** (1-5 % of Oat COM), a filtrate
- **aurafirm S** (1-2 % of Oat COM), a clear serum

PROFILING

aurafirm P is a creamy liquid with a high concentration of fermented oat bran. **aurafirm P** shows a selective recovery of a disrupted microbiome, boosting the diversity of the microflora in a selective way, favouring useful species such as *S. epidermidis*, giving rise to healthier skin. It also benefits the skin by improving smoothness, radiance, and complexion.

PREBIOTICS

Molecules that promote the growth of beneficial microorganisms on the skin and provide a healthy and balanced diet or skin microbiota. Prebiotic molecules in **aurafirm P** are:

Oat Beta-Glucan

aurafirm P contains beta-glucan, that are long-chain carbohydrates naturally present in oats, which can form a thin film on the skin and keep it moisturised. Research shows that oat beta-glucans can nourish the skin's beneficial bacterial strains, and therefore stimulate the growth of bacteria. As prebiotics are defined as high fibre food for live bacteria, oat beta-glucans also behave like prebiotics on the skin.³

aurafirm P	
Oat Beta-Glucan*	1%

Starch

Oat COM contains starches, naturally present in oats. Starch is highly hydrophilic and help to hold in water, which enhances the moisturising abilities of **aurafirm P**. Improvement in starch digestibility during fermentation is due to the breakdown of starch oligosaccharides. The enzymes bring about cleavage of amylase and amylopectin to maltose and glucose.¹

aurafirm P	
Starch*	10%

Antioxidants

Most phenolic compounds in oats are insoluble-bound phenolics that are covalently bonded to the structural components of the cell wall. The crude enzymes from fermented have had the ability to hydrolyse the bound between phenolics and cell wall macromolecules, leading to the increase of the soluble phenolic content³. The fermentation of Oat COM increases the antioxidant capacity of **aurafirm P**.

*Indicative Typical values

PREBIOTICS (CONT.)

Ectoin:
Ectoin is a natural substance which is produced by bacteria to protect against extreme conditions. It promotes hydration of the epidermis by maintaining the correct water balance and prevents its dehydration. As a result, the skin looks and feels smooth and soft.

PROBIOTICS

Live microorganisms which when administered in adequate amounts confer a health benefit by strengthening the skin microbiome. Probiotic components in **aurafirm P** are:

Lactobacillus Bacteria

Lactobacillus bacteria can inhibit pathogen attachment to epidermal cells of the skin, by blocking the site of attachment and attracting bacteria of the same or similar species.⁴ Data shows that heat-killed bacteria, their fractions, or purified components have probiotic effects with advantages over live probiotics.

aurafirm P	
Lactobacillus bacteria*	200,000 bacterial count/ml

Diacetyl

Strains of *Lactobacillus* bacteria can produce diacetyl. It has the potential to exhibit dermal antimicrobial activities, with greater sensitivity against gram-negative bacteria and fungi as compared to gram-positive bacteria.⁴

POSTBIOTICS

Range of metabolites produced by live bacteria during the fermentation process that help to regulate the composition of the skin microbiome ecosystem. Postbiotic molecules in **aurafirm P** are:

AHAs:

Strains of *Lactobacilli* can produce α-hydroxy acids (AHAs) to exhibit pH-adjustments and antibacterial activity against most dermal pathogenic bacteria. AHAs can exfoliate the uppermost layer of the skin. AHAs have the ability to hydrate the skin, improve the stratum corneum barrier function and enhance the production of ceramides by keratinocytes. **aurafirm P** contains hydroxy acids, particularly lactic acid.

aurafirm P	
Organic acids*	4,000 mg/kg

Amino Acids

Amino acids, which are important to the metabolic activity of the living epidermis, are essential for maintaining the integrity of the skin barrier, protein synthesis and nutrient absorption. During fermentation, proteins are digested by microbial proteases and peptidases resulting in amino acids.¹ **aurafirm P** supplies the skin with essential amino acids.

aurafirm P	
Amino acids* of which	17,500 mg/kg
Glutamic acid*	4,000 mg/kg
Aspartic acid*	1,500 mg/kg
Leucine*	1,500 mg/kg

Bioactive Peptides

The fermentation process produces peptides that help the skin’s cellular renewal. Peptides have high bioavailability and are involved in the modulation of cell proliferation, cell migration, inflammation and protein synthesis and regulation.

CONCLUSION

Fermentation is the enzymatic decomposition and utilisation of nutrients, particularly carbohydrates, by microbes. The process of fermentation enhances the accessibility of actives in Oat COM and this is responsible for the development and improvement of **aurafirm P**, a fermented product. The levels of bioactive compounds can be modified during fermentation by the metabolic activity of lactic acid bacteria and enzymes derived from oat (amylases, xylanases, and proteases). The fermentation process induces structural breakdown of oat cell walls leading to the liberation and synthesis of various bioactive compounds. As the skin is incapable of breaking down large molecules, fermentation allows greater bioavailability of potentially protective and reparative molecules. This bioavailability is because proteins are broken down into peptides and amino acids, many of which are small enough to penetrate past the stratum corneum.^{1,2} Oat COM, a colloidal oatmeal, contains proteins, polysaccharides, and vitamin B. It has been widely accepted as a prebiotic for gut health and data suggests benefits for the skin too as demonstrated in our studies. In addition, Oat COM exhibits prebiotic benefits to enhance growth of healthy bacteria on the skin, its composition makes it an optimal starting material for fermentation.

BACKGROUND

Fourier-transform infrared spectroscopy (FT-IR) was used to profile and create a molecular fingerprint for **aurafirm P**.

METHOD

FT-IR analyses molecules that absorb light in the infrared region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in a compound. The absorption spectrum obtained from the FT-IR analysis indicates the presence of various chemical bonds and functional groups in the sample. FT-IR was used to characterise **aurafirm P** due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infrared range.

RESULTS

The spectrum obtained from **aurafirm P** has very strong peaks at 3300 and 1050 cm^{-1} corresponding to O-H and C-O bonds. There is a minor peak at around 1720 cm^{-1} which represents the double bond C=O, meaning there is a relatively low concentration of carboxylic acid in **aurafirm P**. This spectrum shows that **aurafirm P** contains a great number of polysaccharides or cellulose.

Figure 1:

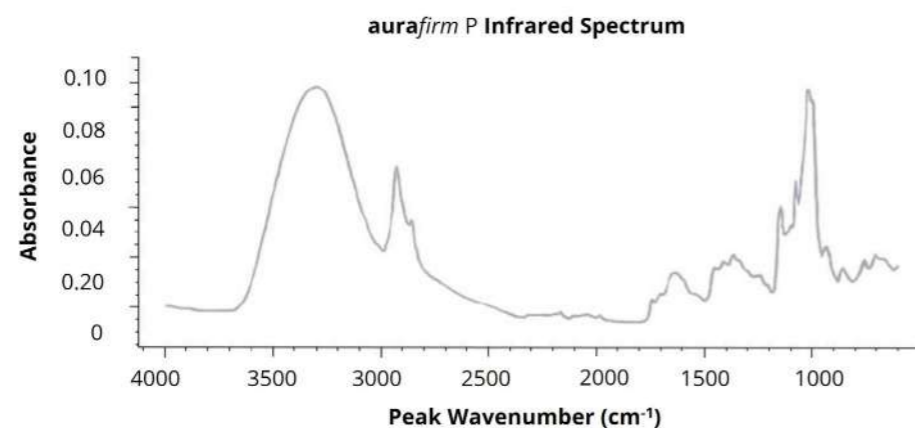


Figure 2:

Wavenumber Assignment of **aurafirm P** Infrared Spectrum

Peak Wavenumber (cm^{-1})	Associated Chemical Bond
3300-3400	O-H
2900-2950	C-C bonds close to a double bond C=O or particularly C-H bond stretching
1610-1720	C=O or C=C
1300-1400	C-H
1050	C-O
860	C=C-H
680-780	Aromatic C-H

CONCLUSION

Similar FT-IR spectrums were obtained from **aurafirm N** and **S** proving that they have a similar content of oat-based material. **aurafirm P** is unique and contains a larger amount of pre-digested oat material.

BACKGROUND

A study was performed to confirm the viability and count the number of *Lactobacillus* bacteria in **aurafirm P** using flow cytometry.

METHOD

Using a flow cytometer, **aurafirm P** was passed through a laser light beam to measure the interaction of its components with the light. Fluorescent markers are bound to the *Lactobacillus* cells and the fluorescence intensity represents the count of bacteria. As with the confocal microscopy, SYTO 9 and propidium iodide were used; the bacteria are dead and will therefore show up red. The concentration of bacteria was calculated using counting beads as a reference. Three replicates were analysed and an average was taken.

RESULTS

Figure 1:
Lactobacillus Bacteria Count in **aurafirm** Grades

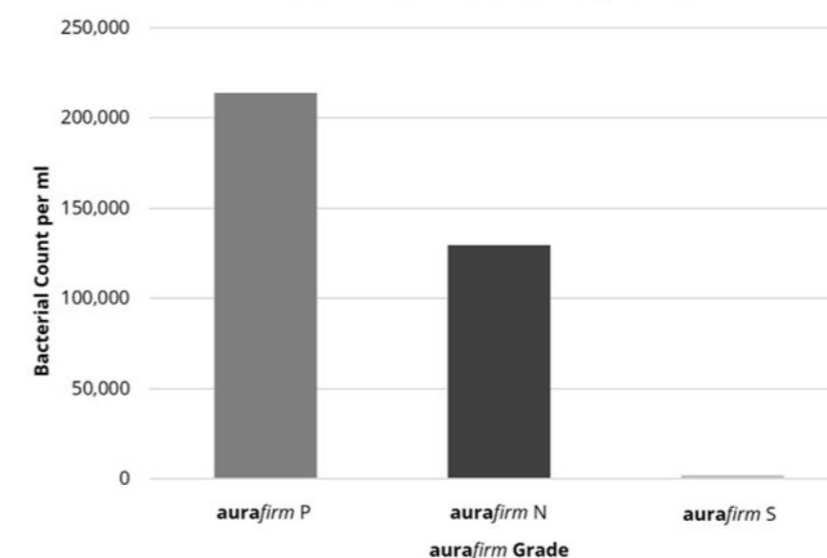
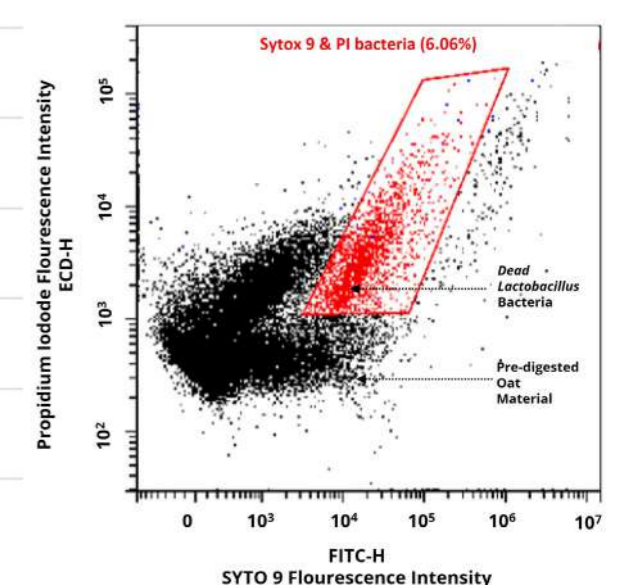


Figure 2:
Visualisation of *Lactobacillus* Bacteria in **aurafirm P**



CONCLUSION

The results show that *Lactobacillus* bacteria, deactivated by the pasteurisation step at the end of the fermentation process, are present in all **aurafirm** grades. However, a clear distinction is seen between **aurafirm P** and the other **aurafirm** grades. The bacterial content in **aurafirm P** is nearly double that in **aurafirm N**. However, the flow cytometry results indicate that 6% of all solid matter is *Lactobacillus* bacteria, which means that **aurafirm P** contains a lot of other solid matter and the bacteria are not free flowing in solution. These results confirm that not only will **aurafirm P** have a probiotic-like effect on the skin, but it also confirms that it contains a high concentration of pre-digested oat material which will act strongly as a prebiotic when applied to the skin.

