Oat cosmetics

aura*firm* N

DATA PACK

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aura*firm*

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Introduction

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

aura*firm* N is a mobile liquid containing a low level of insoluble fermented oat bran giving rise to a cloudy suspension when shaken. **aura***firm* N contains a broad spectrum of water-soluble molecules produced by the fermentation process which condition the skin and boost microflora diversity. This product is ideal for low viscosity lotions, toners and serums.

FERMENTATION

aura*firm* N 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 a filtered paste, **aura***firm* P – 5 to 10% of Oat COM, and a filtrate, **aura***firm* N – 1 to 5% of Oat COM. The filtrate can be further processed to create a clear serum, **aura***firm* S – 1 to 2% of Oat COM.

PREBIOTICS

Molecules that promote the growth of beneficial microorganisms on the skin and provide a healthy and balanced diet for skin microbiota.

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 oats had the ability to hydrolyse the bound between phenolics and cell wall macromolecules, leading to the increase of the soluble phenolic content[1]. The fermentation of Oat COM increased the antioxidant capacity of **aura***firm* N.

ECTOIN

Ectoin is a natural substance which is produced by bacteria to protect against extreme conditions. It promotes hydration by maintaining the correct water balance in skin meaning the skin appears smooth and soft preventing dehydration of the epidermis.

PROBIOTICS

Live microorganisms which when administered in adequate amounts confer a health benefit by strengthening the skin microbiome.

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.[2] Data shows that heat-killed bacteria, their fractions or purified components have probiotic effects with advantages over live probiotics.

DIACETYL

Strains of Lactobacillus bacteria can produce diacetyl. It has potential to exhibit dermal antimicrobial activities, with greater sensitivity against Gram-negative bacteria and fungi as compared to Gram-positive bacteria.[2]

Introduction (Cont.)

POSTBIOTICS

Range of metabolites produced by live bacteria during the fermentation process that help to regulate the composition of the skin microbiome ecosystem.

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. **aura***firm* N contains hydroxy acids, particularly Lactic acid.

AMINO ACIDS

Amino acids, which are important to the metabolic activity of the living epidermis, are essential in maintaining the integrity of the skin barrier, for protein synthesis and nutrient absorption. During fermentation, proteins are digested by microbial proteases and peptidases resulting in amino acids.[3] **aura***firm* N will supply the skin with essential amino acids which protect skin from free-radical damage and reduce signs of ageing.

BIOACTIVE PEPTIDES

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

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 **aura***firm* N, 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[3,4].

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.

aura*firm* N Profile: FT-IR Analysis

BACKGROUND

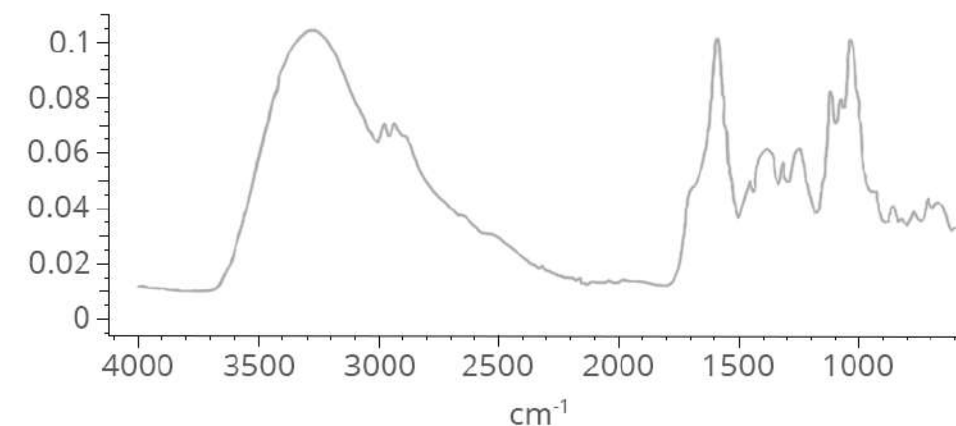
Fourier–transform infrared spectroscopy (FT-IR) was used to profile and create a fingerprint for **aura***firm* N.

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 **aura***firm* N 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.

aura*firm* N is a complex sample that cannot be easily resolved.

RESULTS



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

Wavenumber Assignment of FT-IR

CONCLUSION

The peaks around 3300 cm^{-1}, 2900 cm^{-1}, 1720 cm^{-1} and 1050 cm^{-1} represent hydroxy carboxylic acids such as lactic acid. This type of compound is the major component of the sample.

The bonds around 1610 cm^{-1} and 860 cm^{-1} are likely to correspond to the preservative Sorbic Acid and the peaks around 680-780 cm^{-1} to the preservative Benzoic Acid.

