

## Introduction:

Fermentation technology is used in various applications such as foods, detergents, medicines in our lives. In particular, fermented foods have a very long history, and the ingredients produced by fermenting plants with microorganisms are delicious, good for health, and have various effectiveness such as improving storage stability. Among them, it has long been known that the combination of rice and yeast is the best for sake brewing, which is a traditional Japanese fermentation technology. In recent years, cosmetic ingredients produced by microbial fermentation from the perspective of the bioconversion of phytochemicals have been researched and developed [1] [2] [3]. Therefore, we have tried to develop a rice fermented liquid for cosmetics because it is well compatible with the skin when applying. So far, it has confirmed that 2-ketoglutaric acid (2-KG), which is efficiently produced by yeast fermentation of rice, has promoting effects on proliferation of epidermal keratinocytes and on mRNA expressions of factors (filaggrin, serine palmitoyltransferase, involucrin) related to the moisturizing and barrier function in *in vitro* tests (reported at the IFSCC Mexico conference 2021). In this study, we conducted a human clinical trial with continuous use of a lotion containing rice fermented liquid and found that the fermented liquid is a promising cosmetic ingredient with the function to retain skin moisture and elasticity.

## Materials & Methods:

### Test sample

The lotion containing 94.5% of rice fermented liquid, 5% of propanediol and 0.5 % of phenoxyethanol or that containing purified water instead of rice fermented liquid as a blank was prepared for this study.

