aura*firm* N

DATA PACK

PROBIOTIC-LIKE ACTIVE WITH A SKIN FIRMING AND PLUMPING EFFECT

DCI: Revision No: 11 Issue Date: 14th July 2022

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CREDENTIALS

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INGREDIENT PROFILE

aura*firm* N is a prebiotic-like oat active with the ability to firm and plump up the skin. The key characteristics of this active ingredient include:

- Probiotic-like effect due to the presence of *Lactobacillus* bacteria
- Various bioactive compounds such as antioxidants and amino acids due to the benefits from the fermentation process
- Hydroxy carboxylic acids such as lactic acid which provides hydration

INTRODUCTION

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

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:

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

PROFILING

aura*firm* N is a mobile liquid which contains a low level of insoluble fermented oat bran which forms a cloudy suspension when shaken. **aura***firm* N contains a broad spectrum of water-soluble molecules produced by the fermentation process which conditions the skin and boosts microflora diversity. This product is ideal for low viscosity formulations.

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 **aura***firm* N are:

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 have the ability to hydrolyse the bond 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 prevents dehydration of the epidermis by maintaining the correct water balance, resulting in smooth and soft appearance of the skin.

PROBIOTICS

Live microorganisms when administered in adequate amounts confer a health benefit by strengthening the skin microbiome. Probiotic-like components in **aura***firm* N 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[2]. Data shows that heat-killed bacteria, their fractions, or purified components have probiotic effects with advantages over live probiotics.

	aura*firm* N
Lactobacillus* bacteria	130,000 bacterial count/ml

*Typical values may vary

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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] barrier recovery.

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 **aura***firm* N are:

AHAs:

Strains of *Lactobacilli* can produce α-hydroxy acids (AHAs) that exhibit pH-adjustments and antibacterial activity against most dermal pathogenic bacteria. AHAs can exfoliate the uppermost layer of the skin. AHAs 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.

	aura*firm* N
Organic acids*	5,000 mg/kg

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 supplies the skin with essential amino acids which protect skin from free-radical damage and reduce signs of ageing.

	aura*firm* N
Amino acids* *of which*	10,800 mg/kg
Glutamic acid*	2,200 mg/kg
Aspartic acid*	1,100 mg/kg
Proline*	700 mg/kg

Bioactive Peptides:

The fermentation process produces peptides that help with the cellular renewal of the skin. 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. Fermentation allows greater bioavailability of potentially protective and reparative molecules. This increase in bioavailability is achieved due to the breakdown of proteins 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.

*Typical values may vary

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BACKGROUND

Fourier–transform infrared spectroscopy (FT-IR) was used to profile and create a molecular 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.

RESULTS

Figure 1:
aura*firm* N **Infrared Spectrum**

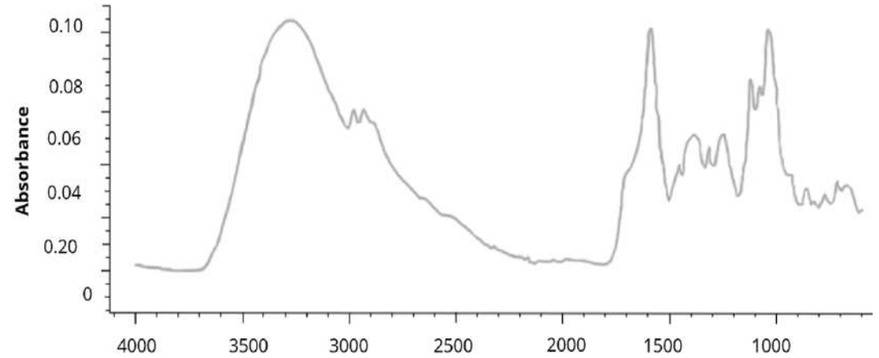

Figure 2:
Wavenumber Assignment of aura*firm* N **Infrared Spectrum**

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

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, Apr 2019.

*Typical values may vary

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Viability Of *Lactobacillus* Bacteria: Flow Cytometry

BACKGROUND

A study was performed to confirm the viability and count of 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 bind are bound to the *Lactobacillus* cells and the fluorescence intensity represents the count of the 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.

Figure 1:
***Lactobacillus* Bacteria in aura*firm* Grades**

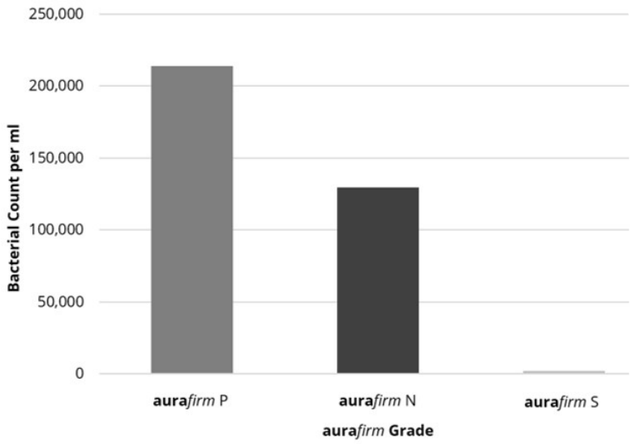
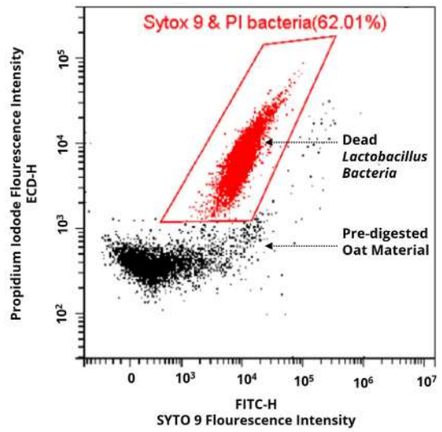


Figure 2:
Visualisation of *Lactobacillus* Bacteria in aura*firm* N



CONCLUSION

The results show that *Lactobacillus* bacteria, deactivated by the pasteurisation step at the end of the fermentation process, is present in all **aura***firm* grades. However, a clear distinction is seen between **aura***firm* N and the other **aura***firm* grades. **aura***firm* N's flow cytometry results indicate that 62% of all solid matter is *Lactobacillus* bacteria, meaning **aura***firm* N contains approximately 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.