Independently tested by Campden BRI, April 2019. Full report available upon request.

Viability of Lactobacillus Bacteria: Flow Cytometry Study

BACKGROUND

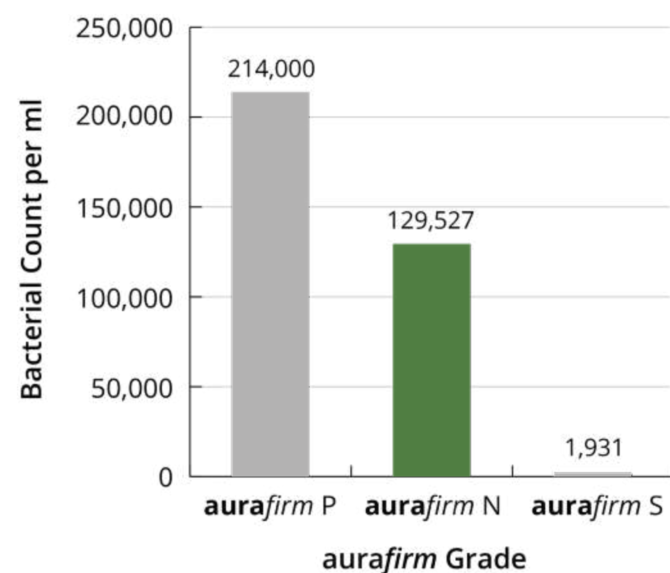
A study was performed to confirm the viability and count the Lactobacillus bacteria in **aura***firm* N using flow cytometry.

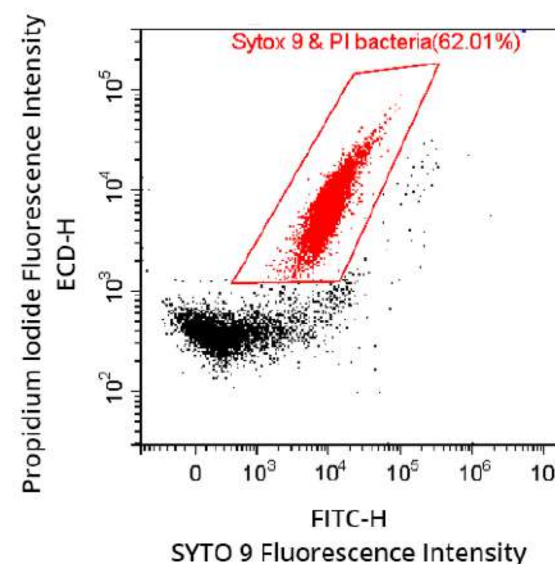
METHOD

Using a flow cytometer, **aura***firm* N 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 was average taken.

RESULTS





Red: Lactobacillus bacteria are identified as dead and account for 62% solid matter in **aura***firm* N
Black: Represents pre-digested oat material

CONCLUSION

The results show that Lactobacillus bacteria, deactivated by the pasteurisation step at the end of the fermentation process, are present in all **aura***firm* grades. However, a clear distinction is seen between **aura***firm* N and the other **aura***firm* grades.

The bacterial content in **aura***firm* N is lower than in **aura***firm* P, however the flow cytometry results indicate that 62.01% of all solid matter is Lactobacillus bacteria, meaning **aura***firm* N contains approx. 0.6% Lactobacillus bacteria in total. Additionally, in **aura***firm* N the bacteria are free flowing in solution hence during application onto the skin, the bacteria are readily available in solution and can immediately interact with the skin microbiome.

Independently tested by The University of York, April 2019. Full report available upon request.

Concentration of Lactobacillus Bacteria: Confocal Microscopy

BACKGROUND

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

METHOD

In order to monitor the viability of Lactobacillus bacteria in **aura***firm*, two fluorescent markers, SYTO 9 green (live bacteria) and propidium iodide red (dead bacteria), were added in solution to **aura***firm* N respectively and incubated at room temperature for 20 minutes. The image analysis was performed on a 780LSM confocal microscope.

RESULTS

20µm

The Lactobacillus bacteria are free flowing, meaning they are readily available when applied. Bacteria are confirmed dead but remain intact so will act similarly to live bacteria when applied to the skin.

CONCLUSION

Lactobacillus bacteria is present in **aura***firm* N – the dye has 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 bacteria are readily available when applied to the skin due to the fact they are free flowing in solution.

Independently tested by The University of York, April 2019. Full report available upon request.

BACKGROUND

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

METHOD

To monitor the influence of **aura***firm* N on the growth and adhesion of three different bacterial communities, 3 cultural plates were cultivated in a 48-wells plate in presence and absence of **aura***firm* N.

