sofw journal
Home & Personal Care Ingredients & Formulations
powered by SOFW

Can Fermentation "Preserve" the Skin?

S. Hettwer, E. Besic Gyenge, B. Suter, B. Obermayer

Can Fermentation "Preserve" the Skin?

S. Hettwer, E. Besic Gyenge, B. Suter, B. Obermayer

abstract

Fermentation preserves food by displacing unwanted microorganisms. Does it work in the same way with skin? Fermented products are becoming increasingly popular in the food sector. Although it is an ancient process for preserving food, fermentation is a very modern topic, including in the cosmetics industry. It is known that certain lactic acid bacteria such as *Lactobacillus sakei* can positively affect atopic dermatitis (taken orally) [1]. Here we show that millet ferment, produced with lactic acid bacteria from sourdough production, can also positively influence atopic skin when applied topically. We demonstrated that bacteria of a healthy skin microbiota can gain a growth advantage and thus lead the skin out of dryness stress. A dermatological assessment of the atopic condition showed a rapid reduction of symptoms even compared to placebo-treated areas.

Introduction

Of course, we cannot ferment our skin, but we can explore the question of why fermentation is a powerful tool to influence the microbiota. Fermentation is one of the oldest preservatives used by humans to preserve food. Microorganisms play a crucial role in this process. Today, fermentation is believed to have played a crucial role in the settlement of humans in the Neolithic period. Initially, cereals were not processed into bread, as was assumed for a long time, but fermented into a beer-like beverage. Here, yeast took over the task of fermentation by converting the starch from the grain into sugar and then fermenting it into alcohol [2]. It was only in times of food abundance that man was able to experiment with other fermentation methods. One of the first is probably the production of sourdough, which is still used today in bread baking and makes the bread more digestible, longer fresh and durable. The oldest sourdough bread found so far is almost 6000 years old and comes from Switzerland [3]. The sourdough uses lactic acid bacteria and yeasts that occur naturally on the cereals. The focus here is not on alcoholic fermentation, but on heterofermentative lactic acid fermentation **(Figure 1)**. In this process, sugars are fermented to carbon dioxide, acetic acid, lactic acid and ethanol. The shelf life is determined less by the alcohol than by the reduction of the pH value. Hence the name "sourdough". A low pH value ensures an environment in which only very few bacteria and virtually no mold can grow. The advantage is that the lactic acid bacteria are not harmful to humans. They colonize the entire "habitat" and displace other bacteria or no longer allow the new settlement of undesirable bacteria. In addition, lactic acid bacteria are probiotic, i.e. they are healthy for the intestinal flora and positively influence the human immune system [4]. It is conceivable that heterofermentative lactic acid fermentation found its way into vegetable fermentation through the knowledge of sourdough production. It is heterofermentative, here e.g. cabbages are preserved (sauerkraut, kimchi) but also carrots or cucumbers.

The yeast fermentation of berries, here in particular the grape berry, to an alcoholic beverage (wine) enabled a subsequent fermentation, namely the acetic acid fermentation. Although this is somewhat simple, it is also delicate, since special hygiene precautions must be taken to prevent acetic acid fermentation from spreading to a winemaker's wine stocks. However, the preservative properties of acetic acid are undisputed and are the basis for e.g. sour pickles and even sour roast, but of course also vinegar as a seasoning.

With the cultivation of dairy cattle and a surplus of milk, the possibility for another form of lactic acid fermentation was discovered: homofermentative lactic acid fermentation. Here, the sugars are fermented only to lactic acid. Other fermentation products do not occur because specific lactic acid bacteria strains that enter the milk from the animal's udder. These strains have a different metabolism than the heterofermentative lactic acid bacteria. Until the discovery of bacteria and microorganisms in the 17th century and thereafter, everything depended on experience in fermenting natural products. In modern food production we can precisely comply with all conditions to arrive at the perfect product. For generations, stable lactic acid bacteria communities have been cultivated in sourdough production. These could be identified using modern genome sequencing methods. Thus, today it is possible to achieve the same result every time with these cultures. However, instead of fermenting only rye and other cereals, today we have all the possibilities to use any plant material containing starch or sugar. Therefore, traditionally used botanical ingredients that have been used in cosmetics for decades can