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Concentration of *Lactobacillus* Bacteria: Confocal Microscopy

BACKGROUND

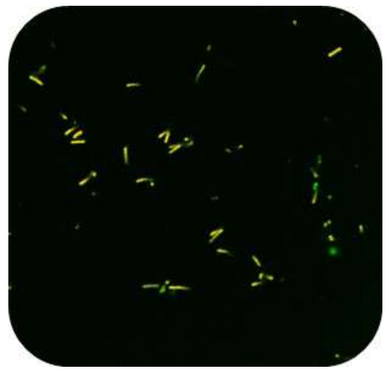
A study was performed to evaluate the concentration and viability of prebiotic *Lactobacillus* bacteria in **aura***firm* N under confocal microscopy.

METHOD

To monitor the viability of *Lactobacillus* bacteria in **aura***firm* N, 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

Figure 1:
Visualisation of the Viability of *Lactobacillus* Bacteria in aura*firm* N (20 µm)



The *Lactobacillus* bacteria are free flowing, which means that they are readily available when applied. Bacteria are confirmed to be dead but still intact. As a result, when applied to the skin, the effects would be similar as that of live bacteria.

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.

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MODE OF ACTION

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

BACKGROUND

This study was performed to understand how **aura***firm* N influences gene expression in the skin.

METHOD

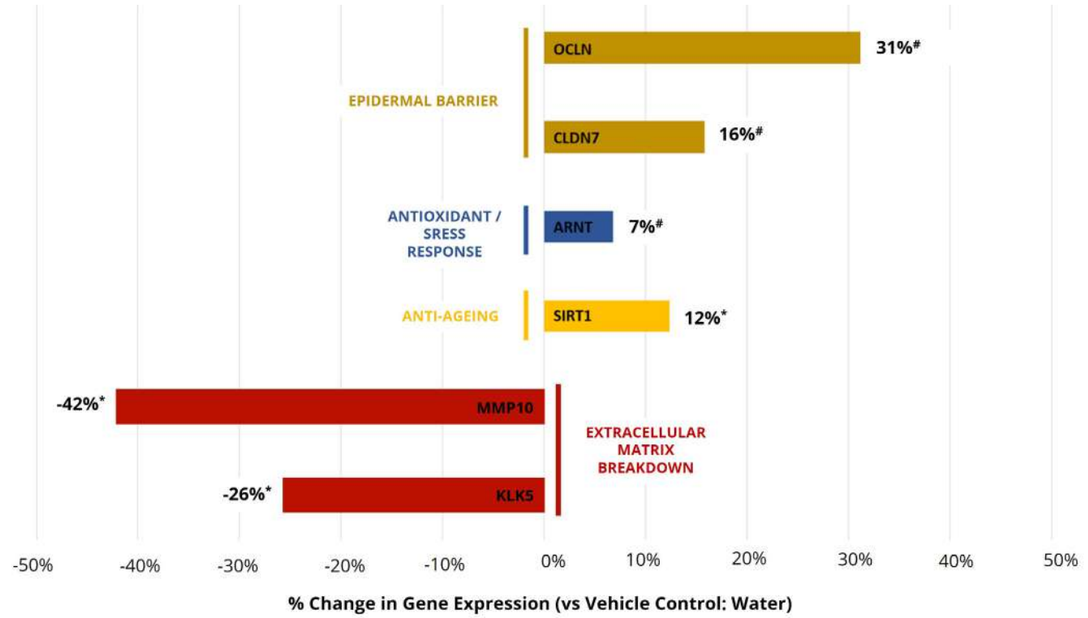
Tissue Model – A 3D in vitro skin model containing epidermal keratinocytes and dermal fibroblasts were used for the study.

Test Groups and Time Points - 2% **aura***firm* N diluted in water and water (vehicle control) were the two test material groups. After 48 hours of application of the test materials (after 24 hours, the tissue models were rinsed and treatment were reapplied), tissues 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. Each gene was measured in duplicate.

RESULTS

Figure 1:
Statistically Significant Changes in Gene Expression After Applying 2% aura*firm* N for 48 Hours



The results show that 2% **aura***firm* N increases the performance of genes that play an important role in skin biology.

Unpaired t-test, Significance *p<0.05 (95%) and #p<0.1 (90%), N=4

Independently tested by Genemarkers Mar 2022

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

Gene Function	Gene ID	Interpretation
Epidermal Barrier	CLDN7	Involves in the formation of tight junctions and maintains skin barrier integrity[1]
	OCLN	Improves tight junction stability and barrier function[1]
Antioxidant / Stress Response	ARNT	Induces anti-oxidant response and regulates inflammatory pathways
Anti-Ageing	SIRT1	Protects collagens from MMP9 degradation after UV exposure[2]
Extracellular Matrix Breakdown	KLK5	Downregulation = Regulates cell shedding (desquamation) (by protecting from degradation proteins which form the extracellular component of cell junctions in the stratum corneum)[3]
	MMP10	Downregulation = Inhibits degradation of collagens, elastin, fibronectin, and proteoglycans[4]

CONCLUSION

Topical application of 2% **aura***firm* N stimulated expression of genes involved in mitigating and preventing extracellular matrix breakdown and epidermal barrier mechanisms. This is extremely relevant when managing major signs of skin ageing:

- Strengthen skin barrier integrity (maintains epidermal barrier function)
- Skin firmness (prevents extracellular matrix breakdown)
- Skin hydration (regulates tight junctions' function and so water retention can occur)

EFFICACY ON SKIN

aura*firm* N is a probiotic-like active with a skin firming and plumping effect. This part of the data pack provides information on the studies performed to evaluate the efficacy of **aura***firm* N on the skin .