Three different bacterial communities, representing the most abundant phylum, were used in the study:

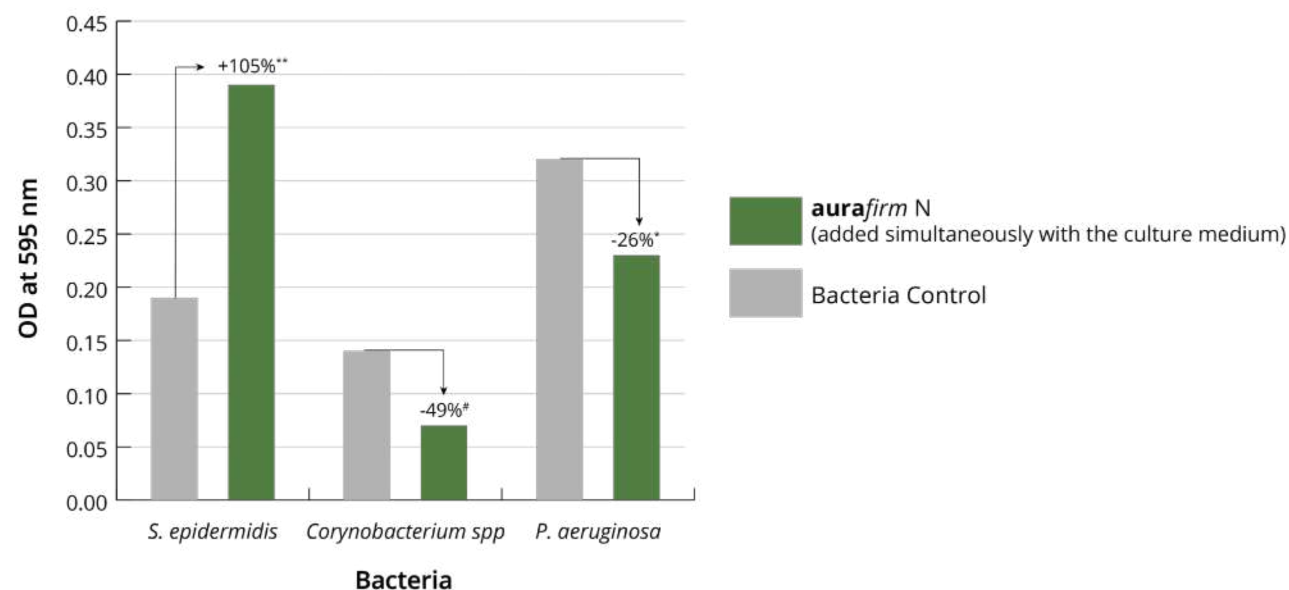
- *Staphyloccus epidermidis* (Firmicute – representing 24% of skin bacteria)
- *Corynobacterium spp.* (Actinobacteria - representing 52% of skin bacteria)
- *Pseudomonas aeruginosa* (Proteobacteria - representing 16% of skin bacteria)

A culture medium with known quantity of each bacteria (colony-forming unit, CFU/ml) was added to the wells of the 3 plates as well as **aura***firm* N, at a concentration of 1% (diluted with culture media), this addition corresponded to the following conditions:

1. Simultaneously with bacteria in the culture medium (during bacterial adhesion)
2. 8h after the culture medium (during the bacterial growth)

After being incubated for 24hrs, 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



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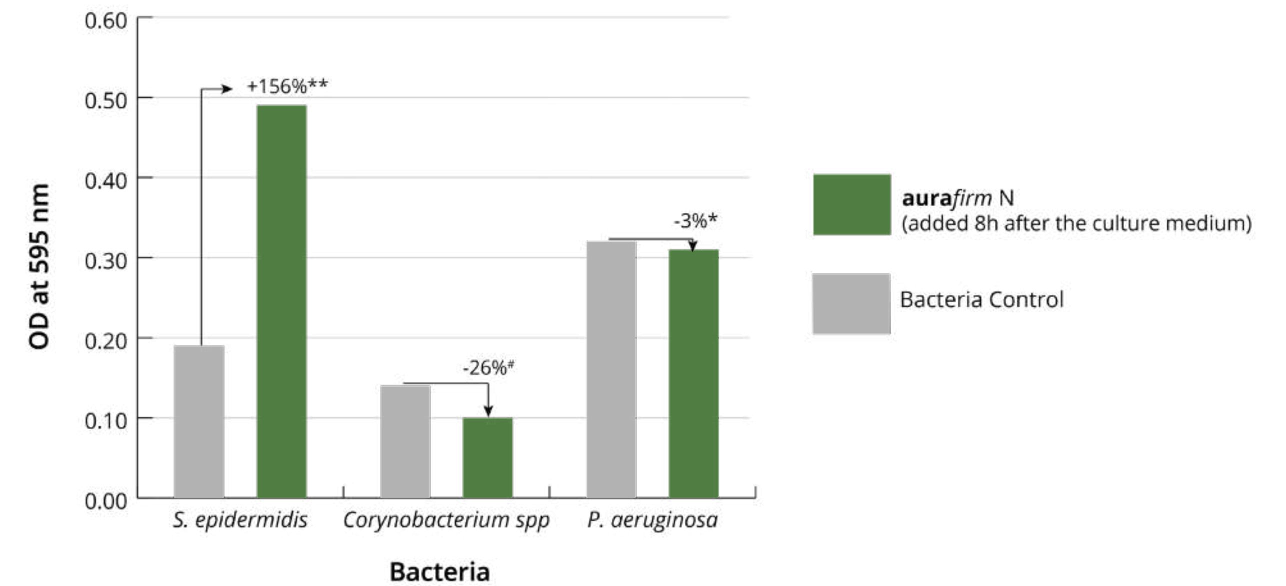
Significant: * = p<0.05 (95%), **= p<0.01 (99%), #= p<0.10 (90%)