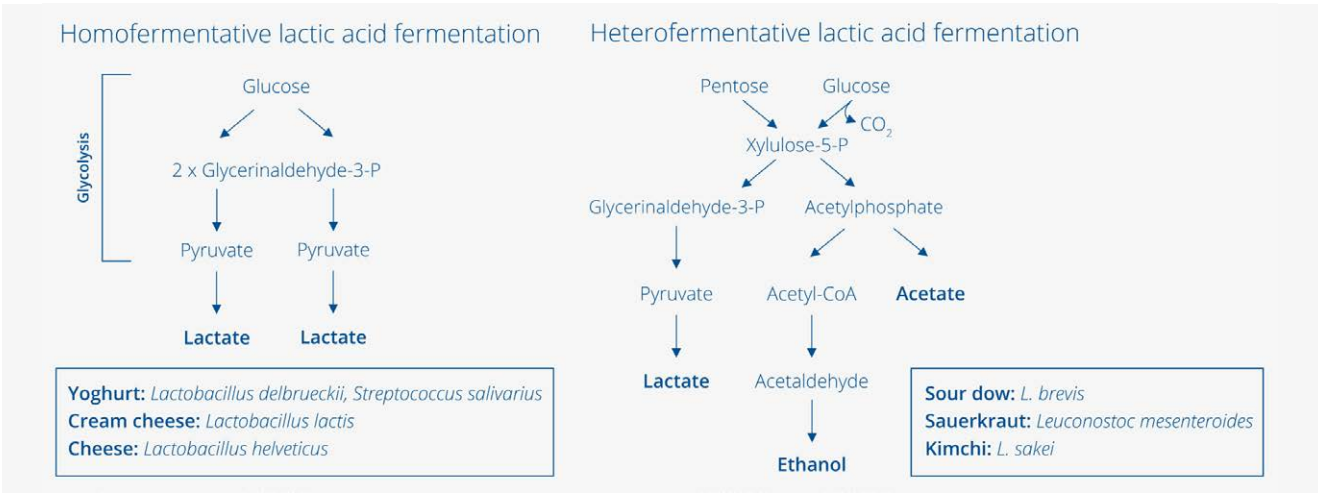

Fig. 1 Homo and hetero fermantative lactic acid fermentation in summary.

be lifted to the next level. An example of this is the golden millet *Panicum miliaceum*, which is particularly promoted for strengthening keratin structures (hair, fingernails), but has overall skin-strengthening effects. Fermentation of the millet seed with a community of specially selected lactic acid bacteria creates a product with completely new properties (DEFENSIL®-PURE, hereinafter "millet ferment").

Material and methods

Extract preparation to obtain DEFENSIL®-PURE: ground millet seed was fermented with a proprietary mixture of lactic acid bacteria used for sourdough production. The fermentation supernatant was filtered and preserved (INCI: Water, Panicum Miliaceum (Millet) Seed Extract, Lactobacillus Ferment, Sodium Benzoate, Potassium Sorbate).

Analysis of saccharides and organic acids by ^{1}H-NMR spectroscopy (Bruker Avance III HD 500 MHz). The same for calcium and magnesium. Silicon analysis by inductively coupled plasma atomic emission spectrometry according to DIN EN ISO 11885:2009-09.

Bacterial growth was determined in liquid cultures. Growth of different strains in buffered chloride-peptone solution (NPP, Biolife 4013952) and transfer of 100 - 1000 cfu/ml into phosphate/citrate buffer (10 mM citrate, 20 mM disodium hydrogen phosphate, 1.09 g/l NaCl, 0.37 g/l KCl, 0.055 g/l $CaCl_2 \cdot 2H_2O$, 0.011 g/l $MgCl_2 \cdot 6H_2O$). Growth of the strains was determined after 24 hours by plating and counting the colony forming units (cfu).

Caspase-1 activation was determined by fluorescence microscopy. Primary human keratinocytes (47-year-old donor with Caucasian skin) were cultured for 24 hours and then treated with a cytokine cocktail (TNF-α, IL-4, IL-5, and IL-13, and *Staphylococcus aureus* toxin) to induce atopic conditions for 6 hours. The success of induction was verified with quantification of TSLP. The active was added at 1% before (24 h), during, or after induction of the atopic condition (6 h). Fluorescence staining with 5-carboxyfluorescein-Tyr-Val-Ala-Asp-fluoromethyl ketone covalently bound to activated caspase-1.