The studies demonstrated that **aura***firm* N displays anti-ageing effects by increasing the thickness of stratum corneum and epidermis resulting in improvement in skin firmness in the short term. These studies also confirm the hydrating properties resulting improvements in skin plumpness in the long term. It has also been proven efficient in maintaining the skin microbiome composition over time. **aura***firm* N would be ideal for skin with early to moderate photoageing.

Skin Microbiome:
Bacterial Adhesion and Growth

IN VITRO

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)
- *Corynebacterium spp.* (Actinobacteria - representing 52% of skin bacteria)
- *Pseudomonas aeruginosa* (Proteobacteria - representing 16% of skin bacteria)

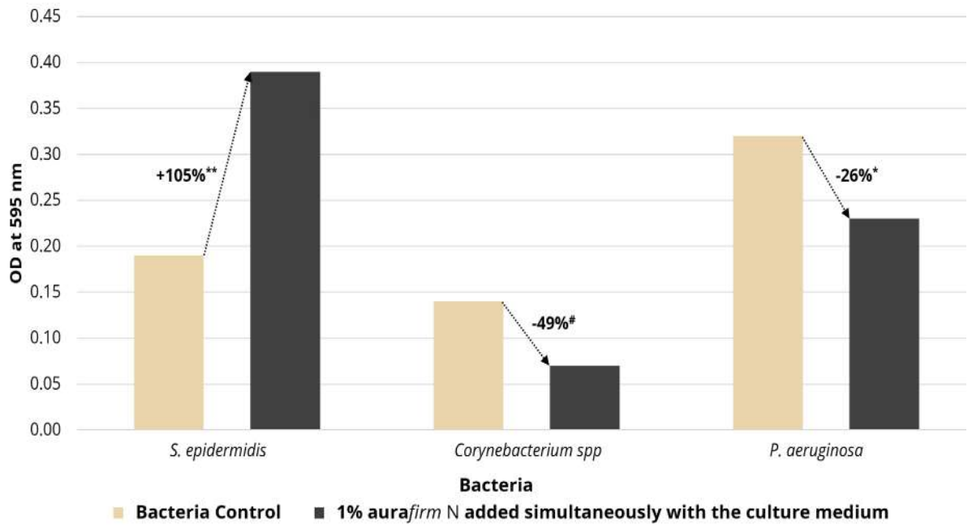
A culture medium with known quantity of each bacterial community (colony-forming unit, CFU/ml) was added to the wells of the 3 plates as well as **aura***firm* N, at a concentration of 1% (diluted with culture media), this addition corresponded to the following conditions:

- 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

Figure 1:
Effect of aura*firm* N on Bacterial Adhesion After 24 Hours



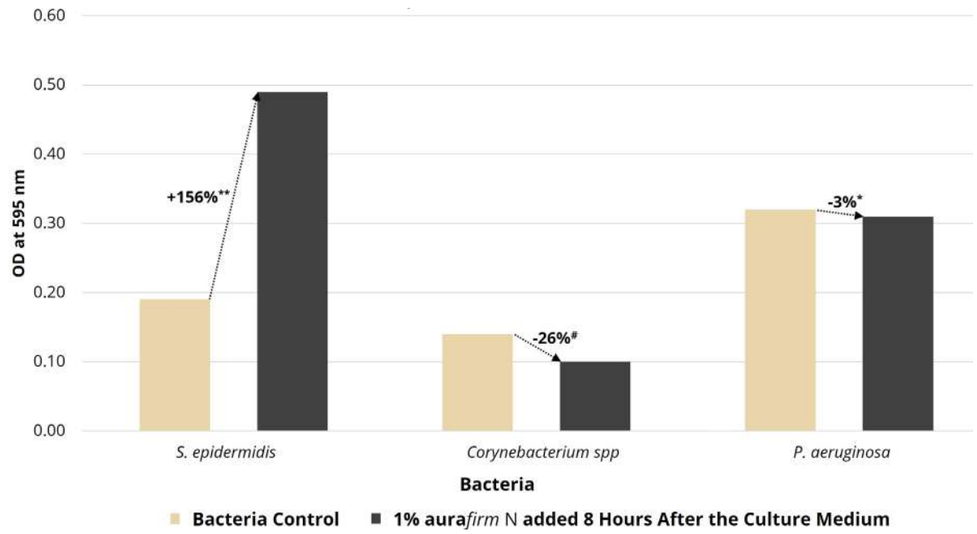
Significant: **= p<0.01 (99%), * = p<0.05 (95%), #= p<0.10 (90%)

Skin Microbiome:
Bacterial Adhesion and Growth

IN VITRO

RESULTS (CONT.)

Figure 2:
Effect of aura*firm* N on Bacterial Growth After 24 Hours



The results show that:

- Application of 1% **aura***firm* N induces a significant increase by 105%** on *S. epidermidis*' adhesion and by 156%** on its growth, after 24 hours.
- Application of 1% **aura***firm* N induces a significant decrease by 49%# on *Corynebacterium spp.* adhesion, after 24 hours.
- Application of 1% **aura***firm* N induces a significant decrease by 26%*on *P. aeruginosa* adhesion, after 24 hours.

CONCLUSION

The results show that **aura***firm* N has a selective effect on the growth of gram-positive and potentially 'pathogenic' bacteria. aurafirm N enhanced the adhesion effect and growth of *S. epidermidis* significantly yet hindered the bacterial adhesion and growth of *Corynebacterium* and *P. aeruginosa*, both considered 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 a 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<0.01 (99%), * = p<0.05 (95%), #= p<0.10 (90%)

Independently tested by Bio EC, Jun 2019.