Independently tested by Bio EC, June 2019. Full report available upon request.

Skin Microbiome: Bacterial Adhesion and Growth Study (In Vitro)



The results show that:

- Application of 1% **aura***firm* N induces a significant increase, +105%**, on *S. epidermidis* adhesion and on its growth, +156%**, after 24h.
- On *Corynobacterium ssp* (which represent 19% of microbes on human skin), **aura***firm* N induces a significant decrease on its adhesion, -49%#.
- On *P. aeruginosa*, **aura***firm* N induces a significant decrease on its adhesion, -26%*.

CONCLUSION

The results show that **aura***firm* N has a selective effect on the growth of gram positive and potentially 'pathogenic' bacteria. **aura***firm* N enhanced the adhesion effect and growth of *S. epidermidis* significantly yet hindered the bacterial adhesion and growth of *Corynobacterium* and *P. aeruginosa*, both considered as pathogenic when growth is not controlled.

Many studies show that common skin diseases are determined by an increase or a decrease of certain bacteria. For example, Psoriasis vulgaris is associated with a combined increase of Actinobacteria and decrease of *Staphyloccus epidermidis*[1]. Therefore, based on the above study **aura***firm* N would assist in helping to fight this skin disease, by rebalancing the skin microbiome.

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 **aura***firm* N will help to rebalance and improve the diversity of common skin bacteria.

Significant: * = p<0.05 (95%), **= p<0.01 (99%), #= p<0.10 (90%)

Independently tested by Bio EC, June 2019. Full report available on request.

BACKGROUND

The skin in its entirety can be defined as a unique ecosystem. Besides the physical barrier provided by the stratum corneum, the skin's resident microbiota represent the first line of defence against external aggression, guarantee protection and act as a biological barrier. Skin microbiota play an essential role in the maintenance of healthy skin.

Following in vitro analysis this study was performed to evaluate the ability of **aura***firm* N 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).

Executive Summary

aura*firm* N has been shown to be beneficial for the skin's natural microbiome

- Defends microbiota from external aggression
- Maintains skin's natural bacterial species' ratios
- Microbiome friendly
- Increases the presence of bacteria commonly lost through ageing
- Rebalances skin's natural pH

METHOD

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 **aura***firm* N on the whole diversity of skin microorganisms, identify major bacterial phylum and evaluate the ratio between major bacterial phylum (skin microbiota balance).

3 Caucasian women aged between 22 to 31, applied 2% **aura***firm* N on their forearms twice-daily (morning/evening) for 10 days. **aura***firm* N was diluted with distilled water in the laboratory of the test facility: test solutions were 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.

The bioinformatic analysis data was carry out by detection and elimination of chimeras and the clustering of sequences in Operational Taxonomic unit (OTU) at 97% homology.

Additionally, pH of the skin was measured for each sampling (Day 0, Day 1 and Day 10), indicating the effect of the test product on the skin microbiota recovery.

RESULTS

The effect of **aura***firm* N on the skin microbiome was analysed using three parameters: alpha diversity, genus profiling and change in skin pH.