The *in-vivo* study was performed in accordance with the Declaration of Helsinki of the World's Medical Association. All study participants signed a written informed consent at the beginning of the study. The active ingredient (3% Panicum Miliaceum (Millet) Seed Extract, Lactobacillus Ferment) or placebo was administered in an emulsion (INCI: Water, Caprylic/Capric Triglyceride, Glyceryl Stearate Citrate, Pentylene Glycol, Cetearyl Alcohol, Glycerin, Sodium Anisate, Sodium Levulinate, Xanthan Gum, Citric Acid, Panicum Miliaceum (Millet) Seed extract, Lactobacillus Ferment, Sodium Benzoate, Potassium Sorbate) was applied to the face and atopic site twice daily for 8 weeks. It was tested on 20 study participants (10 verum, 10 placebo). Skin hydration was determined by corneometry, transepidermal water loss by TEWAmetry. The severity of the atopic site was determined dermatologically using a 100 mm analog scale.

Results

To obtain the millet ferment, the ground millet seed was broken down in a multi-culture fermentation process. Various lactic acid bacteria are used in a stable ratio to form an ecosystem that effectively displaces competitors and optimally breaks down nutrients. The ecosystem comes from a sourdough base that has been sampled and preserved over decades and must be kept stable by carefully monitoring and adjusting the culture parameters.

A comparison was made between non-fermented millet extract and fermented millet extract. Analysis of (poly)saccharides and AHA acids (alpha-hydroxy acids: malic acid, lactic acid) as well as acetic acid and ethanol showed complete degradation of polysaccharides in the ferment and their conversion into the fermentation products of a hetreofermentative lactic acid fer-

mentation **(Table 1)**. The acidic pH between 4 and 5 reflects the accumulation of organic acids. Interestingly, fermentation significantly increased the content of important calcium and magnesium. In the case of magnesium by more than double, and in the case of calcium by 9 times. A similar phenomenon was also observed in the fermentation of moringa leaves [5]. The content of bioavailable silicon remained constant.

Ingredient	before fermentation	after fermentation
Polysaccharides	253 mg/l	0 mg/l
Glucose	452 mg/l	0 mg/l
Malic acid	15 mg/l	63 mg/l
Lactic acid	0 mg/l	1379 mg/l
Acetic acid	110 mg/l	194 mg/l
Ethanol	0 mg/l	1747 mg/l
Mg	18.2 mg/l	40.9 mg/l
Ca	6.3 mg/l	56.6 mg/l
Si	9.0 mg/l	10 mg/l

Tab. 1 Comparison of ingredients before and after fermentation. Polysaccharides and sugars are completely converted into organic acids and ethanol. Minerals can be released more effectively.

To study the effects of the active ingredient towards the skin microbiota, representatives of bacterial strains found on the skin were used. *L. sakei* was used as a representative of the Lactobacili, which is found, for example, in the nose, but is best known for its isolation from kimchi, the Korean sauer-

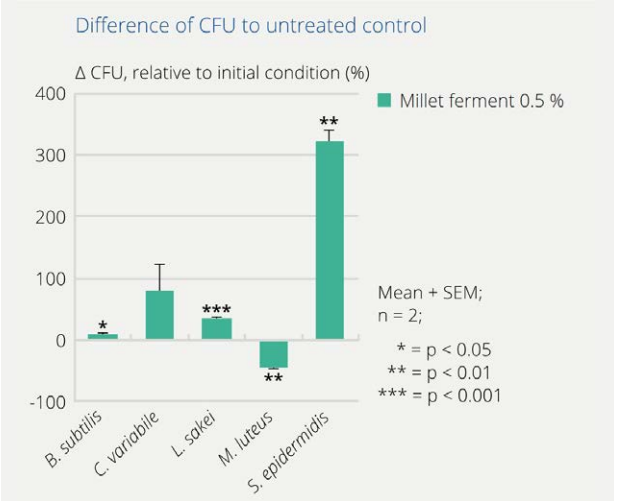

Fig. 2 Promotion of the growth of various skin germs. *Staphylococcus epidermidis* is strongly positively regulated.