BACKGROUND

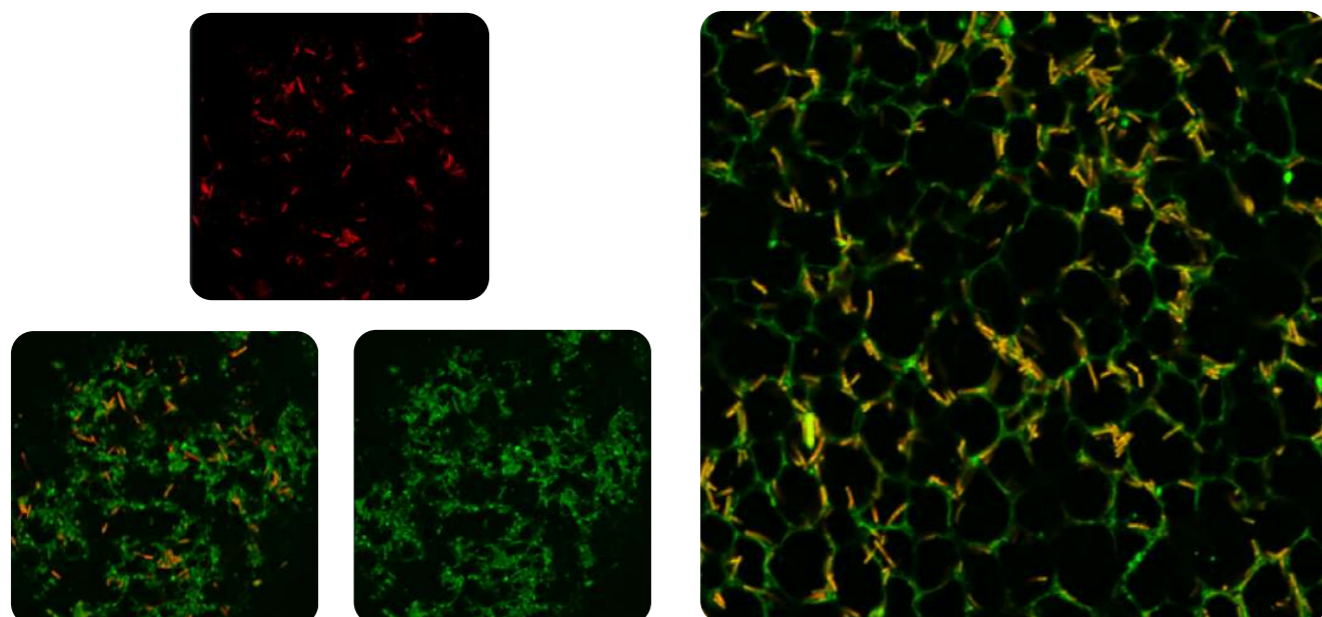
A study was performed to evaluate the concentration and viability of prebiotic *Lactobacillus* bacteria in **aurafirm** P under confocal microscopy.

METHOD

To monitor the viability of *Lactobacillus* bacteria in **aurafirm** P, two fluorescent markers, SYTO 9 green (live bacteria) and propidium iodide red (dead bacteria), were added in solution to **aurafirm** P respectively and incubated at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 20 minutes. The image analysis was performed on a 780LSM confocal microscope.

RESULTS

Figure 1:
Visualisation of the Viability of *Lactobacillus* Bacteria in **aurafirm** P (20 μm)



Bacteria are confirmed dead but remain intact, meaning that they have not been lysed and will act similarly to live bacteria when applied to the skin

The *Lactobacillus* bacteria are sat on an oat-based matrix, meaning they are not as easily accessible to the skin bacteria and will be slowly released onto skin during application

CONCLUSION

Lactobacillus bacteria is present in **aurafirm** P. The dye confirmed that these bacteria are no longer alive and have been killed during the pasteurisation stage, however the microscope clearly shows that bacteria are still withholding their shape and the cell wall and membranes are intact. This confirms that the bacteria have not been lysed and will act similarly to live bacteria when applied to the skin. The orientation of the bacteria in **aurafirm** P is unique - the *Lactobacillus* bacteria are 'sat' on an oat-based matrix. This orientation will have a significant impact on the probiotic-like effect that **aurafirm** P will have on the skin microbiome when topically applied as the bacteria are not as easily accessible to the skin bacteria and are therefore slowly released onto the skin during application.

BACKGROUND

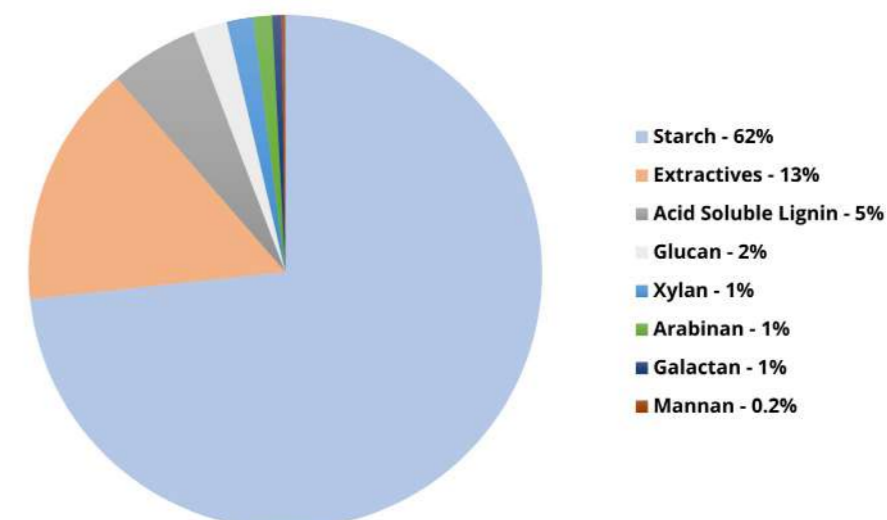
The skin microbiome is defined as all the micro-organisms living on the human skin. The microbial development of these organisms is characterised by their growth. This means the increase in cell size and cell mass during the development of a bacteria. Bacteria require basic parameters for their energy generation and cellular biosynthesis. The growth of bacteria is affected by physical and nutritional factors.

METHOD

The analytical procedure of this study was first to determinate ash content. **aurafirm** P was hydrolysed using sulfuric acid to determine the lignocellulosic sugars (glucan, xylan, arabinan, galactan, mannan, rhamnan) content. The hydrolysate is diluted and analysed by Ion Chromatography (Dionex ICS-3000 system) and Near Infrared Spectroscopy (FOSS XDS NIR). The chemical bonds that can be analysed, at infrared wavelengths, are only C-H, O-H and N-H.

RESULTS

Figure 1:
Lignocellulosic Composition of **aurafirm** P Biomass



The results show that **aurafirm** P is full of polysaccharides (long chain sugars) which attract water and help to lubricate the skin and correct water imbalances. Polysaccharides can form a protective film on the surface of the stratum corneum, avoiding the penetration of external molecules.¹

CONCLUSION

This study shows the benefits of **aurafirm** P composition for skin microbiota, which will provide a healthy and balanced diet for skin microbiota. Glucan or other carbon sources encourage the growth of bacteria as they are an available source of energy for the cutaneous flora. Consequently, the skin barrier reinforcing activity of **aurafirm** P is due to the polysaccharides pre-digested oat molecules that are known to have a prebiotic action. These molecules have the capability of forming a skin-protecting film (by protecting bacteria) and maintain stable environmental conditions.

MODE OF ACTION

We have used sophisticated technology to undertake a gene expression analysis. This part of the data pack displays the genes stimulated and consequently their potential effects on the skin mechanisms produced by *aurafirm* P.

BACKGROUND

This study was performed to understand how *aurafirm* P influences gene expression when applied topically on the skin.

METHOD

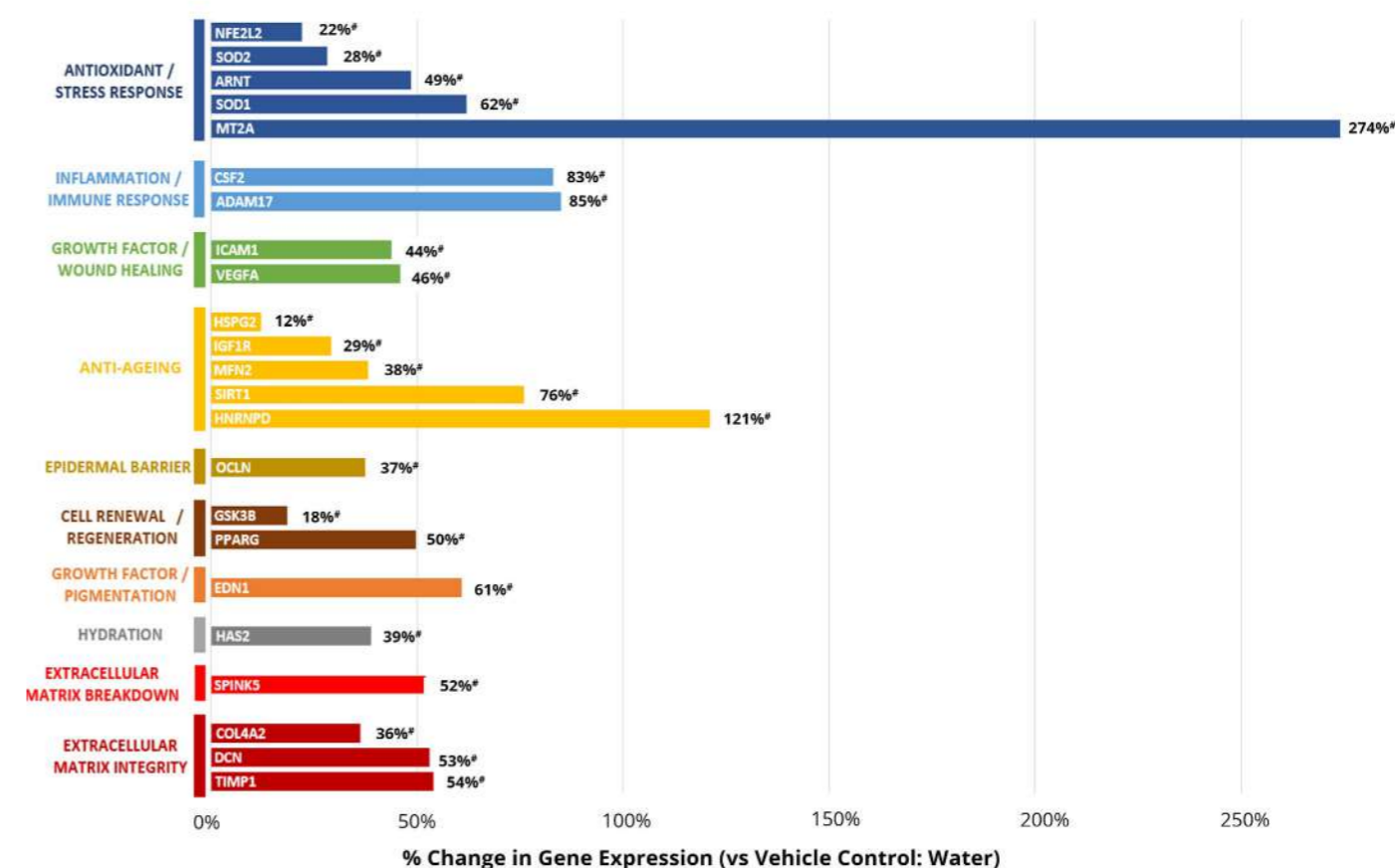
Tissue Model – A 3D in vitro skin model containing epidermal keratinocytes and dermal fibroblasts were used for the study.