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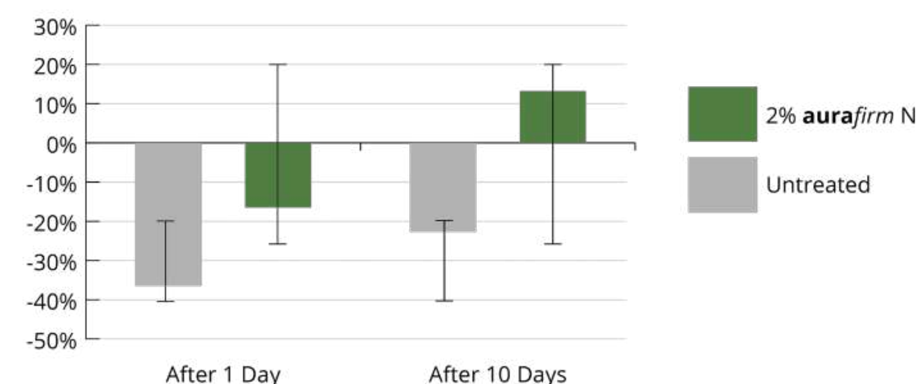
Alpha Diversity

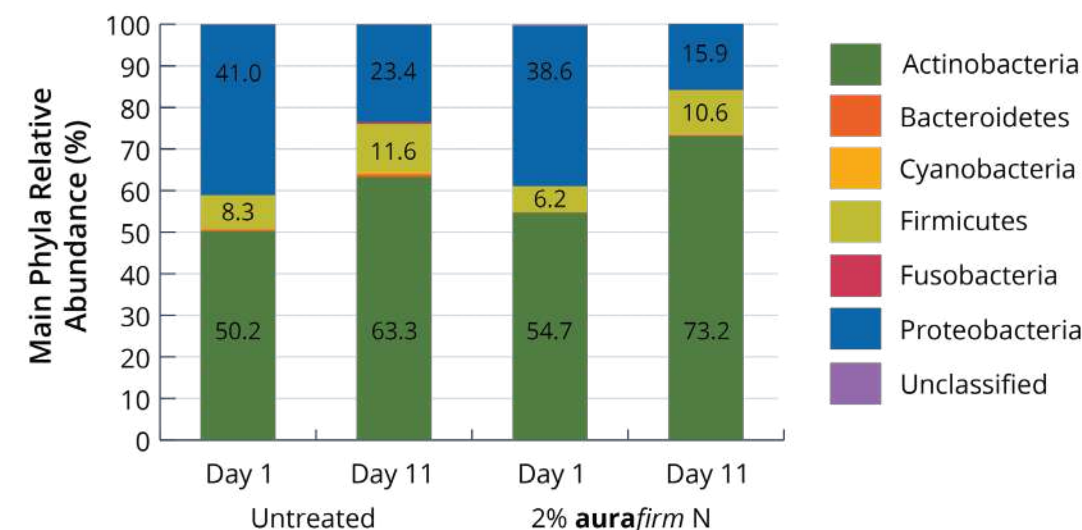


The Shannon index increases as both the richness (the number of species present) and the evenness (their relative abundances) of the community increase. It is well known that an increased biodiversity results in a healthy ecosystem, therefore an increase in Shannon index indicates a healthier skin microbiome[2].

Results show that, after 1 day skin microbiota diversity is reduced due to external aggression, however, with the application of **aura***firm* N, the reduction is less. After 10 days of application of **aura***firm* N, we observe an increase in the skin microbiota diversity.

2% aura*firm* N increases biodiversity of the skin microbiota.