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Effect of aura*firm* N on Skin Microbiota

IN VIVO

BACKGROUND

The skin in its entirety can be defined as a unique ecosystem. Besides the physical barrier provided by the stratum corneum, the resident microbiota of the skin represents the first line of defence against external aggression, guarantees protection and acts as a biological barrier. Skin microbiota plays an essential role in the maintenance of healthy skin. Following the 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).

METHOD

Product Treatment

3 Caucasian women, aged between 22 to 31, applied 2% **aura***firm* N on their forearms twice-daily (morning/evening) for 11 days.

Assessment of Skin Microbiome

DNA Extraction - 2% **aura***firm* N was diluted with distilled water in the laboratory of the test facility. The 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.

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 **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).

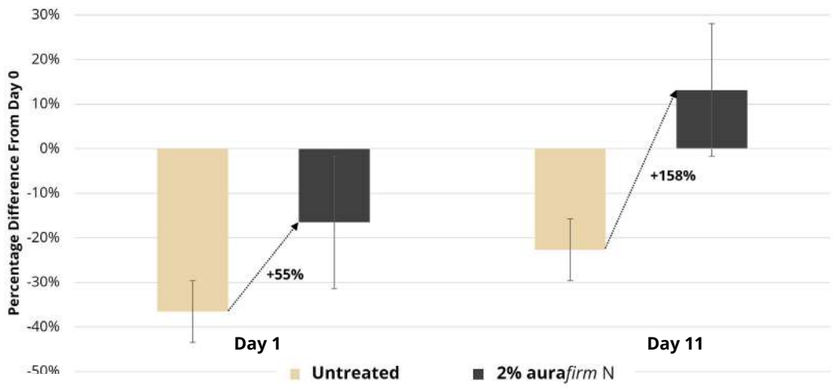
Data Analysis - The bioinformatic analysis data was carried out by detection and elimination of chimeras and the clustering of sequences in Operational Taxonomic unit (OTU) at 97% homology.

Measurement of Skin pH

Additionally, pH of the skin was measured using a pH meter, at Day 1 and Day 11, indicating the effect of the test product on the skin microbiota recovery.

RESULTS: ALPHA DIVERSITY

Figure 1:
Evolution of Alpha Diversity (Shannon Indexes)



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Effect of aura*firm* N on Skin Microbiota

IN VIVO

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 microbiome[2]. Results show that, after Day 1, skin microbiota diversity is reduced due to external aggression, however, with the application of **aura***firm* N, the reduction is less. After 11 days of application of **aura***firm* N, we observe an increase in the skin microbiota diversity.

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

RESULTS: GENUS PROFILING

Figure 2:
Protection of Skin Microbiota Balance with Application of aura*firm* N

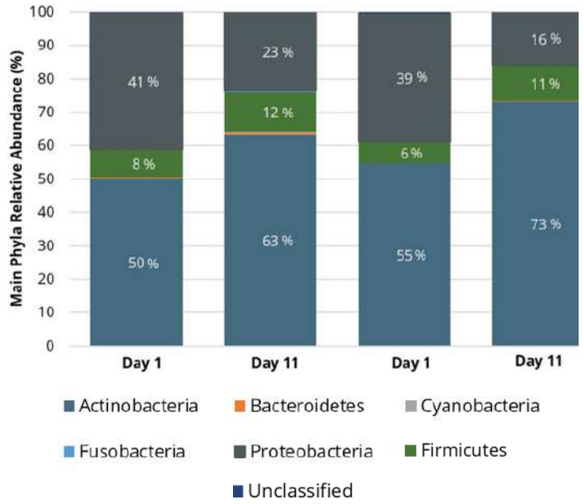
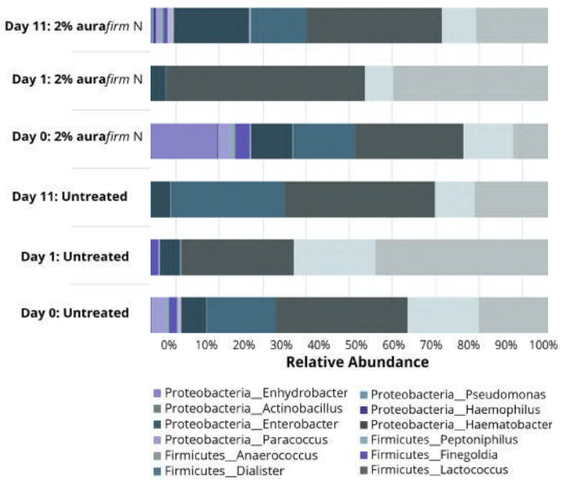


Figure 3:
Effect of aura*firm* N on the Cutaneous Microbiota Over Time



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 2% **aura***firm* N does not induce a detrimental effect on the bacteria on the skin and can therefore be considered 'skin microbiome friendly'. It was observed that the application of 2% **aura***firm* N increased the Actinobacteria abundance by 34% in comparison to untreated, 26%. A reduction in Actinobacteria phylum is commonly associated with skin ageing, therefore by increasing its abundance, 2% **aura***firm* N protects the skin against ageing, maintains a balanced microbiome by avoiding dysbiosis and protects the skin microbiome composition over time[2]. After 11 days of application, 2% **aura***firm* N helps in maintaining the natural ratios of the skin microbiome and is ideal for daily use as a 'microbiome-friendly' active.

2% aura*firm* N increases the presence of bacteria lost through ageing.