Test Groups and Time Points - 1% *aurafirm* P diluted in water and water (vehicle control) were used as the test material groups. After 48 hours of application of the test materials (after 24 hours, the tissue models were rinsed and treatment was reapplied), the tissue samples were collected and analysed (RNA isolation and cDNA synthesis).

Gene Expression Analysis - This study included 4 biological replicates per Test Group. The gene expression analysis was performed using a qPCR-based array that contain genes that regulate a range of skin functions. Each gene measurement was duplicated.

RESULTS

Figure 1:
Statistically Significant Changes in Gene Expression After Applying 1% *aurafirm* P for 48 Hours



The results show that 1% *aurafirm* P increases the performance of genes that play an important role in skin biology.

The gene functions and their interpretations are summarised in the following table:

Gene Function	Gene ID	Interpretation
Antioxidant and Stress Response	SOD1&2	Binds and destroys superoxide radicals ¹
	ARNT	Induces antioxidant response and regulates inflammatory pathways
	MT2A	Lowers free radical activity, oxidative damage and inflammation ²
Inflammation and Immune Response	CSF2	Aids in wound healing by regulation of keratinocyte proliferation and differentiation ³
	ADAM17	Aids in wound healing, key regulator of epidermal growth factor receptor signaling
Growth Factor and Wound Healing	VEGFA	Promotes wound repair by regulating angiogenesis, epithelial growth and migration ⁴
Anti-Ageing	MFN2	Reduces mitochondrial damage ⁵
	SIRT1	Protection of collagen from MMP9 degradation after UV exposure ⁶
	HNRNPD	Protective effects on telomeres Maintains normal ageing ⁷
Extracellular Matrix Breakdown	SPINK5	Improves skin barrier function and reduced inflammation in skin
Extracellular Matrix Integrity	DCN	Enables wound healing and regeneration

CONCLUSION

The topical application of **aurafirm** P can stimulate the expression of genes involved in mitigating and preventing many antioxidant and ageing mechanisms, with relevance to major signs of facial stress response and ageing:

- Skin protection against oxidative damage
- Skin radiance (lowers free radical activity and promotes cell renewal)

EFFICACY ON SKIN

aurafirm P is a prebiotic active that protects and maintains a healthy microbiome. This part of the data pack provides information on the studies performed to evaluate the efficacy of **aurafirm** P on the skin.

Our studies demonstrated that **aurafirm** P is a microbiome-friendly ingredient which maintains the natural ratio of the cutaneous microbiota. It has the ability to combat oxidative damage by increasing the total antioxidant capacity after UV exposure. It also combats the oxidative damage induced by air pollution which results in an improvement of the skin radiance and texture.



BACKGROUND

An in vitro study was performed to evaluate the effects of **aurafirm P** on the growth and adhesion of microorganisms. The bacterial communities used in this study represent cutaneous microflora.

METHOD

To monitor the influence of **aurafirm P** on the growth and adhesion of three different bacterial communities, three culture plates were cultivated in a 48-wells plate in presence and absence of **aurafirm P**. Three different bacterial communities, representing the most abundant phyla, were used in the study:

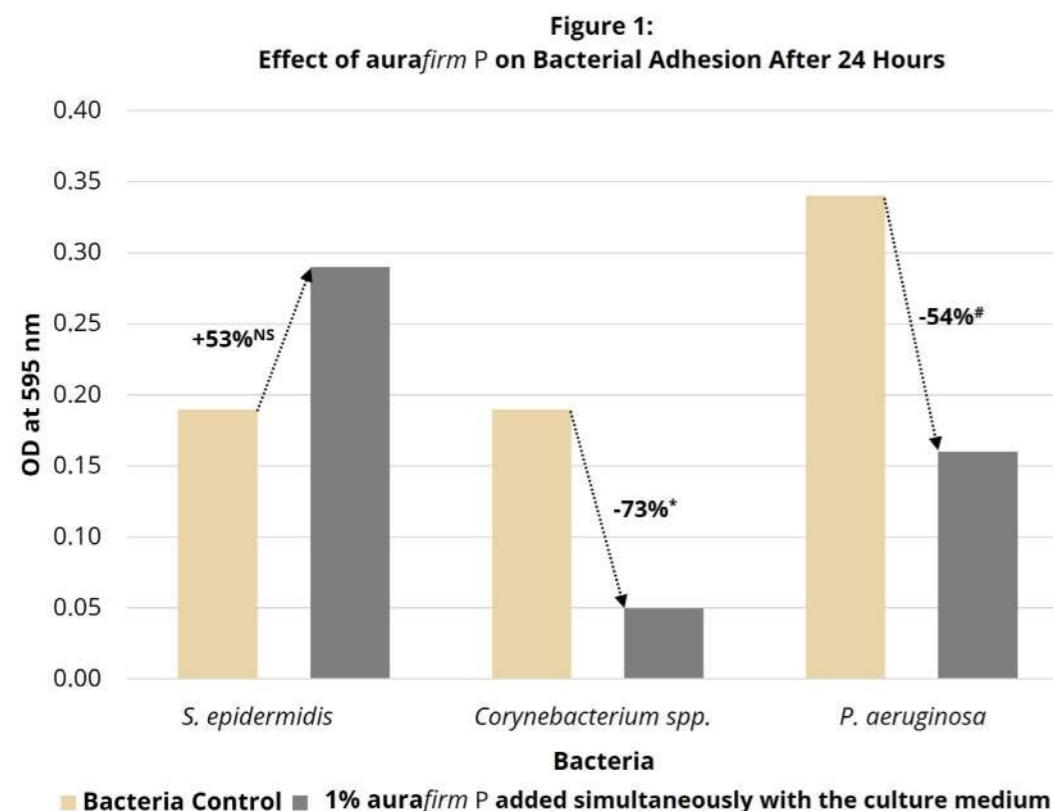
- *Staphylococcus epidermidis* (Firmicute – representing 24% of skin bacteria)
- *Corynebacterium spp.* (Actinobacteria - representing 52% of skin bacteria)
- *Pseudomonas aeruginosa* (Proteobacteria - representing 16% of skin bacteria)

A culture medium with known quantity of each bacteria (colony-forming unit, CFU/ml) was added to the wells of the three plates as well as **aurafirm P**, at a concentration of 1% (diluted with culture media), this addition corresponded to the following conditions:

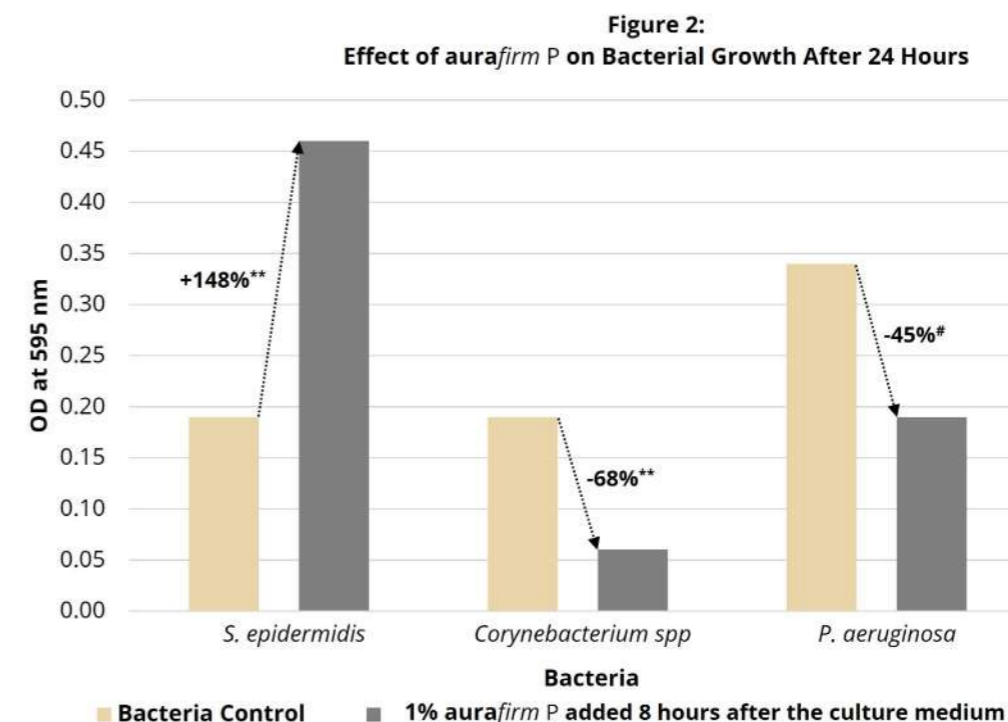
- Simultaneously with bacteria in the culture medium (during bacterial adhesion)
- 8 hours after the culture medium (during the bacterial growth)

After being incubated for 24 hours, the solutions were taken from the wells and optical density (OD) was measured, with a spectrophotometer at 595 nm, to evaluate the quantity of planktonic bacteria.

RESULTS



RESULTS (CONT.)



The results show that after 24 hours:

- Application of 1% **aurafirm P** induced a significant increase on the growth of *S. epidermidis* by 148%**.
- Application of 1% **aurafirm P** induced a significant decrease on the adhesion of *Corynebacterium spp.* by 73%* and significantly reduced its growth by 68%**.
- Application of 1% **aurafirm P** induced a significant decrease on the adhesion of *P. aeruginosa* by 54%# and significantly decreased its growth by 45%#.

CONCLUSION

The results show that **aurafirm P** has a selective effect on the growth of 'good' bacteria and potentially 'pathogenic' bacteria. **aurafirm P** enhanced the adhesion effect and growth of *S. epidermidis* significantly yet hindered the bacterial adhesion and growth of *Corynebacterium* and *P. aeruginosa*, both considered as pathogenic when growth is not controlled. These two potentially pathogenic bacteria will increase sebaceous gland activity which stimulates sebum secretion. An abundance of these bacteria can lead to atopic dermatitis or acne. It is reported that *S. epidermidis* can protect the skin by producing lactic acid which lowers the pH of the skin and controls the growth of pathogens. It also regulates the skin by helping to constrain inflammation and repairs it by speeding up the barrier function recovery and enhancing tight junctions¹. Interestingly, **aurafirm P** had a stronger antimicrobial effect on 'bad' bacteria compared to **aurafirm N**, this is due to the 'probiotic' type effect that the deactivated *Lactobacillus* has on this bacteria and the difference in the orientation of this bacteria between P and N. The skin microbiome is a matter of balance: the higher the diversity is, the healthier the skin will be. The above results have proven that **aurafirm P** will help to rebalance and significantly improve the diversity of common skin bacteria.