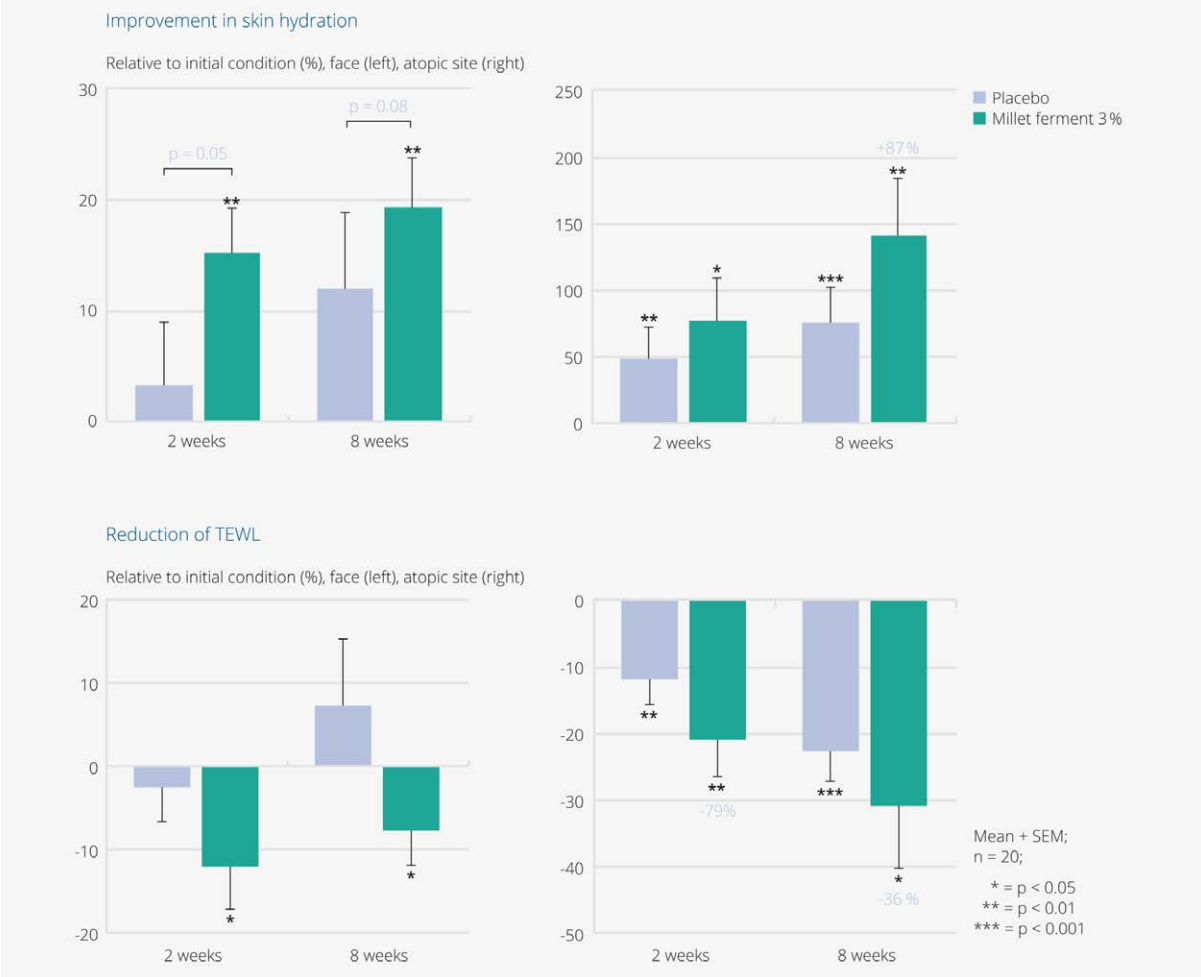

Fig. 3 Skin hydration and transepidermal water loss on the face and atopic sites. The active ingredient can increase skin hydration at atopic sites by up to 140%.

kraut. It was determined how the growth of the bacteria behaves when exposed to a buffer having approximately the composition of human sweat (citrate/phosphate buffer with mineral salts, see Material and Methods) in the presence of the active. It was found that the growth of *Staphylococcus epidermidis* in particular, was stimulated **(Figure 2)**.

This germ is crucial for a healthy skin flora by limiting or even preventing the growth of undesirable microbiota, such as *S. aureus*, a potential trigger of atopic dermatitis. Similarly, it limits excessive growth of *Bacillus subtilis*, a commensal skin germ that is significantly but only slightly increased in our experiment. Based on current research, neither explicit good nor bad properties are attributed to *B. subtilis*. Nevertheless, it is known that there is quorum sensing between it and *S. epidermidis*, with *S. epidermidis* having the upper hand. *Bacillus subtilis* can be responsible for an unpleasant foot odour. The promotion of the growth of *Corynebacterium variabile* and *Lactobacillus sakei* suggest that the agent may promote a microbiota found on somewhat moister skin regions. As a consequence, the milieu could change from dry skin to slightly more moist skin. *Micrococcus luteus*, another commensal germ also known as an "air germ", is negatively regulated. This bacterium can create malodour.