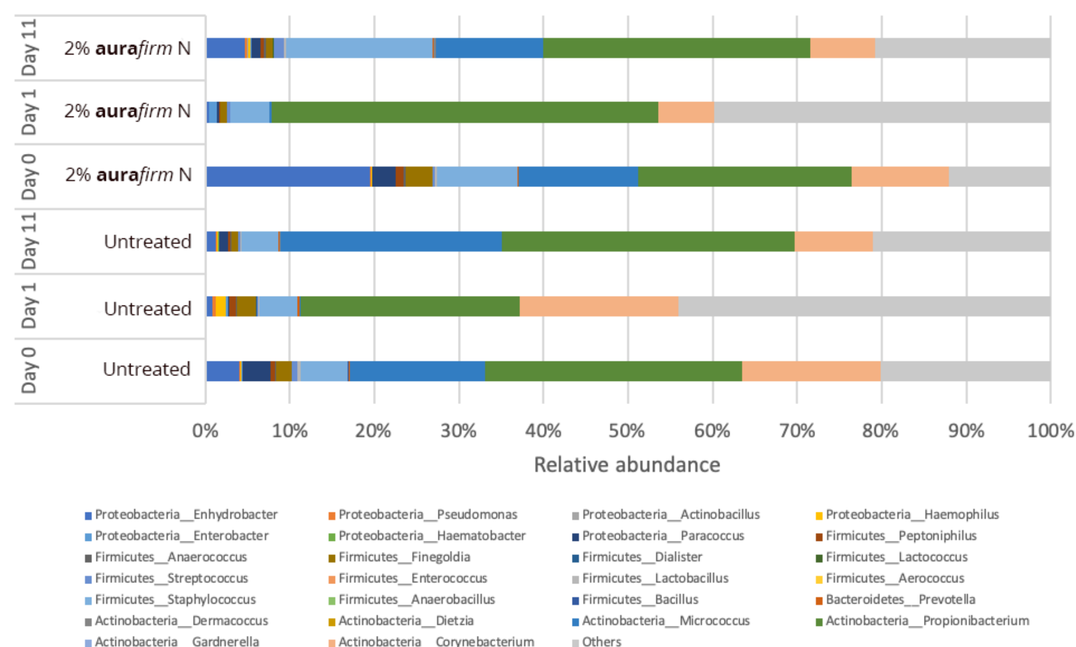
Genus Profiling



A balanced skin microbiota is characterised by high diversity of species and by an equilibrium in the quantitative ratios between the different bacteria. A closer look at the relative abundance of the main phyla present on the skin shows that application of **aura***firm* N does not induce a detrimental effect on the bacteria on the skin and can therefore be considered 'skin microbiome friendly'.

It is observed that the application of **aura***firm* N increased the Actinobacteria abundance by 34% in comparison to untreated, 26%. A reduction in Actinobacteria phyla is commonly associated with skin ageing, therefore by increasing its abundance, **aura***firm* N is protecting the skin against ageing, maintaining a balanced microbiome, avoiding dysbiosis and protecting the skin microbiome composition over time.[2]

Effect of aura*firm* N on the Composition of the Cutaneous Microbiota Over Time



After 10 days of application, 2% aura*firm* N helps to maintain the natural ratio of the skin microbiome and is ideal for daily use as a 'microbiome friendly' active. It also increases the presence of bacteria lost through ageing.

Skin pH

Healthy skin has a slightly acidic environment: this is on average between 5.0 and 5.5. The acidic environment is created by the hydrolipidic film, which provides a natural defence of the skin. A high 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's microbiome[3].

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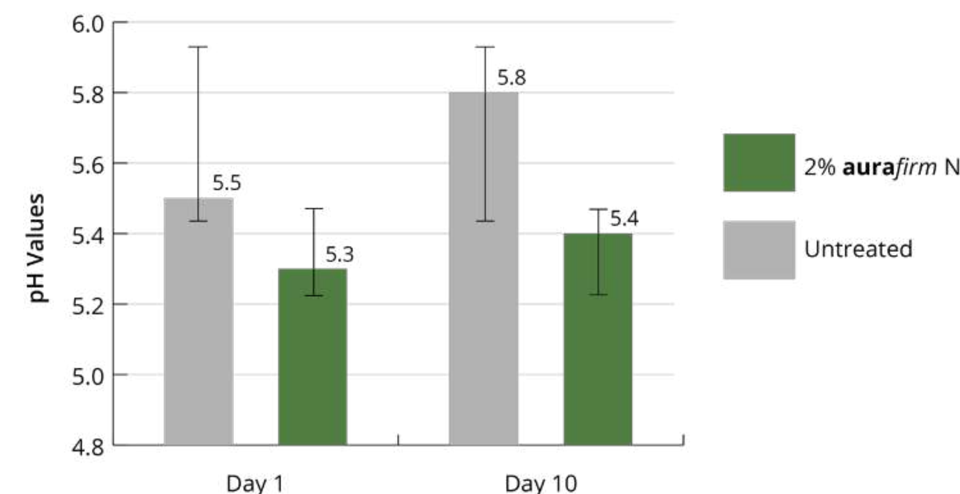
Oat cosmetics

Effect of aura*firm* N on Skin Microbiota (In Vivo)

Balance of the Skin pH



The results show that the use of 2% of **aura***firm* N maintains the skin pH better than untreated. This is in part due to lactic acid, a product of Lactobacillus fermentation, present in **aura***firm* P which helps rebalance the skin pH.

The results show that the use of 2% of aura*firm* N rebalances the skin pH and leads to a healthy skin microbiome.

CONCLUSION

Balancing the skin microbiota is essential to get healthier skin. It is due to microbiome disturbances that the skin experiences (oxidative) stress, and thus an increase in ageing parameters[2]. Taxonomic composition analysis on aged skin vs young skin shows a decrease in Actinobacteria abundance on the older skin[4].

Results show that the application of 2% **aura***firm* N provides favourable conditions for the microbiome. Lifestyle and environmental stress disturb the skin microflora, thus applying **aura***firm* N daily will promote a healthy balance of skin microbiome and help to increase the concentration of bacteria commonly lost through ageing.