BACKGROUND

The skin is naturally enveloped in its own microbiome, and these are unique to each individual, with no two persons' microbiome being identical. The skin's microbial biodiversity is heavily influenced by many external and internal factors such as host physiology, environment, lifestyle habits, immune system and ageing which impacts its evolution. Following in vitro analysis, this study was performed to evaluate the ability of *aurafirm* P to maintain diversity and balance of the skin microbiota by the determination of the taxonomical composition of microbiota and the calculation of their respective alpha diversity indexes (Observed OTUs, Chao1 and Shannon indexes).

METHOD

Product Treatment

3 Caucasian women aged between 22 to 31, applied 2% *aurafirm* P on their forearms twice a day, both morning and evening for 11 days.

Assessment of Skin Microbiome

DNA Extraction - 2% *aurafirm* P was diluted with distilled water in the laboratory of the test facility and fresh test solution was prepared every day to avoid microbial contamination. Samples were prepared by swabbing the skin surface (6 hours after the application of products) and then rinsed with phosphate-buffered saline to collect bacteria from the surface. A standardised protocol was used for extraction of RNA from swabs.

Gene Sequence Analysis - For the identification of skin bacteria, a 16S rRNA gene sequence analysis (the Amplicon method)¹ was used. The generated data was compared to a DNA database for taxonomic classification and more than 300 different species of bacteria were identified. This allows us to register the impact of 2% *aurafirm* P on the whole diversity of skin microorganisms, identify major bacterial phyla and evaluate the ratios between major bacterial phyla (skin microbiota balance).

Data Analysis - The bioinformatic analysis data was carried out by detection and elimination of chimeras and the clustering of sequences in Operational Taxonomic Unit (OTU) at 97% homology.

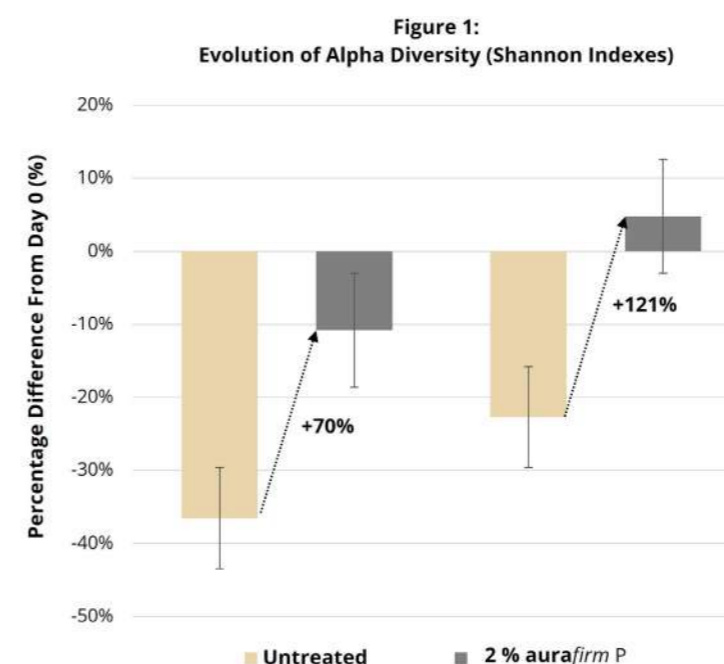
Measurement of Skin pH

Additionally, the pH of the skin was measured using a pH meter, Day 1 and Day 11, to represent the effect of 2% *aurafirm* P on the skin microbiota recovery.

RESULTS:

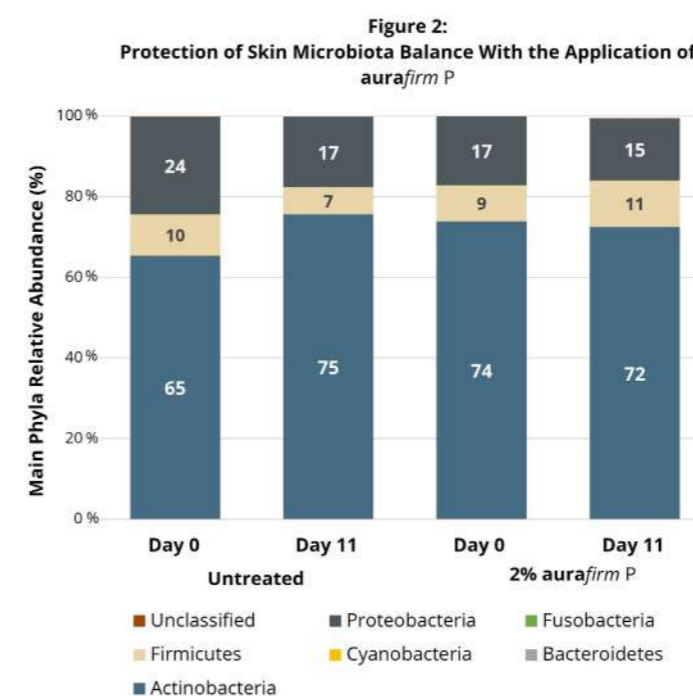
The effect of *aurafirm* P on the skin microbiome was analysed using three parameters: Alpha Diversity, Genus Profiling and Skin pH.

RESULTS: ALPHA DIVERSITY



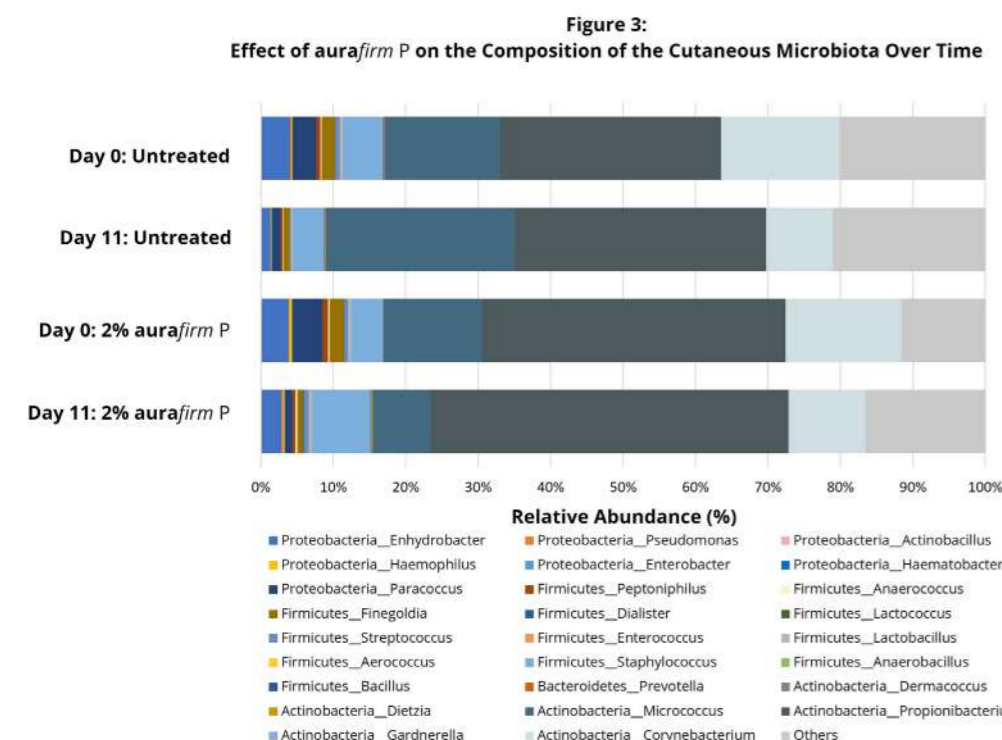
The Shannon index increases as both the richness (the number of species present) and the evenness (their relative abundances) of the community increase. It is well known that an increased biodiversity results in a healthy ecosystem, therefore an increase in Shannon index indicates a healthier skin microbiome². Results show that, at Day 1, skin microbiota diversity is reduced due to external aggression. However, with the application of 2% *aurafirm* P the reduction is less. At Day 11, 2% *aurafirm* P increases in the skin microbiota diversity. For the untreated sample, the skin microbiome composition is still altered at Day 11. 2% *aurafirm* P increases biodiversity of the skin microbiota.

RESULTS: GENUS PROFILING



A closer look at the relative abundance of the main phyla present on the skin shows that application of 2% *aurafirm* P helps to maintain the natural ratio of bacteria on the skin, therefore helping to maintain a healthy microbiome, as compared to untreated.

RESULTS: GENUS PROFILING (CONT.)

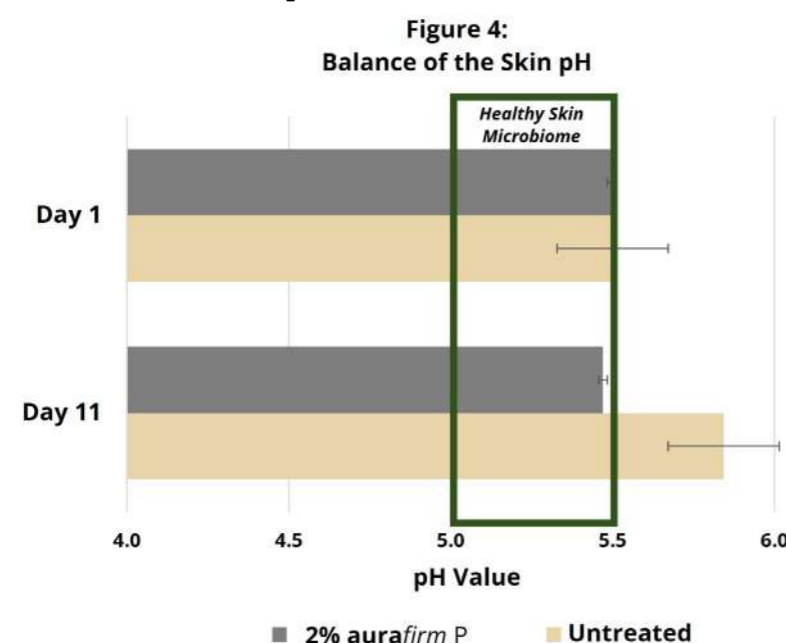


2% *aurafirm* P helps to maintain the natural ratios of the skin bacteria and maintain a healthy skin microbiome, as compared to untreated.

CONCLUSION

The equilibrium between protective and pathogenic species of microorganisms in the skin microbiota is important. This balance can be easily disrupted by osmotic stresses, changing of pH, low or high temperature or the use of strong cleansers. The alteration of skin microbiota may lead to dysbiosis which has been associated with skin disorders as inflammation, atopic dermatitis, psoriasis and acne³. Results show that the application of *aurafirm* P maintains the natural ratios of the cutaneous microbial population and protects the balance of a healthy skin microbiome. *aurafirm* P can be considered a 'microbiome-friendly' ingredient.