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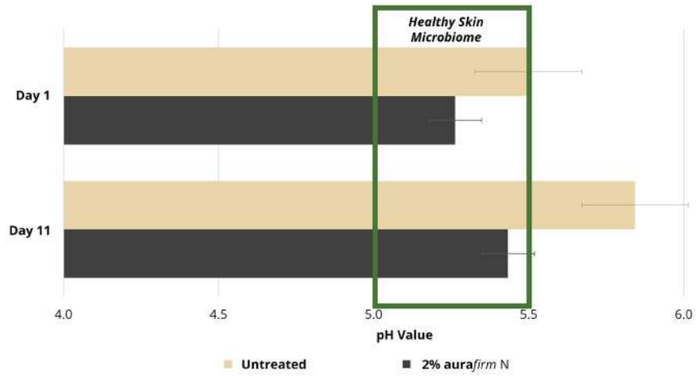
Effect of aura*firm* N on Skin Microbiota

IN VIVO

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 acts as a natural defence for the skin. A high pH leads to barrier dysfunction and a favourable environment for the growth of *S. aureus* and *S. pyogenes,* contributing to dysbiosis of the skin's microbiome[3].

Figure 4:
Balance of the Skin pH



The results show that the use of 2% **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* N which helps in rebalancing the skin's pH.

2% aura*firm* N rebalances the skin pH and leads to a healthy skin microbiome.

CONCLUSION

Balancing the skin microbiota is essential for getting 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 2% **aura***firm* N daily will promote a healthy balance of skin microbiome and help in increasing the concentration of bacteria commonly lost through ageing.

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EX VIVO

Anti-Ageing: Epidermal Structure Improvement

BACKGROUND

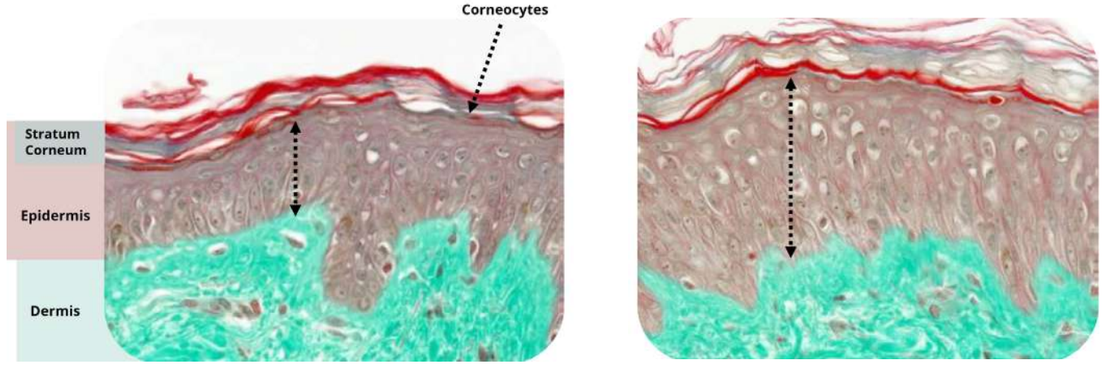
An independent ex vivo study was carried out to evaluate the effects of **aura***firm* N on the epidermal and dermal structures of living human skin explants. Two specially designed finished products (a commercial anti-ageing cream and a commercial anti-ageing cream with retinol) were tested in the same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

METHOD

An abdoplasty from a 40-year-old woman was divided and prepared into 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 based on 1mg per cm^2 to the explants using a small spatula on Day 0 (D0), Day 1 (D1) and Day 5 (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

Figure 1:
Microscopical Observation of Skin Morphology With and Without aura*firm* N



Untreated At Day 0 **2% aura*firm* N At Day 5**

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 colouration. 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 a 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 desquamated , meaning that the process of detachment of external layers of corneocytes has taken place.

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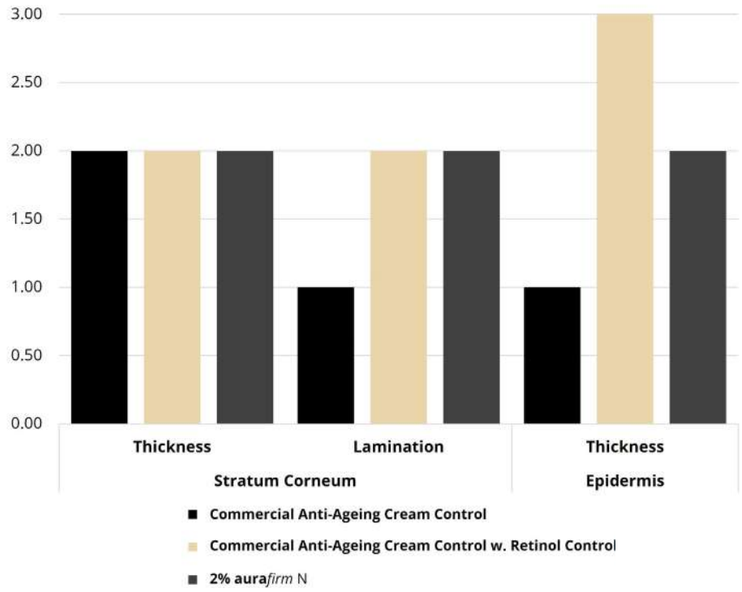
RESULTS (CONT.)

Figure 2:
Improvement in Skin Morphology At Day 5



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. Results on Day 5 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.

CONCLUSION

The increase in epidermis thickness and stratum corneum thickness and lamination confirms **aura***firm* N anti-ageing activity. As demonstrated in 'Genes Modulation and Biological Activity' , **aura***firm* N regulates the genes involved in the regulation of the extracellular matrix. Studies have demonstrated that stratum corneum thickening is due to the inhibition of KLK5[1].