BACKGROUND

An independent ex-vivo study was created with the aim of evaluating effects of **aura***firm* N on the epidermal and dermal structures of living human skin explants. Two specially designed finished products (a commercial anti-ageing cream and a commercial anti-ageing cream with retinol) were tested in the same way for comparative purposes, as well as a placebo.

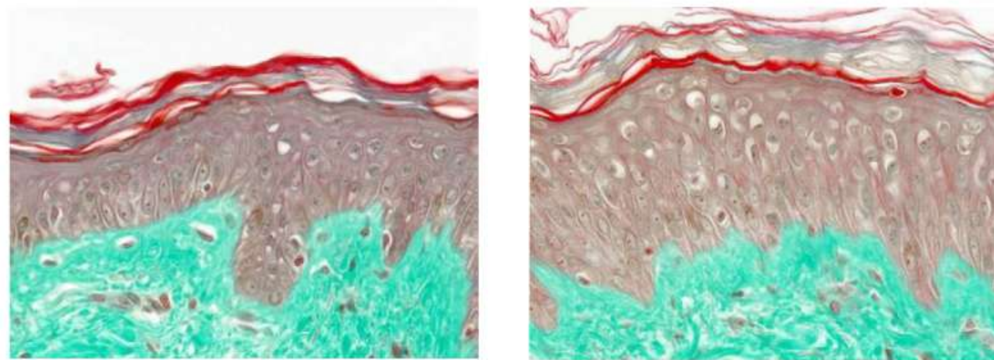
METHOD

An abdoplasty from a 40-year old woman was divided and prepared into 81 skin explants of an average diameter of 11mm and kept in survival in BEM culture medium.

aura*firm* N was added to a CMC aqueous gel and topically applied on the basis of 1mg per cm^2 to the explants using a small spatula on Day 0 (D0), D1 and D5. On D0 and D5 explants were collected and stored for analysis.

The general morphology study of epidermal and dermal structures was performed on slides stained according to the Trichome Masson Protocol.

RESULTS



Placebo   2% **aura***firm* N

5μm - thin histological cryo sections stained according to trichome masons protocol showing the stratum corneum.

Thanks to the images we can observe that Eosin, the acidic dye, binds keratins of the corneocytes which provides a strong red coloration. The red lines represent these dense layers of corneocytes. Staining is stronger at basal layers of the stratum corneum as layers are more compact and the pH is more basic.

Corneocytes are keratinocytes without nucleus in their last stage of differentiation and maturation. Corneocytes are interconnected with corneodesmosomes, forming the layers of the cells. The lack of layers above the red lines indicates that these layers have been desquamed, meaning that the process of detachment of external layers of corneocytes has taken place.

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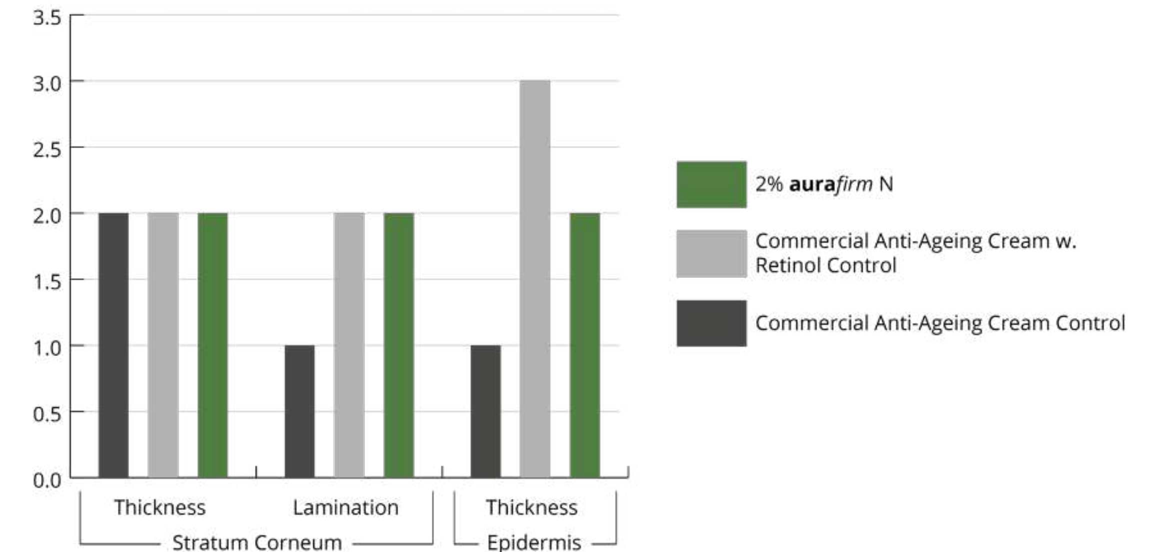
Independently tested by Bio EC, Mar 2018. Full report available on request.