RESULTS: SKIN pH



Healthy skin has a slightly acidic environment, averaging between 5.0 and 5.5. The acidic environment is created by the hydrolipidic film, which provides a natural defence for the skin. A higher pH leads to impaired barrier dysfunction and a favourable environment for the growth of *S. aureus* and *S. pyogenes*, contributing to dysbiosis of the skin microbiome.³

The results show that the use of 2% *aurafirm* P maintains the skin pH. This is in part due to lactic acid - a product of *Lactobacillus* fermentation, present in *aurafirm* P which helps in rebalancing the skin pH. 2% *aurafirm* P rebalances skin pH and leads to a healthy skin microbiome.

BACKGROUND

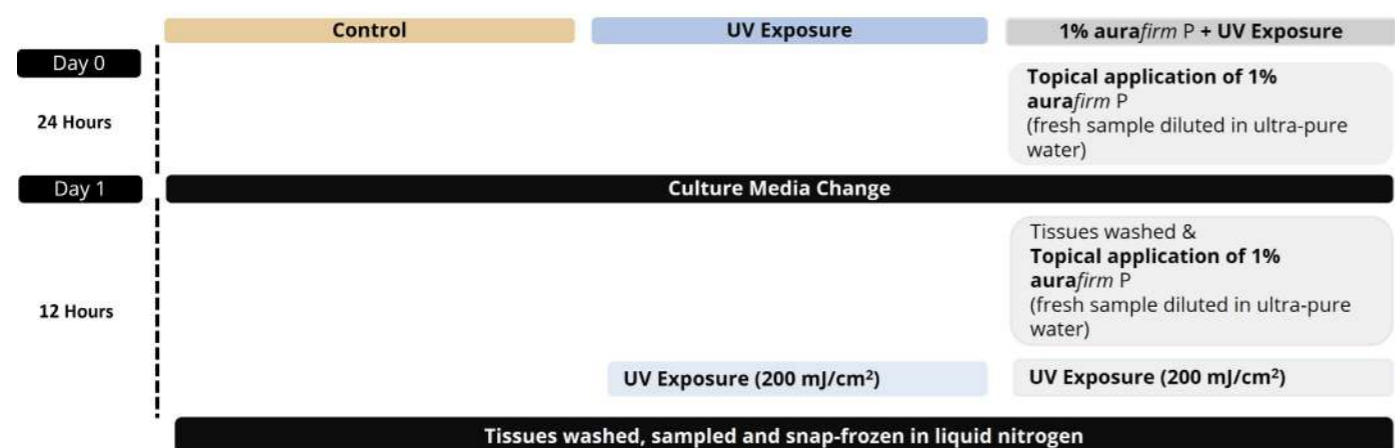
Exposing the skin to air pollution can cause oxidation reactions which lead to the formation of reactive oxygen species (ROS) and subsequent inflammation. Increased ROS in the skin can cause protein carbonylation (damaged proteins) and contribute to accelerated skin ageing. The study was conducted to understand how *aurafirm* P will combat oxidative damage when exposed to UV light.

METHOD

Tissue Model

A 3D in vitro skin model containing epidermal keratinocytes and dermal fibroblasts were used for the study.

Tissues, Treatments and Sampling



Protein Expression Analysis Using ELISA

ELISA (Enzyme Linked Immunosorbent Assay) is a technique used to detect the presence of antigens in biological samples. ELISA uses antibodies to detect a target antigen, using highly specific antibody-antigen interactions and colorimetric methods for detection.

Evaluation of ROS Prevention by Total Antioxidant Capacity (TAC) ELISA Assay

TAC was determined using a colourimetric assay (Abcam, Cambridge, UK, ab65329) based on the Cu²⁺ conversion to Cu⁺ by antioxidants and, to the following release of a colourimetric probe, proportional to the total antioxidant power. Recorded absorbances are interpolated with the standard curve of Trolox, a known antioxidant, and the total antioxidant capacity expressed as nm equivalents.

RESULTS

TAC is the measure of the number of free radicals scavenged.

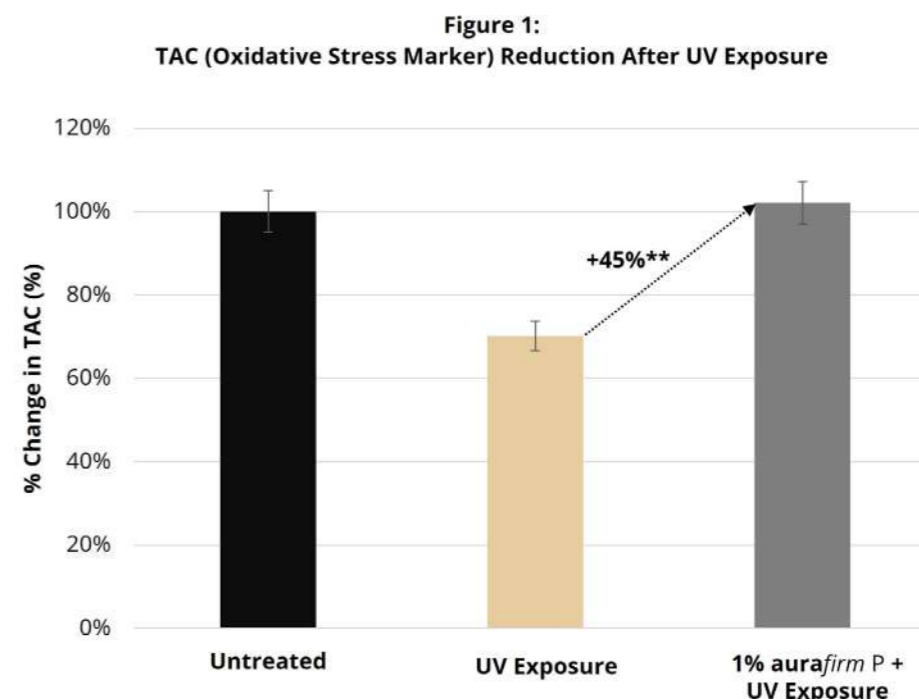


Figure 1 demonstrates that UV exposure has a significant detrimental effect on antioxidant capacity (reduction of TAC by 42%* compared to untreated). 1% *aurafirm* P, applied before UV exposure, significantly increases TAC. 1% *aurafirm* P, counteracts the effect of UV exposure and acts as a free radical scavenger which will neutralise ROS.

CONCLUSION

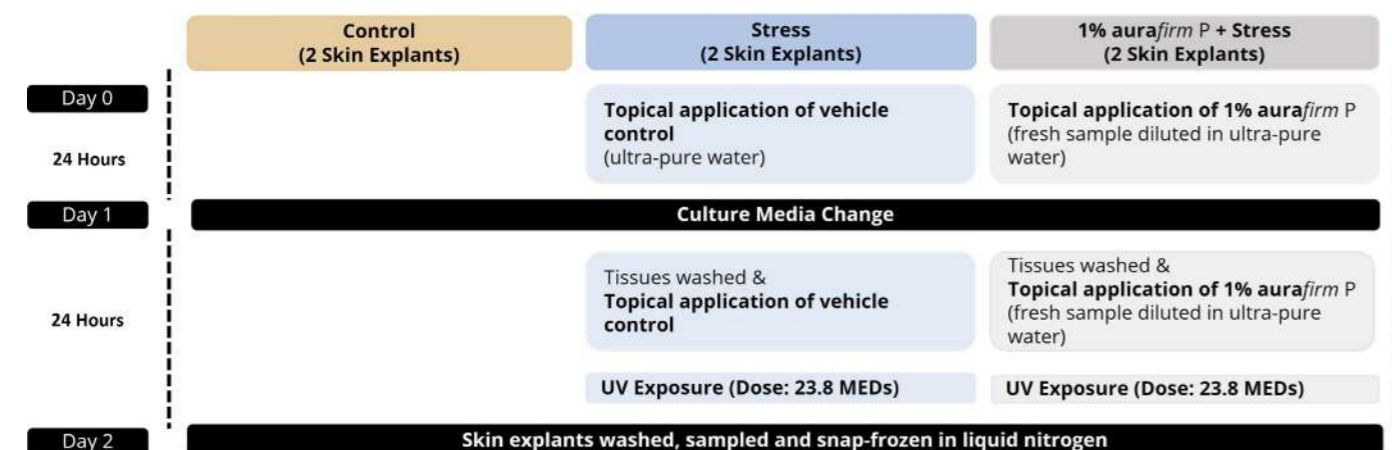
UV irradiation is known to damage endogenous antioxidant systems and trigger a cellular feedback mechanism to cope with the external damage. Antioxidants play an important role in preventing the formation and scavenging free radicals and other oxidising species. By increasing the total antioxidant capacity, *aurafirm* P has the ability to combat oxidative damage (induced by UV exposure).

BACKGROUND

UV light penetrates the skin damaging DNA and other biomolecules via the production of ROS. Increased levels of ROS cause oxidation of DNA bases, leading to the formation of lesions such as 8 hydroxy-2-deoxyguanosine (8-OHdG), a highly mutagenic lesion. ROS are constantly generated in skin cells but are usually neutralised by a network of non-enzymatic and enzymatic antioxidants. As demonstrated in the previous study, *aurafirm* P increases Total Antioxidant Capacity (TAC), after UV exposure. This ex vivo study was conducted to evaluate the consequences of the increase demonstrated in the previous study, and evaluate how *aurafirm* P will protect the skin from oxidative stress when exposed to UV and IR light.

METHOD

Explants, Treatments and Sampling (for a 46-year-old female Caucasian donor)



*UV Exposure Method – Stimulation with UV light (500 mJ/cm²) used a Solar Simulator oA1 Oriel. This mimicks natural sun exposure, which includes an appropriate ratio of UVB, UVA, visible and infra-red light.

Protein Expression Analysis Using ELISA

ELISA (Enzyme Linked Immunosorbent Assay) is a technique used to detect the presence of antigens in biological samples. ELISA uses antibodies to detect a target antigen, using highly specific antibody-antigen interactions and colourimetric methods for detection.

Evaluation of Oxidative DNA Damage by 8-OHdG

Samples were collected and measured by an enzyme-linked immunosorbent assay (ELISA) kit #ab201734 (Abcam, Cambridge, MA, USA). The ELISA utilises an 8-hydroxy-2-deoxyguanosine coated plate and an HRP-conjugated antibody (which recognises both free 8-OHdG and DNA-incorporated 8-OHdG) for detection.

RESULTS

8-OHdG is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species and is an established marker of oxidative stress. Hydroxylation of guanosine occurs in response to both normal metabolic processes and a variety of environmental factors (i.e., Solar UV, IR and visible light, environmental pollution, psychological stress). This has a negative impact on the appearance of skin.

RESULTS (CONT.)