It was shown that 1% of the millet ferment resulted in a significant reduction of caspase-1 expression in a skin model that was exposed to *S. aureus* toxin and a cytokine cocktail to induce an atopic state. Caspase-1 is part of the inflammasome and leads to apoptotic processes (mainly pyroptosis) and strong inflammatory responses. In a preventive application, the expression of this cell death-inducing enzyme was pushed to basal levels. A limit-significant reduction was also observed with immediate (-71%; p = 0.07) or curative application (-61%; p = 0.08) (n = 6, not shown).

Application of the active ingredient to atopic patients in the non-treatment interval showed a skin moisturizing and barrier-strengthening effect both on the face (non-atopic area) and directly on the atopic site **(Figure 3)**. However, while skin hydration in the face increased by about 20% after 8 weeks, it increased by 140% on the very dry atopic site compared to the baseline condition. Transepidermal water loss was similar, but not quite as pronounced. On the face, TEWL was reduced by a maximum of 12%, and on the atopic site by more than 30%

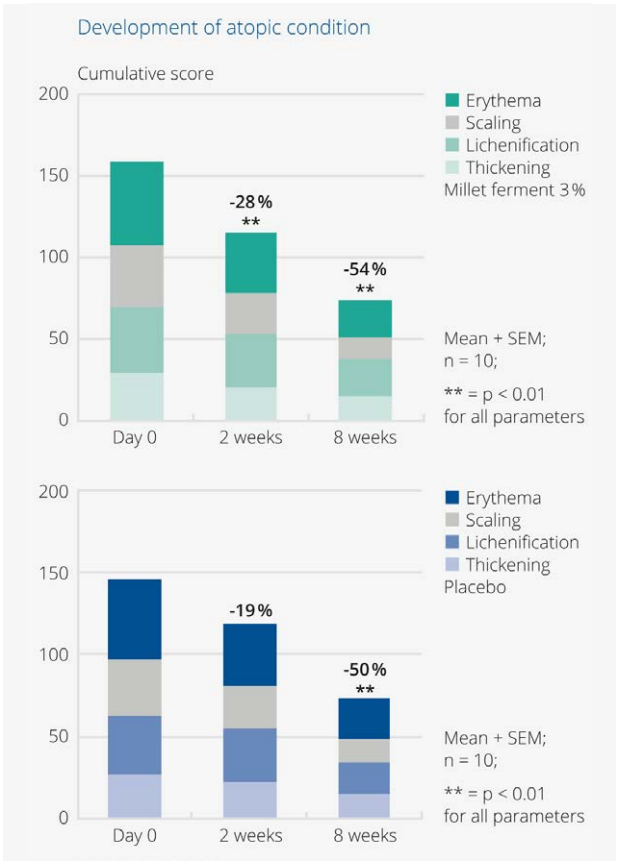

Fig. 4 Dermatological assessment of atopic conditions. Symptoms of atopic dermatitis are highly significantly reduced with active ingredient after 2 weeks, significantly faster than with placebo.

compared to the initial condition. Placebo had no significant effect on the face; at the atopic site, the effect was significantly better with the active substance. This is also reflected in the dermatologist's evaluation of the atopic lesions **(Figure 4)**: the parameters redness, scaling, lichenification and thickening were each reduced highly significantly with the active substance after just 2 weeks, by a total of 28%, which was not the case with placebo. After 8 weeks, the placebo and active ingredient were similar in their effects, with an advantage for the active. The atopic lesions visibly decreased and the appearance of the facial skin improved significantly **(Figure 5)**.

Fig. 5 Improvements in facial skin (left) and atopic sites (right) with the active ingredient.

Discussion

The active ingredient is of probiotic origin, i.e. the end product of a bacterial lactic acid fermentation of millet seed. The filtered clear culture supernatant contains the fermentation products but no living components of the lactic acid bacteria. Thus, the compound can be classified as postbiotic. Its action is likely to function via correction of the very dry skin environment in atopic dermatitis, where the postbiotic fermentation products may play a crucial role: application may optimize the pH and metabolome of the skin for a healthy skin microbiota. It is known that metabolites of lactic acid fermentation can suppress undesirable germs and promote the growth of desirable ones. Furthermore, it has already been shown in a 3D epidermal model that fermentation products from lactic acid fermentation have a positive influence on epidermal health, e.g. reducing TEWL and increasing the moisture content of the stratum corneum [6], analogous to the results from our *in vivo* study.