BACKGROUND

As we age, the dermis layer starts to thin and loses its elasticity. This is the natural process of skin ageing, where less skin cells are created and renewed. The skin's production of elastin and collagen, proteins that help maintain skin elasticity and firmness, decreases. This means the skin becomes less supple and firm, and therefore more likely to wrinkle or cause fine lines. To follow, 'Anti-Ageing Activity: Epidermal Structure Improvement' (which confirms the increase in the thickness of the epidermis and stratum corneum), this study was designed to assess the ability of **aura***firm* N to improve skin firmness, over 7 days, in comparison to a placebo.

METHOD

Product treatment

Over 7 days, 15 Caucasian women, aged between 40 to 68 years old, with mixed type of skin (sensitive and non-sensitive) applied 2% **aura***firm* N in a facial cream and a placebo facial cream on their forearms. The application was carried out twice a day, both morning and evening. Skin measurements were taken at Day 0 (before application of the treatment) and at Day 7.

Measurement of Skin Firmness

A measurement of the firmness of the skin surface was performed by cutometer dual 580 MPA on the forearms area (negative pressure under 450mbar). By applying a pressure in the plane perpendicular to the surface, it produces a mechanical deformation. The ability of the skin to resist suction, represents the firmness of the skin (parameter F4). The smaller F4, the more the skin resists the suction (the more the skin is firm).

The following formulation was used in this study:

Phase	Trade Name	INCI Name	%w/w
A	Purified Water BP	Aqua	74.25
A	Amaze Nordic Barley	*Hordeum Vulgare* Powder	5.00
A	Sodium Gluconate Food Grade USP	Sodium Gluconate	0.10
B	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.00
C	Surfac MCTG	Caprylic/Capric Triglyceride	12.50
C	Cetiol MM	Myristyl Myristate	4.00
C	Cutina GMS V	Glyceryl Stearate	0.80
C	Carnauba Wax SP-63	Copernicia Cerifera Wax	0.35
D	**aura***firm* N	**Aqua, *Avena Sativa* (Oat) Kernel Extract, *Lactobacillus* Ferment, Sodium Benzoate, Potassium Sorbate**	**2.00**

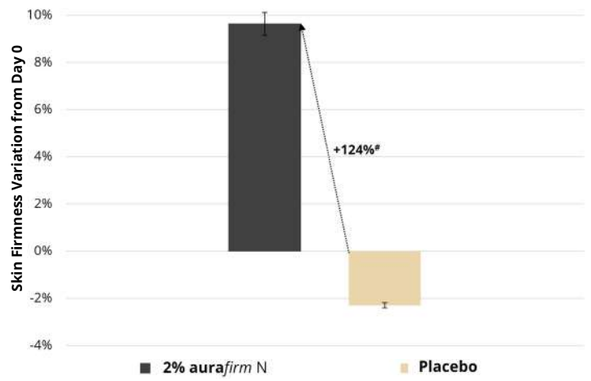
*Placebo formulation was identical minus 2% **aura***firm* N – remaining % was made up with water.

Skin Firmness

IN VIVO

RESULTS

Figure 1:
Improvement in Skin Firmness At Day 7



After 7 days of application, 2% **aura***firm* N significantly increases skin firmness by 10% compared to Day 0. Results showed skin firmness increase of up to 37% for one participant.

CONCLUSION

aura*firm* N improves skin firmness after 7 days of treatment by preventing sagging of the skin. The improvement in skin flexibility is due to the increase in the thickness of the epidermal skin layers, as demonstrated in 'Anti-Ageing: Epidermal Structure Improvement'.

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Significant: #= p<0.1 (90%)

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Skin Hydration

EX VIVO

BACKGROUND

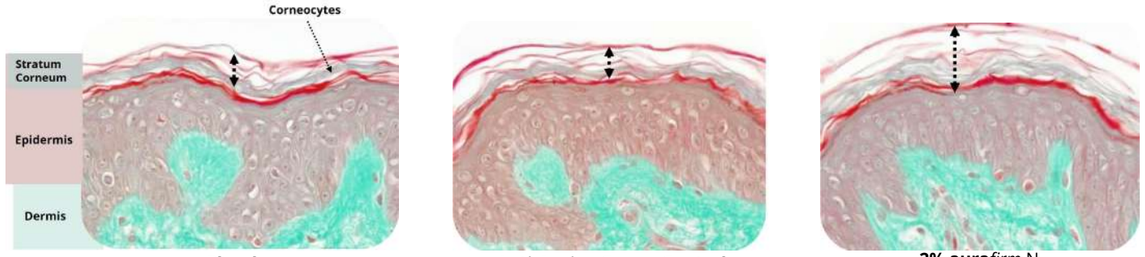
An independent ex vivo study was created with the aim of evaluating the hydrating effects of **aura***firm* N on the epidermal and dermal structures of living human skin explants. A commercially available finished product (a commercial anti-ageing cream) was tested in the same way for comparative purposes,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

METHOD

An abdoplasty from a 40-year old woman was divided and prepared into skin explants of an average diameter of 11mm and kept in survival in BEM culture medium. 2% **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

Figure 1:
Microscopical Observation of Skin Morphology Without aura*firm* N**, With Anti-Ageing Cream Control and With aura***firm* N:**



Placebo
After 24 Hours

Anti-Ageing Cream Control
After 24 Hours

2% aura*firm* N
After 24 Hours

Corneocytes are keratinocytes without a 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 desquamated, meaning that the process of detachment of external layers of corneocytes has taken place.