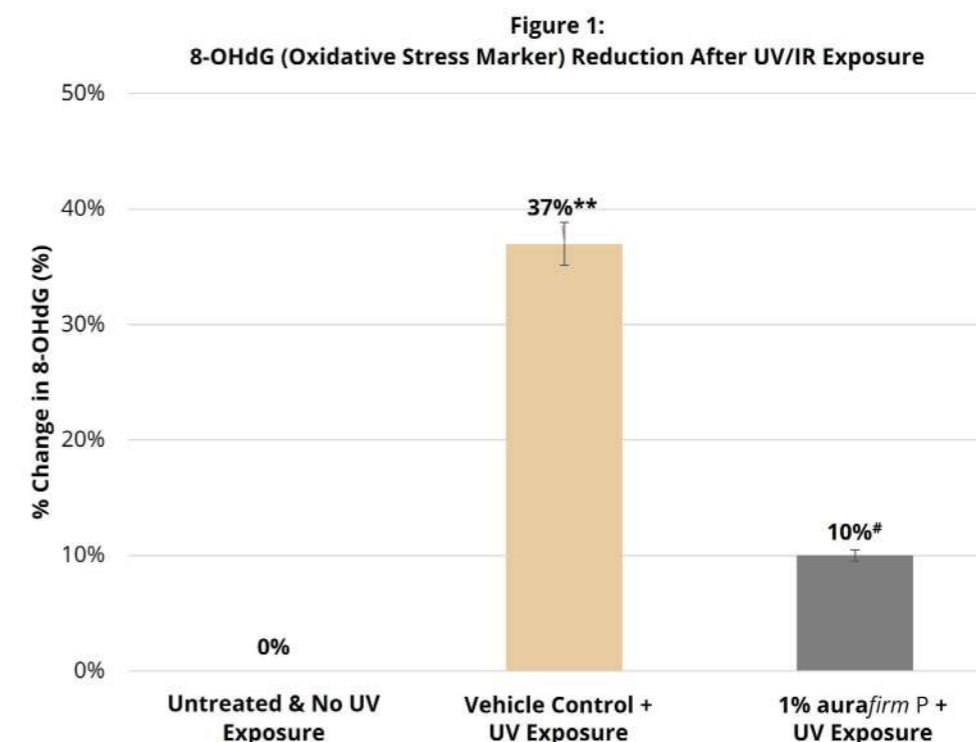


Figure 1 demonstrated that UV/IR light has a detrimental effect (reduction of 8-OHdG expression levels compared to untreated) and induced oxidative stress. Topical application of 1% *aurafirm* P, before induced oxidative stress, resulted in a significant reduction of 8-OHdG expression levels.

CONCLUSION

Skin, as the outermost barrier to external aggressors, is in direct contact with various air pollutants such as UV and IR exposure. These pollutants will cause oxidative stress and DNA damage within the skin and lead to premature skin ageing. *aurafirm* P has the ability to counteract the formation of reactive oxygen species and combat oxidative damage induced by air pollution.

BACKGROUND

The skin ageing process results in modification of the organisation of skin lines (tension lines). They pass through a homogeneous isotropic state (lines oriented in all directions), for a young person, then through a state where the furrows gradually disappear, to finally give place to a state where deep furrows persist, leading to the formation of highly oriented wrinkles. An independent study was created and undertaken to evaluate the short-term effects of **aurafirm P** versus a placebo on skin texture.

METHOD

Product Treatment

10 women aged between 45 to 60 years old, with dry skin, applied 2% **aurafirm** P and a placebo on each half of the face respectively. Skin measurements were taken before application of the treatment and 1 hour after application.

Measurements of Cutaneous Microrelief

A skin texture analysis was undertaken using SkinEvidence, an imaging device containing standardised illumination with normal and polarised light together for a quantitative analysis for skin surface. Cutaneous microrelief was assessed with the anisotropy index, which enables the effect of a product on the topography of the skin relief to be measured. This index was calculated by analysing the grey levels of images taken using the SkinEvidence video probe.

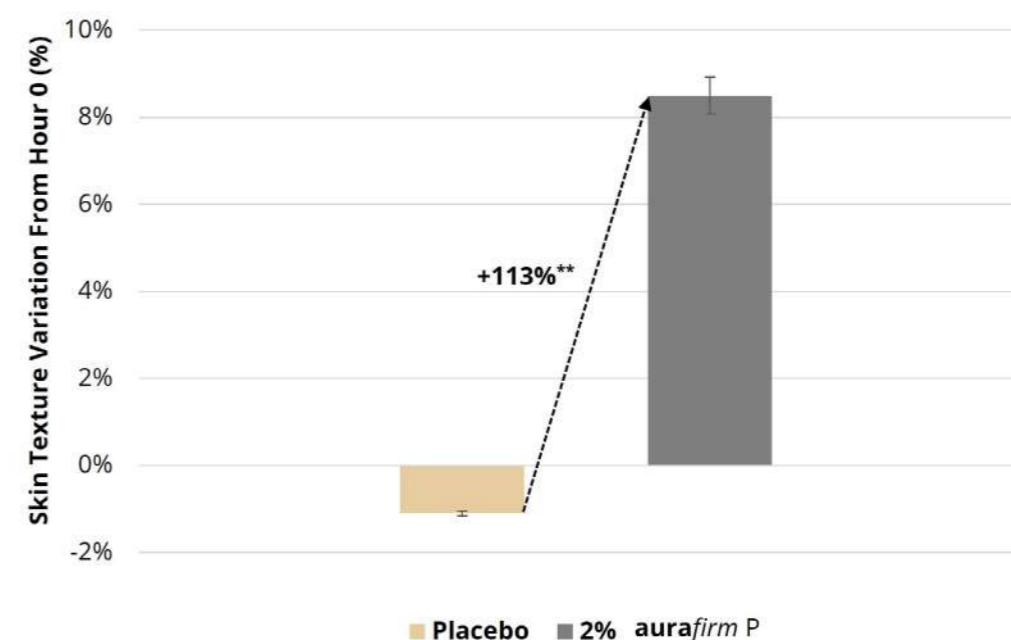
The following formulation was used in this study:

Phase	Trade Name	INCI Name	% w/w
A	Purified Water BP	Aqua	93.000
A	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.000
B	Sepiplus 400	Polyacrylate-13, Polyisobutene, Polysorbate 20	4.000
C	aurafirm P	Aqua, <i>Lactobacillus</i> Ferment, <i>Avena sativa</i> Kernel Extract, Sodium Benzoate, Potassium Sorbate	2.000

*Placebo formulation was identical minus 2% **aurafirm** P – remaining % was made up with water.

RESULTS

Figure 1:
Immediate Improvement in Skin Texture



aurafirm P showed a rapid decrease of 8.5% in skin roughness within 1 hour of application when compared to placebo.

CONCLUSION

aurafirm P improves skin texture. Due to skin beneficial molecules present in **aurafirm** P, like amino acids and antioxidants, it will hydrate and smooth the skin.



BACKGROUND

Skin radiance is affected by the smoothness of skin texture, the smoother the skin, the greater the reflectivity. Rough skin texture can trap and disperse light, leaving the skin looking dull and tired. An independent study was created to evaluate the rapid and short-term effects of **aurafirm P** versus a placebo on skin radiance.

METHOD

Product Treatment

Over a period of 7 days, 15 women aged between 45 to 60 years old with dry skin applied 2% **aurafirm P** and a placebo on each half of the face respectively. The application was carried out twice a day, both morning and evening. Skin measurements were taken on Day 0 (before application of the treatment), after 1 hour and on Day 7.

Measurements of Skin Colour Intensity

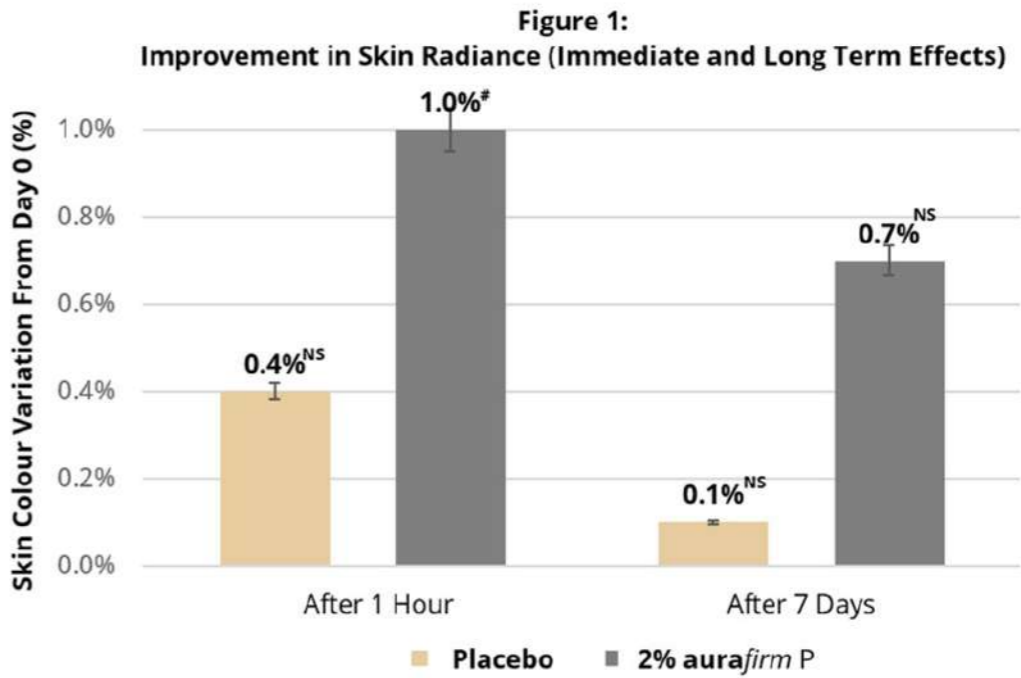
The measurement of skin colour intensity was performed by colourimetry through chromameter (Spectrocolorimeter CM2600D™). Parameter L* was analysed during this study, which determines the brightness (or luminance) from zero (black) to 100 (white).

The following formulation was used in this study:

Phase	Trade Name	INCI Name	% w/w
A	Purified Water BP	Aqua	93.000
A	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.000
B	Sepiplus 400	Polyacrylate-13, Polyisobutene, Polysorbate 20	4.000
C	aurafirm P	Aqua, Lactobacillus Ferment, Avena sativa Kernel Extract, Sodium Benzoate, Potassium Sorbate	2.000

*Placebo formulation was identical minus 2% **aurafirm P** – remaining % was made up with water.

RESULTS



The Chromameter measured a significant increase of 1%, after 1 hour of using the product containing 2% **aurafirm P**, compared to placebo. Skin radiance was also improved after 7 days of use.

CONCLUSION

aurafirm P significantly improves the radiance of the complexion immediately after application.

aurafirm P will,

- Help to stimulate the skin cell regeneration process (as demonstrated in 'Gene Modulation and Biological Activity', **aurafirm P** upregulates - GSK3B and PPARG genes which play a role in cell renewal).
- Lock moisture into the skin (as demonstrated in 'Gene Modulation and Biological Activity', **aurafirm P** upregulates HAS2, a critical enzyme-producing hyaluronic acid, which has a unique capacity to bind and retain water molecules).
- Improve skin radiance (**aurafirm P** upregulates the synthesis of a number of several antioxidant genes such as NFE2L2, SOD1, SOD2, MT2A, which will have the ability to protect the skin against free radicals).

BACKGROUND

An independent self-assessment study was created, with the aim of evaluating the rapid and short-term effects of **aurafirm** P on overall skin health.

METHOD

Product Treatment

20 women aged between 45 and 60 years old with dry skin were provided with a product containing 2% **aurafirm** P and the application was carried out twice, daily on one half of the face

Subjective Evaluation

Assessment of the sensation felt, efficacy and cosmetic quality of the product was performed through a self-assessment questionnaire completed on Eval & Go by participants after 1 hour, 24 hours and at Day 7 of product application during the study.