In-depth analysis of the stratum corneum, in terms of thickness and lamination and the thickness of the epidermis provide the most accurate anti-ageing results. The day 5 results show that **aura***firm* N induced an increase in epidermal thickness (hypertrophic acanthosis – increase in size of the keratinocytes) which denotes skin firming and plumping properties.

aura*firm* N matched or outperformed the commercial anti-ageing cream control in all areas. **aura***firm* N matched the commercial anti-ageing cream with retinol control in all areas bar epidermal thickness.

Independently tested by Bio EC, Mar 2018. Full report available upon request.

BACKGROUND

An independent ex vivo study was created with the aim of evaluating the hydrating effects of **aura**_firm_ N on the epidermal and dermal structures of living human skin explants. A commercially available finished product (a commercial anti-ageing cream) was tested in the same way for comparative purposes, as well as a control.

METHOD

An abdoplasty from a 40-year old woman was divided and prepared into 81 skin explants of an average diameter of 11mm and kept in survival in BEM culture medium.

aura_firm_ N was compared as a single ingredient in a very basic preparation, with no skin penetration enhancers.

Hydration measurements were recorded after 3 hours and 24 hours by analysing the general morphology of the epidermal and dermal structures according to the Trichome Masson Protocol.

RESULTS

5μm - thin histological cryo sections stained according to trichome masons protocol showing the stratum corneum.

Thanks to the images we can observe that Eosin, the acidic dye, binds keratins of the corneocytes which provides a strong red coloration. The red lines represent these dense layers of corneocytes. Staining is stronger at basal layers of the stratum corneum as layers are more compact and the pH is more basic.

Corneocytes are keratinocytes without nucleus in their last stage of differentiation and maturation. Corneocytes are interconnected with corneodesmosomes, forming the layers of the cells. The lack of layers above the red lines indicates that these layers have been desquamed, meaning that the process of detachment of external layers of corneocytes has taken place.

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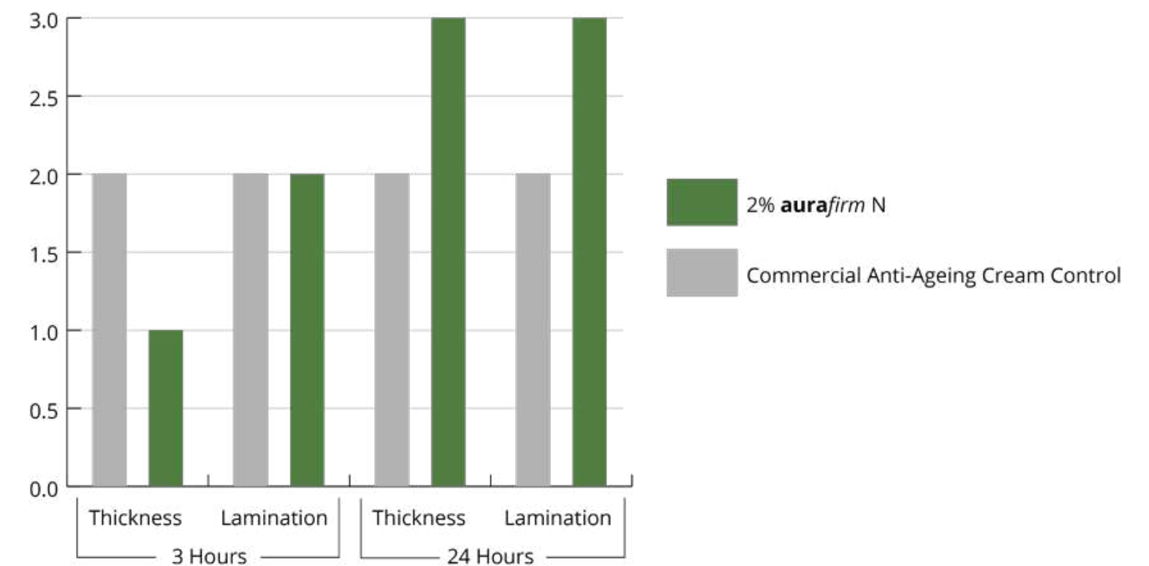

Hydration was measured through in-depth analysis of the stratum corneum, in terms of thickness and lamination and the thickness of the epidermis.

After 24 hours, **aura**_firm_ N had induced a moderate increase in epidermal thickness and lamination and outperformed the commercial anti-ageing cream product.

Independently tested by Bio EC, Mar 2018. Full report available upon request.

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Introduction Pg 1-2

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