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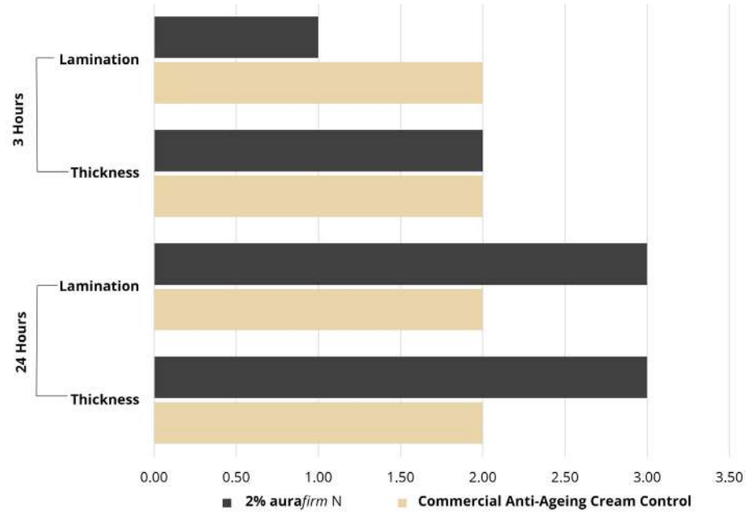
RESULTS

Figure 2:
Improvement in Skin Morphology After 3 Hours and 24 Hours



Hydration was measured through an 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 induced a moderate increase in epidermal thickness and lamination and outperformed the commercial anti-ageing cream product.

CONCLUSION

The increase in stratum corneum thickness and lamination confirms hydrating properties of **aura***firm* N. As demonstrated in 'Gene Modulation and Biological Activity', **aura***firm* N regulates genes involved in the improvement of the integrity of the tight junctions which contribute to the skin barrier function. This function allows the maintenance of hydration of the skin through the regulation of tight junctions by improving water retention.

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BACKGROUND

As we age, the skin loses volume, and skin complexion begins to look dull and tired. The skin becomes drier, which contributes to a less plump look and can make age-related issues more visible. To follow the 'Skin Hydration study', this study was designed to assess the ability of **aura***firm* N to improve skin plumpness, over 28 days, in comparison to a placebo.

METHOD

Product Treatment

Over 28 days, 22 Caucasian women, aged between 40 to 68 years old, with mixed type of skin (sensitive and non-sensitive), applied 2% **aura***firm* N in a facial cream and a placebo facial cream on their forearms. The application was carried out twice a day, both morning and evening. Skin measurements were taken at day 0 (before application of the treatment), then at Day 7 and at Day 28.

Assessment of Skin Plumping Effect

The Plumping effect was evaluated by the dermatologist on a 4-points scale. The scale runs from grade 0 to grade 3. Grade 0 represents the absence plumping effect and grade 3 represents a higher plumping effect.

Subjective Evaluation

The acceptability of the products was measured using a subjective evaluation of the efficacy, after the application of the products .

Dermatological Assessment of Tolerance

A dermatological assessment was performed at Day 28, to evaluate possible adverse symptoms that could appear with the use of 2% **aura***firm* N.

The following formulation was used in this study:

Phase	Trade Name	INCI Name	%w/w
A	Purified Water BP	Aqua	74.25
A	Amaze Nordic Barley	*Hordeum Vulgare* Powder	5.00
A	Sodium Gluconate Food Grade USP	Sodium Gluconate	0.10
B	Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.00
C	Surfac MCTG	Caprylic/Capric Triglyceride	12.50
C	Cetiol MM	Myristyl Myristate	4.00
C	Cutina GMS V	Glyceryl Stearate	0.80
C	Carnauba Wax SP-63	Copernicia Cerifera Wax	0.35
D	**aura***firm* N	**Aqua, *Avena Sativa* (Oat) Kernel Extract, *Lactobacillus* Ferment, Sodium Benzoate, Potassium Sorbate**	**2.00**

*Placebo formulation was identical minus 2% **aura***firm* N – remaining % was made up with water.

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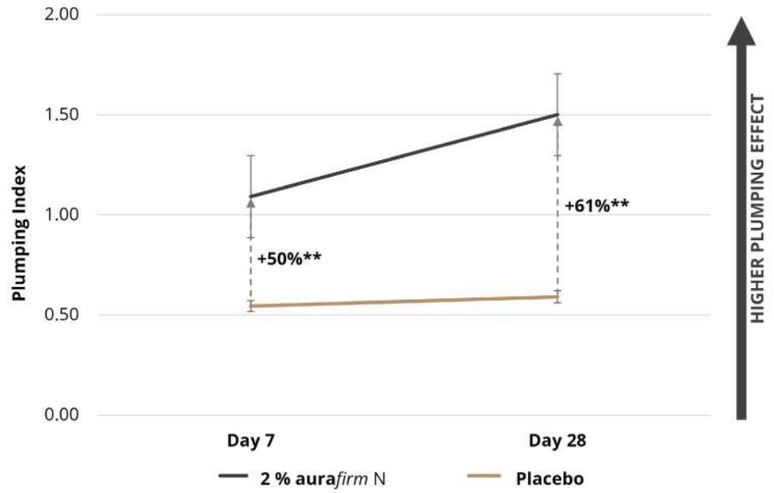
IN VIVO

RESULTS: SKIN PLUMPING EFFECT

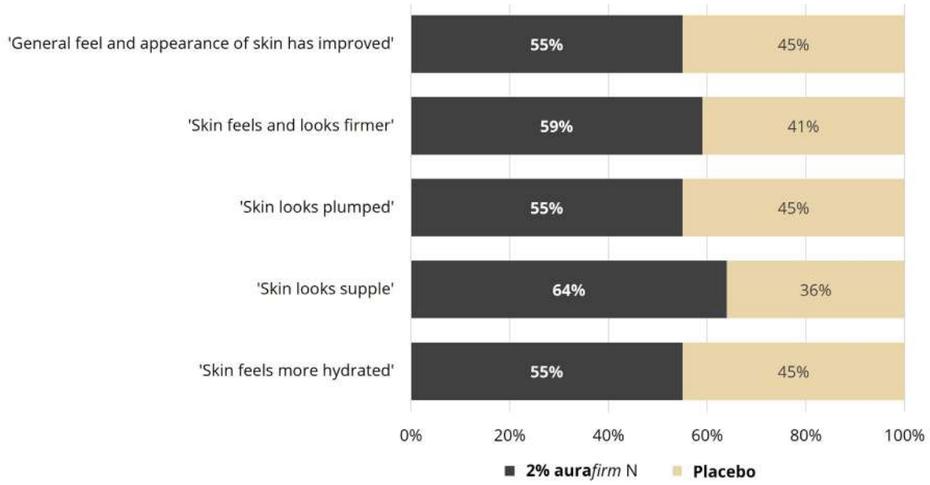
Figure 1:
Improvement in Skin Plumping At Day 28