The following formulation was used in this study:

Phase	Trade Name	INCI Name	% w/w
A	Purified Water BP	Aqua	93.000
A	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.000
B	Sepiplus 400	Polyacrylate-13, Polyisobutene, Polysorbate 20	4.000
C	aurafirm P	Aqua, Lactobacillus Ferment, Avena sativa Kernel Extract, Sodium Benzoate, Potassium Sorbate	2.000

*Placebo formulation was identical minus 2% **aurafirm** P – remaining % was made up with water.

RESULTS

Results have been recorded where participants scored impressions of the product at 70% or higher.

Organoleptic Properties	T + 1 Hour	T + 24 Hours	T + 7 Days
Global appreciation of product	✓	✓	✓
Feeling of moisturisation	✓	✓	
Feeling of suppleness	✓		
Feeling of firmness	✓		✓
Feeling of comfort	✓	✓	✓
Feeling of radiance		✓	✓

The organoleptic qualities of the product containing 2% **aurafirm** P were a feeling of comfort throughout the study period, rapid feelings of moisturisation, suppleness, firmness, and comfort within the first hour, followed by a feeling of radiance, firmness and comfort after 7 days.

RESULTS (CONT.)

Product Efficacy	T + 1 Hour	T + 24 Hours	T + 7 Days
Improved skin feel	✓	✓	✓
Skin is more comfortable	✓	✓	✓
Skin is nourished	✓	✓	✓
The skin is more supple and elastic		✓	✓
The skin is brighter		✓	✓
The skin is moisturised		✓	
Unified complexion		✓	✓
Skin looks more uniform		✓	
Feeling of moisturisation lasts throughout the day		✓	

The product efficacy results from the questionnaire highlight an improved skin feel from 1 hour to Day 7, the skin feels more comfortable and is nourished throughout. There are noticeable positive effects 24 hours after application.

CONCLUSION

The consumer perception study showed that after using **aurafirm** P, more than 70% of the participants agreed that the skin feel improved and there was a positive effect on various parameters such as moisturisation, suppleness, firmness, radiance, firmness, and comfort.

BACKGROUND

The constant use of harsh cleansers disrupts the natural bacterial ecosystem of the skin. As soap generally has a much higher pH than the skin, its use can often lead to deterioration in the skin microbiota, and excessive handwashing leaves large open spaces on the surface of the skin which present potential new territories for colonising bacteria. This skin degradation can lead to a decrease in barrier function, skin dehydration and an increase in desquamation, meaning the integrity and composition of the skin microbiota may ultimately be compromised. A hand washing trial was performed to assess the ability of *aurafirm* P to protect and hydrate the skin. The participants were asked to compare a hand cream containing 2% *aurafirm* P and a placebo cream.

METHOD

Product Treatment

Over 28 days, 35 participants – men and women aged between 20-60 with different skin types (oily, dry and combination) had to regularly wash their hands at least 5 times a day for 20 seconds or more with antibacterial soap. After doing this for 7 days and applying no product, the participants were asked to apply the hand cream containing 2% *aurafirm* P for 7 days and the placebo for 7 days. To ensure the trial was fair, 50% of participants applied the placebo first and 50% applied *aurafirm* P first.

Subjective Evaluation

Assessment of the efficacy of the product was performed through a self-assessment questionnaire. Data was analysed by AGR systems in real time (Ayton System Software).

The following formulation was used in this study:

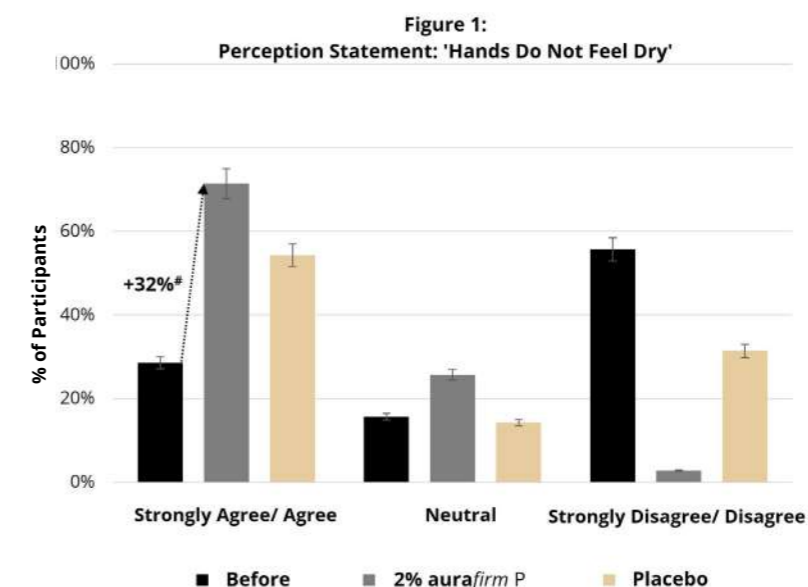
Phase	Trade Name	INCI Name	% w/w
A	Purified Water BP	Aqua	66.300
A	Amaze Nordic Barley	<i>Hordeum Vulgare</i> Flour	5.000
A	Versene NA2 Crystals	Disodium EDTA	0.050
B	Makilene GC	Butylene Glycol	3.000
B	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.000
C	Floraesters	Jojoba Esters	10.500
C	Emulsun	Hydrogenated Sunflower Seed Oil Polyglyceryl-3 Esters, Cetearyl Alcohol, Hydrogenated Sunflower Seed Oil, Glyceryl Esters, Sodium Stearoyl Lactylate	5.000
C	Cetiol MM	Myristyl Myristate	4.000
C	AAK Sweet Almond Oil	<i>Prunus Amygdalus Amara</i> Kernel Oil	2.000
C	Cutina GMS V	Glyceryl Stearate	0.800
C	Carnauba Wax SP- 63	<i>Copernicia Cerifera</i> Wax	0.350
D	<i>aurafirm</i> P	Aqua, <i>Lactobacillus</i> Ferment, <i>Avena sativa</i> Kernel Extract, Sodium Benzoate, Potassium Sorbate	2.000

*Placebo formulation was identical minus 2% *aurafirm* P – remaining % was made up with water.

RESULTS

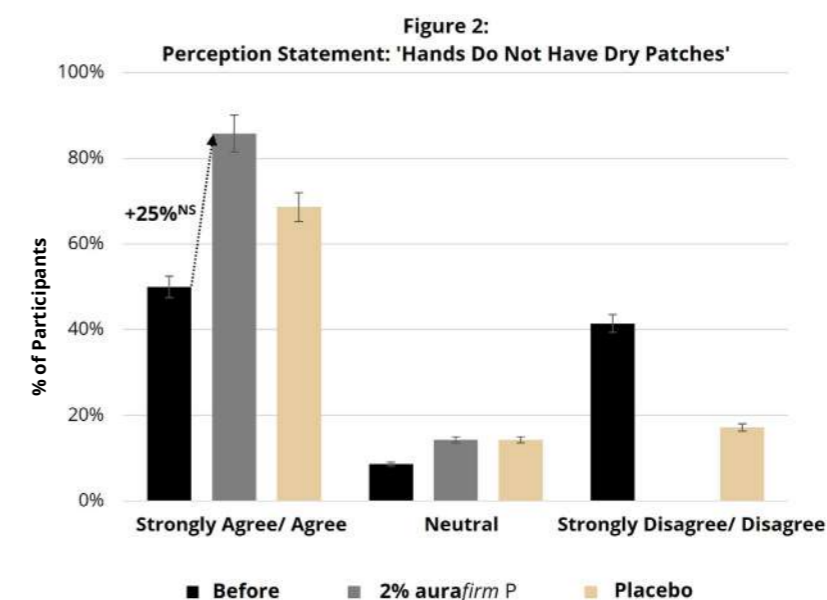
For the following section the participants were asked identical questions regarding the condition of their skin after 1 week of excessive hand washing (before applying the product), after applying *aurafirm* P and after applying the placebo.

Effect on Skin Dryness:



After applying 2% *aurafirm* P for 7 days, only 3% of participants reported having dry skin (though 0% strongly disagreed with the perception statement) compared to 56% reporting dryness before use. 31% of participants reported their hands feeling dry after using the placebo for 7 days.

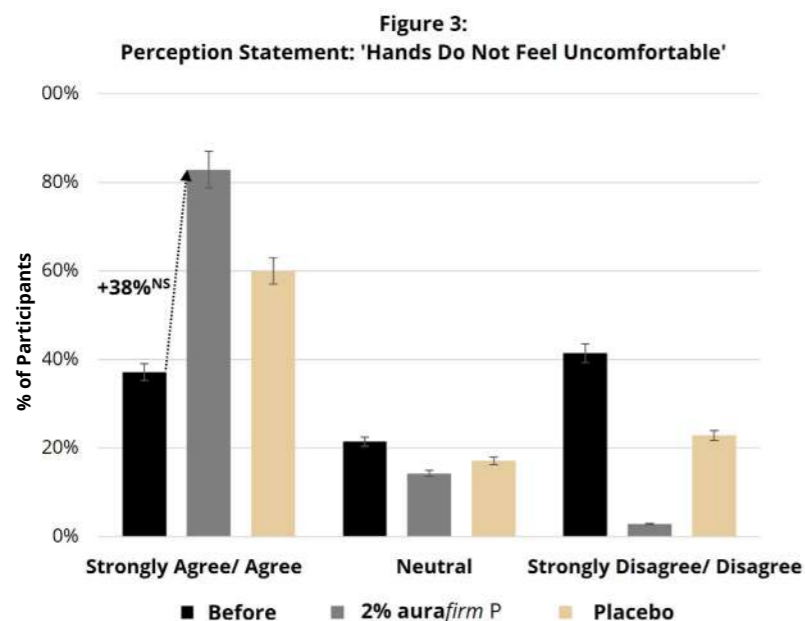
Effect on Skin Damage:



41% of participants reported their hands having visible dry patches before application of *aurafirm* P. 100% of these participants reported that the dry patches to have disappeared after application of 2% *aurafirm* P.

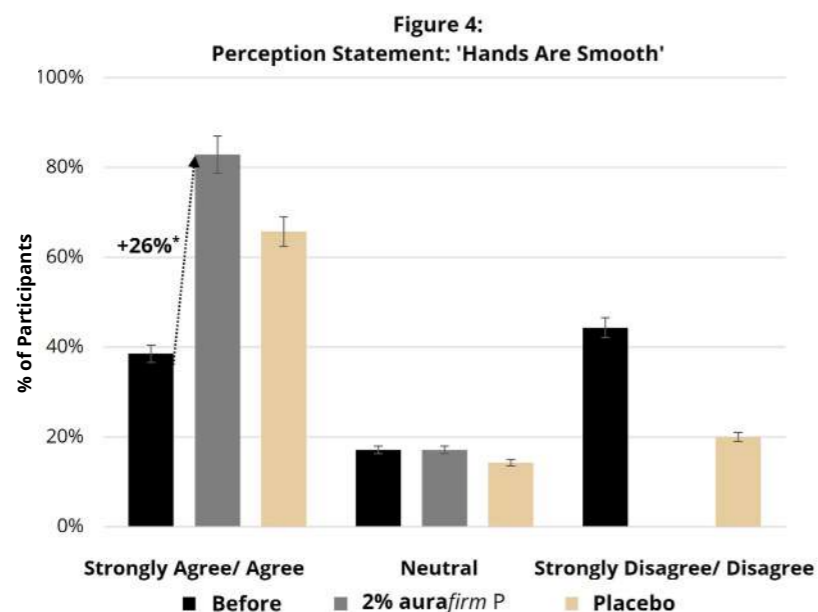
RESULTS (CONT.)