Overall, it can be said that the skin microbiota is positively regulated by the active ingredient, in particular *S. epidermidis*, a germ that can displace undesirable microbes or prevent their colonization. The promotion of more moisture-loving germs such as *C. variabilis* and Lactobacilli suggests that the active ingredient promotes an appropriate skin environment, which is of great importance in atopic dermatitis with extremely dry skin areas. The question arises whether the active ingredient itself creates a moister skin environment (significant increase in skin moisturization and reduction in TEWL) or whether this is first brought about by a correction of the skin microbiota. This would require extensive studies on the microbiome and its temporal development over the entire course of the study, which unfortunately was not possible at the time of the study due to the Corona pandemic. It is a fact that the active substance alleviates the symptoms of atopic skin (redness, scaling, lichenification, thickening) more quickly. A more acidic skin environment and fermentation products from lactic acid fermentation have already been described as beneficial for the care of atopic skin [7-9]. The good effect of the placebo is not surprising, since the formulation used a base that should be suitable for people with atopic dermatitis to achieve appropriate compliance.

The difference between our millet ferment and other fermented cosmetic actives lies in the slow and highly controlled fermentation process similar to sourdough bread. In bread production, this leads to a more digestible product without intolerances, as can be the case with the breads of the wholesale and bakery industry that are optimised for speed. A 9-fold improvement in calcium concentration was shown compared to the unfermented millet extract. The slow and controlled fermentation favours the complete degradation of polysaccharides and thus the release of bound calcium [5]. By that and the use of special alpine millet as the basis for our active ingredient, we take a very sustainable approach, in line with the trend of increasingly popular natural foods. Perfect for providing the right care for sensitive skin such as that of people having atopic skin.

In summary, the active ingredient rapidly moisturizes dry skin areas, strengthens the skin barrier and can compensate for deficiencies, especially on atopic skin. It alleviates atopic symptoms and is particularly well suited for sensitive and dry skin. o return to the initial question: A postbiotic cosmetic active ingredient can indeed "preserve" the skin by making it more resistant to external influences, as evidenced by increased skin hydration and an improved skin barrier.

Bibliography

[1] Rather IA, Kim BC, Lew LC, Cha SK, Lee JH, Nam GJ, et al. Oral Administration of Live and Dead Cells of Lactobacillus sakei proBio65 Alleviated Atopic Dermatitis in Children and Adolescents: a Randomized, Double-Blind, and Placebo-Controlled Study. Probiotics Antimicrob Proteins 2021, 13: 315-326.

2] Legras J-L, Merdingolu D, Cornuet J-M, Karst F. Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history. Molecular Ecology 2007, 16: 2091-2102.

[3] Papadimitriou K, Zoumpopoulou G, Tsakalidou E. Chapter 6 - Sourdough Bread. In: Innovations in Traditional Foods. Edited by CM G: Woodhead Publishing; 2019.

[4] Divella R, G DEP, Tufaro A, Pelagio G, Gadaleta-Caldarola G, Bringiotti R, et al. Diet, Probiotics and Physical Activity: The Right Allies for a Healthy Microbiota. Anticancer Res 2021, 41: 2759-2772.

[5] Dai J, Tao L, Shi C, Yang S, Li D, Sheng J, et al. Fermentation Improves Calcium Bioavailability in Moringa oleifera leaves and Prevents Bone Loss in Calcium-deficient Rats. Food science & nutrition 2020, 8: 3692-3703.

[6] Otsuka M, Tamane T, Tokudome Y. Effect of Lactic Fermentation Products on Human Epidermal Cell Differentiation, Ceramide Content, and Amino Acid Production. Skin Pharmacol Physiol 2021, 34: 103-114.

[7] Lee NR, Lee H-J, Yoon NY, Kim D, Jung M, Choi EH. Application of Topical Acids Improves Atopic Dermatitis in Murine Model by Enhancement of Skin Barrier Functions Regardless of the Origin of Acids. Annals of dermatology 2016, 28: 690-696.

[8] Panther DJ, Jacob SE. The Importance of Acidification in Atopic Eczema: An Underexplored Avenue for Treatment. Journal of clinical medicine 2015, 4: 970-978.

[9] Kim MS, Kim WG, Chung HS, Park BW, Ahn KS, Kim JJ, et al. Improvement of atopic dermatitis-like skin lesions by Platycodon grandiflorum fermented by Lactobacillus plantarum in NC/Nga mice. Biol Pharm Bull 2012, 35: 1222-1229.

contact

Stefan Hettwer, PhD
Emina Besic Gyenge, PhD
Brigit Suter
Barbara Obermayer

RAHN AG, Dörflistrasse 120,
8050 Zürich, Switzerland