After 7 days of continuous use, the treatment with 2% **aura***firm* N provide a significant plumping effect of 50% higher than the placebo treatment. And after 28 days of continuous use, the treatment with 2% **aura***firm* N provided a significant plumping effect which was 61% higher than the placebo treatment .

RESULTS: SUBJECTIVE EVALUATION

Figure 2:
Subjective Evaluation of the Efficacy of 2% aura*firm* N **At Day 28**



The consumer perception study shows that with 2% **aura***firm* N, 55% of the participants noticed that the general feel and appearance of their skin has improved.

(Significant: **=p<0.01 (99%))

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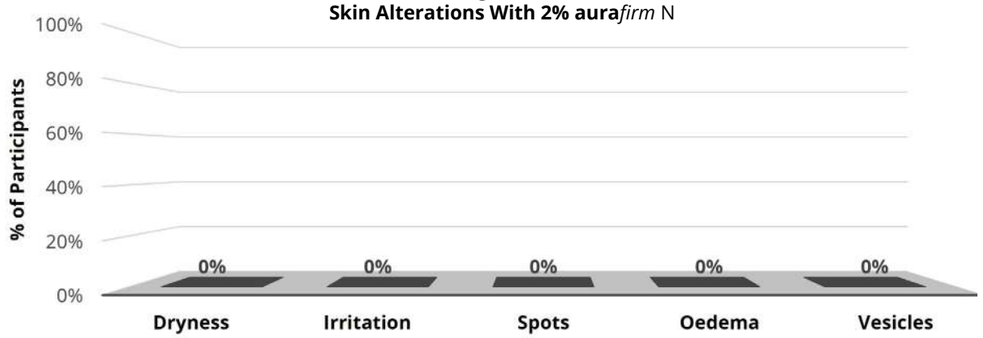
Skin Plumpness

IN VIVO

RESULTS: DERMATOLOGICAL TOLERENCE

Figure 3:
Skin Alterations With 2% aura*firm* N



After 28 days of use of 2% **aura***firm* N, none of the participants had a feeling of skin dryness, skin irritation, or the appearance of spots, or the sensation of oedema.

CONCLUSION

aura*firm* N improves skin plumpness in short-term as well as in the long-term. Due to the plumping effect, the skin appears less tired and more youthful.

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Consumer Perception Evaluation

BACKGROUND

A study was undertaken to assess the perception of **aura***firm* N amongst a group of consumers.

METHOD

Perception evaluation was carried out with a group of 22 Caucasian Women, aged between 40 to 68 years old. Participants were provided with 2% **aura***firm* N in a formulation to apply, twice a day (morning and evening) for 28 days. At the end of the 28-day study, participants completed an evaluation form where they scored the performance of the product with the following perception statements:

- 'Preventing signs of ageing as part of my everyday skincare routine is important for me'
- 'Using natural skincare products is important for my skin wellness'
- 'Improved look and feel of my skin helps to improve my self-esteem'

RESULTS

Perception Statement: 'Preventing signs of ageing as part of my everyday skincare routine is important for me'

Completely Agree	Agree	Neither Agree nor Disagree	Disagree	Completely Disagree
77%	23%	0%	0%	0%

Perception Statement: 'Using natural skincare products is important for my skin wellness'

Completely Agree	Agree	Neither Agree nor Disagree	Disagree	Completely Disagree
63%	32%	5%	0%	0%

Perception Statement: 'Improved look and feel of my skin helps to improve my self-esteem'

Completely Agree	Agree	Neither Agree nor Disagree	Disagree	Completely Disagree
68%	32%	0%	0%	0%

CONCLUSION

The consumer perception study shows that after 28 days of product use, 100% of the participants perceived that preventing signs of ageing as part of their everyday skincare routine is important for them. 95% of participants perceived that using natural skincare products is important for their skin wellness and texture. 100% of participants perceived that their self esteem increases with the improvement in the look and feel of their skin.

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CREDENTIALS

We conclude the data pack with the following credentials of our active fermented oat ingredient. The HRIPT test has demonstrated **aura***firm* N's hypoallergic and non-irritant capacity.

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Human Repeat Insult Patch Test

IN VIVO

BACKGROUND

A Human Repeat Insult Patch Test (HRIPT) was carried out to determine the cutaneous irritation (contact dermatitis) and sensitisation (contact allergy) potential of **aura***firm* N 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, and 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 test subject (the participant) 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, **aura***firm* N was applied at 100%.

RESULTS

aura*firm* N 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

aura*firm* N 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".

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aura*firm* Profile Pg 1-6

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Appendix

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

Gene ID	Gene Name	Associated Function(s) in Skin
ARNT	Aryl hydrocarbon receptor	Antioxidant / Stress Response
CLDN7	Claudin 7	Epidermal Barrier
KLK5	Kallikrein related peptidase 5	Extracellular Matrix Breakdown
MMP10	Matrix metallopeptidase 10	Extracellular Matrix Breakdown
OCLN	Occludin	Epidermal Barrier
SIRT1	Sirtuin 1	Anti-Ageing

GET IN TOUCH

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

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