Effect on Skin Comfort:



3% of participants reported their hands feeling uncomfortable after using 2% *aurafirm* P (though 0% strongly disagreed with the perception statement) compared to 23% when using the placebo and 41% before using any product.

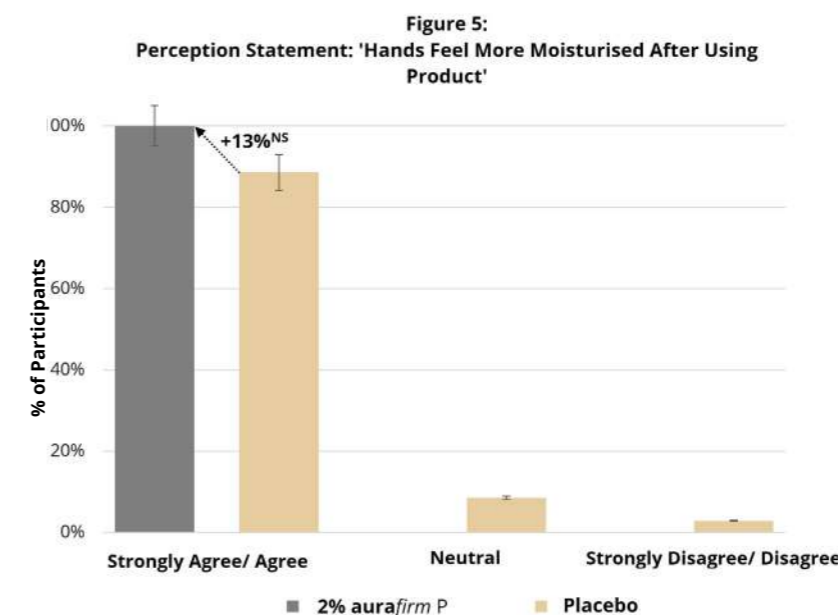
Effect on Skin Texture:



83% of participants reported their hands being smooth after using 2% *aurafirm* P compared to 66% when using the placebo and 39% before using any product.

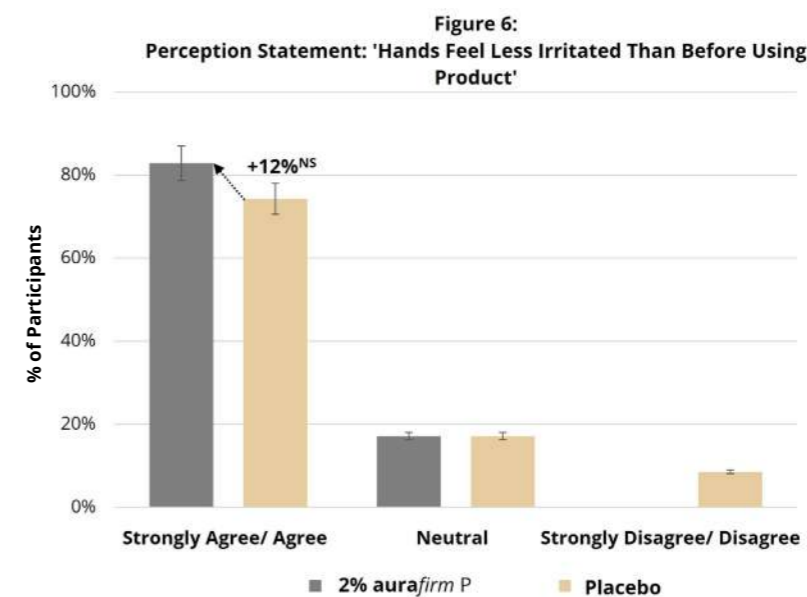
RESULTS (CONT.)

Effect on Skin Hydration:



After applying the 2% *aurafirm* P hand cream for 7 days, 100% of the participants reported their hands feeling more moisturised.

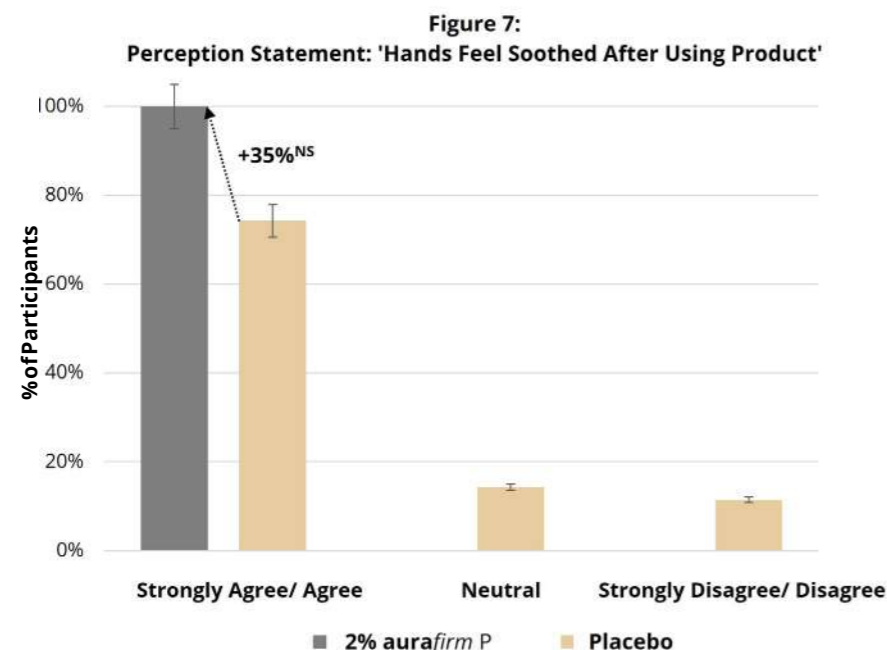
Effect on Skin Irritation:



22% more participants reported their hands feeling less irritated after using 2% *aurafirm* P, compared to the placebo.

RESULTS (CONT.)

Effect on Skin Condition:



After applying 2% *aurafirm* P hand cream for 7 days, 100% of the participants reported their hands feeling more soothed.

CONCLUSION

Skin, when subjected to excessive washing and exposure to hand sanitisers may have less microbial diversity or an unbalanced microbiome. This results in a compromised skin barrier which may allow the ingress of irritants that can cause inflammation, flare-ups and itching. Crucially, however, the balance of the microflora can be restored through the use of probiotic, prebiotic, and postbiotic substances. *aurafirm* P possesses the ability to repopulate a disrupted microflora and rebalance it selectively in favour of useful organisms such as *S. epidermidis* - a key bacterium in a healthy microbiome which helps to reduce the skin pH by increasing the production of lactic acid and producing antimicrobial peptides which help to repair damaged skin

CREDENTIALS

We conclude the data pack with the following credentials of our active fermented oat ingredient. The HRIPT test has demonstrated the hypoallergic and non-irritant capacity of *aurafirm* P.



*Product image for illustration purposes only, actual product may vary

Significant: *= $p < 0.05$ (95%); #= $p < 0.1$ (90%) and NS: Non Significant

BACKGROUND

A Human Repeat Insult Patch Test (HRIPT) was carried out to determine the cutaneous irritation (contact dermatitis) and sensitisation (contact allergy) potential of **aurafirm P**, when applied to the skin of healthy participants.

METHOD

The study consisted of 52 participants (male and female, aged 20-78) and 3 phases: induction, in which 10 patches were repetitively applied over the course of 21 days; incubation, a rest period; and revealing, a challenge phase. Repeated contact with a potential allergen in the formula, if present, generates a series of immunological reactions in the body of the participants and induces a visible reaction on the application site. Any reactions were observed, recorded, and evaluated by a dermatologist to confirm the allergenicity of the product and hence the product's safety.

Repeated Skin Contact Test (Induction Phase): Prior to applying the patches, the test area - upper back, between the two shoulder blades - was carefully examined. A patch containing the test products and the control was applied to the test area and left in contact with the skin for 48 hours. When this first patch was removed at the laboratory 48 hours after application, the observation area was rinsed with water, dried, and examined for any skin changes. Following the examination, a new patch with fresh test product was applied. The test products were applied on the selected zones every second day, over 21 consecutive days.

Rest Period (or Incubation Phase): After the completion of the Induction Phase, a Rest Period of 10 to 14 days took place.

Challenge Phase (or Revealing Phase): The application site used during the Challenge Phase was different to the one used in the Induction Phase. For this phase, the patch was removed at the laboratory 48 hours after application. The test site was cleaned and examined for any signs of intolerance or irritation. Throughout the study, **aurafirm P** was applied at 100%.

RESULTS

aurafirm P did not produce any signs of cutaneous irritation or skin sensitisation. That is, no participants showed presence of oedema, vesicles, blisters, or ulcerations or reported immediate or delayed reactions such as redness, irritation, itching or other sensations.

CONCLUSION

aurafirm P can be considered both hypo-allergenic and non-irritant. Furthermore, given the control provided by a dermatologist during the study, the test products may also bear the claim "tested under the control of a dermatologist" or "dermatologically-tested".

Introduction Pg 1-4

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Alphabetical list of Standard Skin Panel Gene IDs, Gene Names and Functions:

Gene ID	Gene Names	Associated Function(s) in Skin
ADAM17	ADAM Metallopeptidase Domain 17	Inflammation / Immune Response
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator	Antioxidant / Stress Response
COL4A2	Collagen Type IV Alpha 2	Extracellular Matrix Integrity
CSF2	Colony Stimulating Factor 2	Inflammation / Immune Response
DCN	Decorin	Extracellular Matrix Integrity
EDN1	Endothelin 1	Growth Factor / Pigmentation
GSK3B	Glycogen Synthase Kinase 3 Beta	Cell Renewal / Regeneration
HAS2	Hyaluronan Synthase 2	Hydration
HNRNP	Heterogeneous Nuclear Ribonucleoprotein D	Anti-Ageing
HSPG2	Heparan Sulfate Proteoglycan 2	Anti-Ageing
ICAM1	Intercellular Adhesion Molecule 1	Growth Factor / Wound Healing
IGF1R	Insulin Like Growth Factor 1 Receptor	Anti-Ageing
MFN2	Mitofusin 2	Anti-Ageing
MT2A	Metallothionein 2A	Antioxidant / Stress Response
NFE2L2	Nuclear Factor, Erythroid 2 Like 2	Antioxidant / Stress Response
OCLN	Occludin	Epidermal Barrier
PPARD	Peroxisome Proliferator Activated Receptor Gamma	Cell Renewal / Regeneration
SIRT1	Sirtuin 1	Anti-Ageing
SOD1	Superoxide Dismutase 1, Soluble	Antioxidant / Stress Response
SOD2	Superoxide Dismutase 2, Mitochondrial	Antioxidant / Stress Response
SPINK5	Serine Peptidase Inhibitor, Kazal Type 5	Extracellular Matrix Breakdown
TIMP1	TIMP Metallopeptidase Inhibitor 1	Extracellular Matrix Integrity
VEGFA	Vascular Endothelial Growth Factor A	Growth Factor / Wound Healing

GET IN TOUCH

For more information about **aurafirm P**, or any other enquires about our offerings at Oat Cosmetics, please contact our Sales team at **sales@oat.co.uk**

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