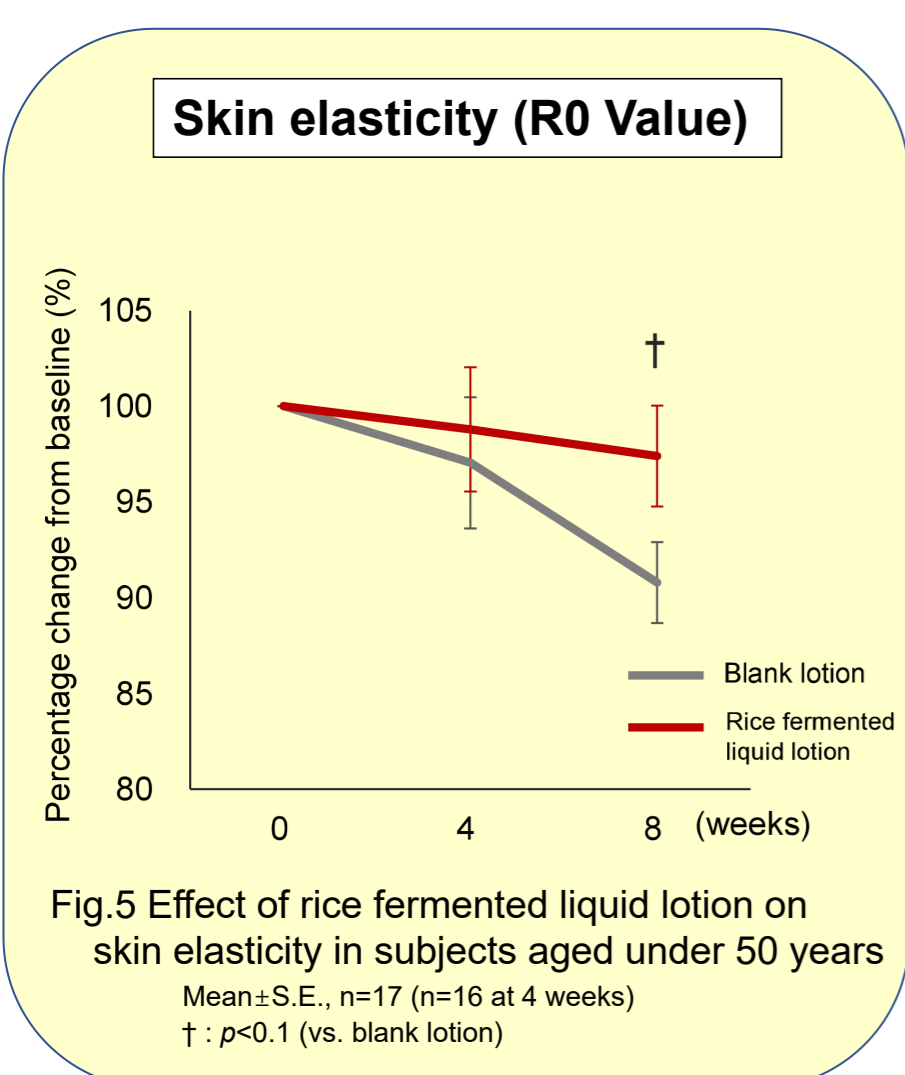
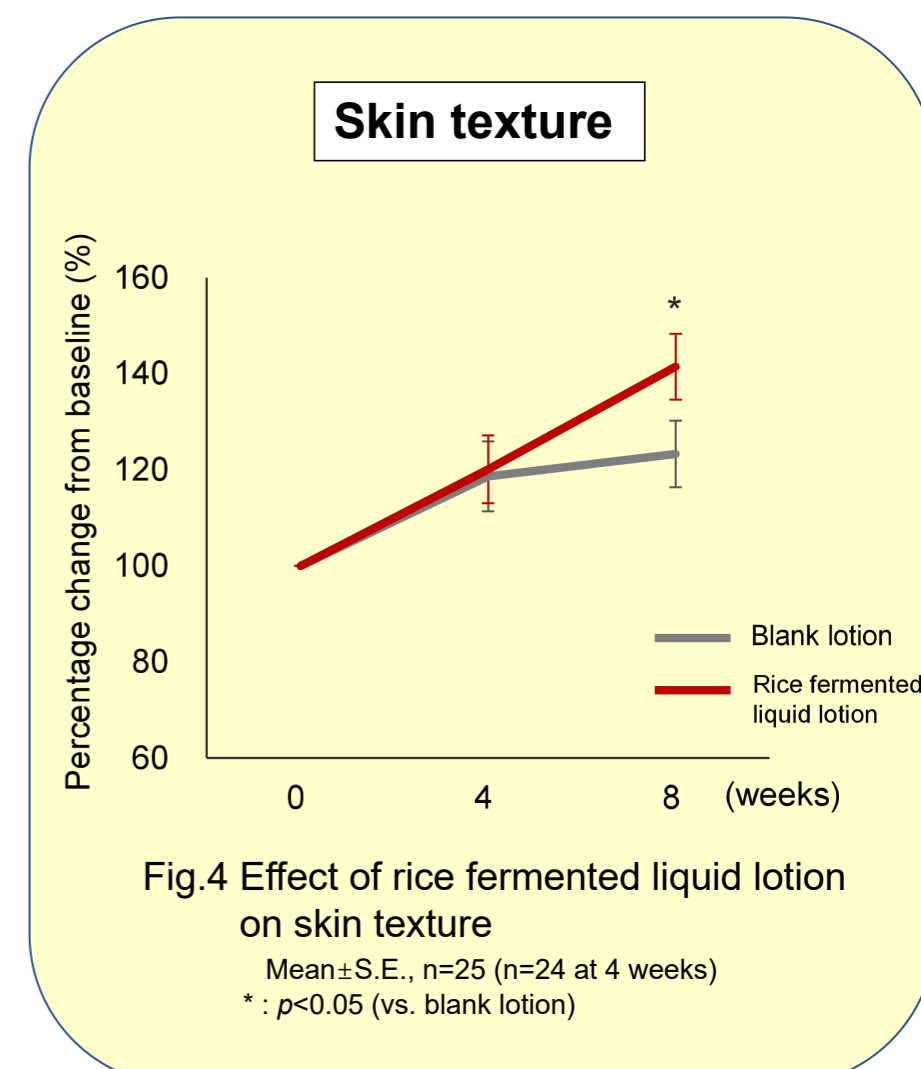
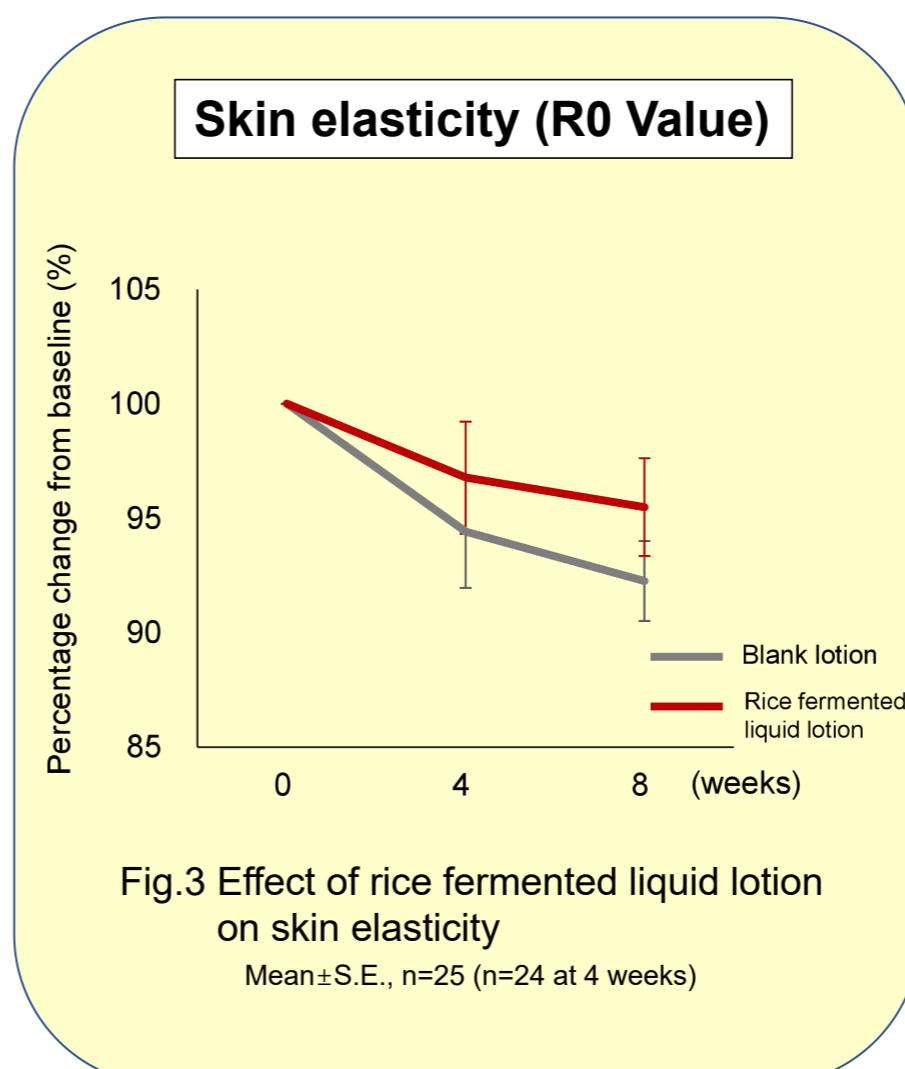
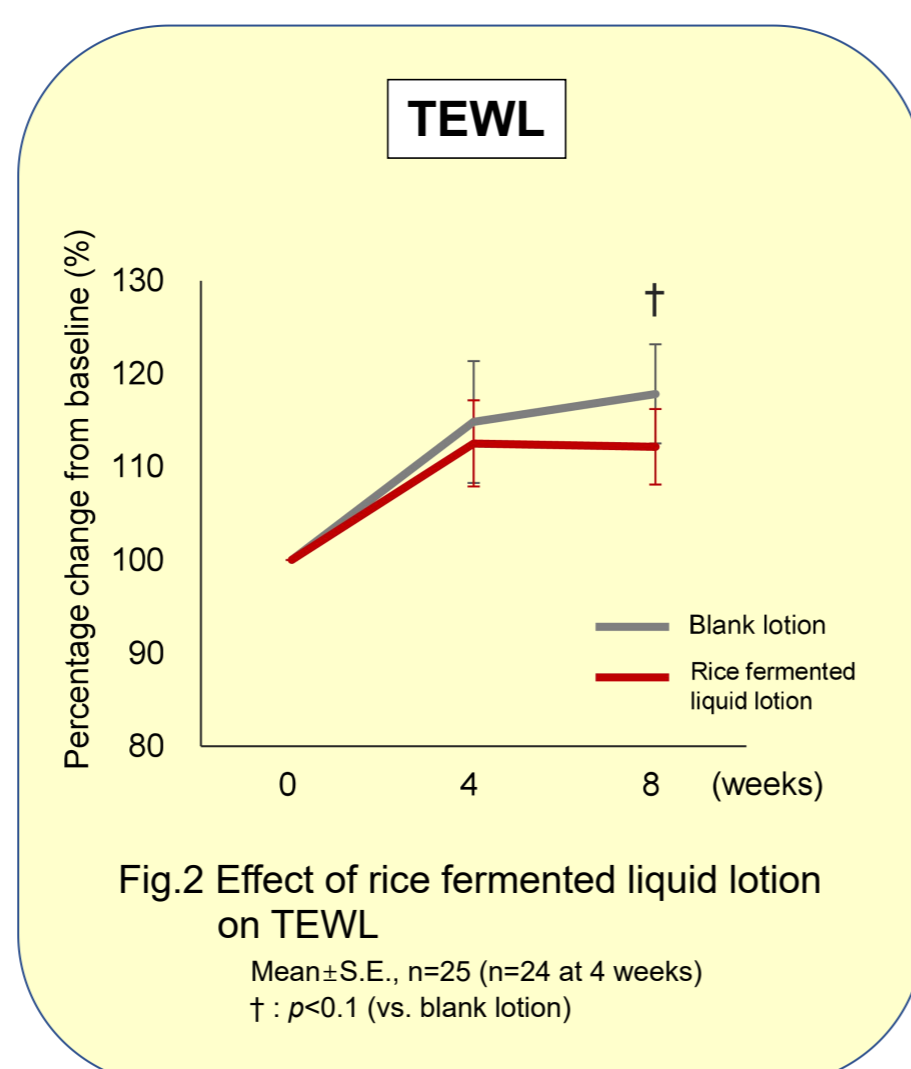
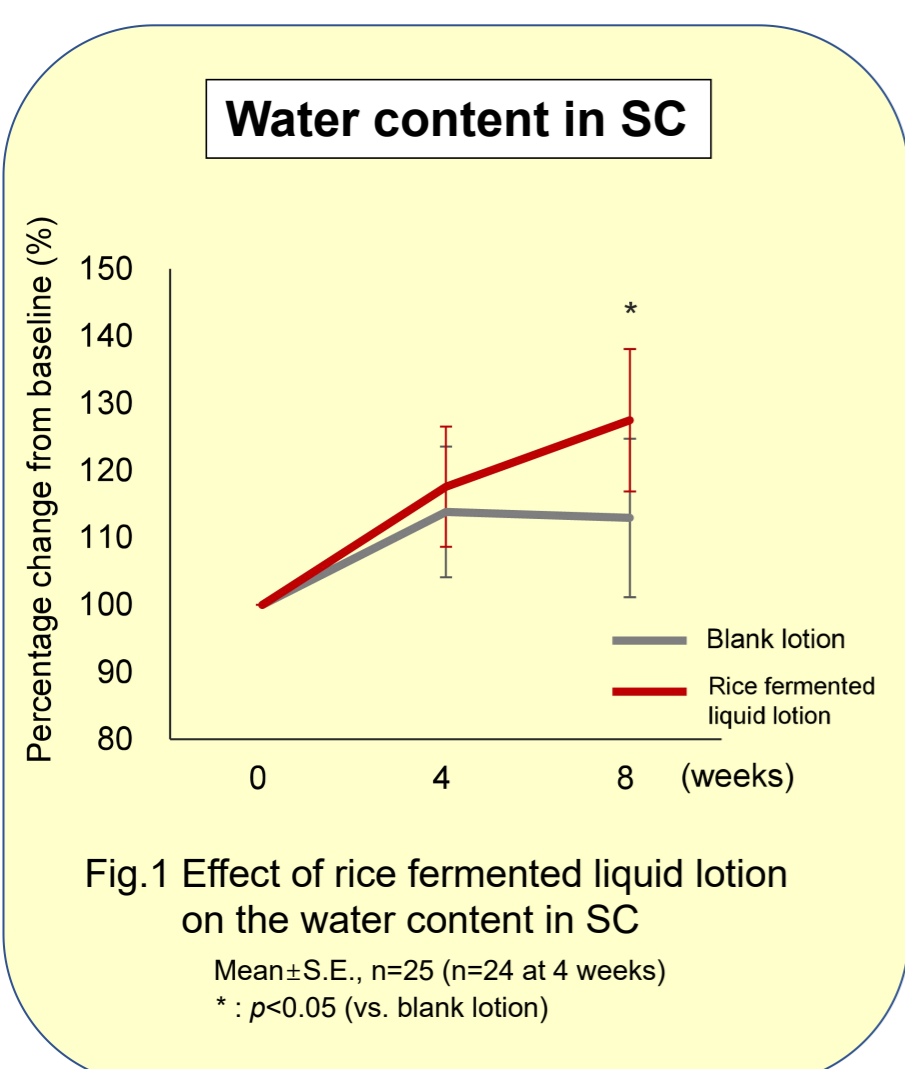
### Test method

Twenty-five healthy Japanese female subjects aged between 24 and 57 (Ave. 44) with low water content in stratum corneum (SC) and little difference between the left and right sides of the face who signed the informed consent were selected. The double-blind, placebo-controlled, left-right randomized clinical trial was conducted by topical application of rice fermented liquid lotion and its blank on each half side of the face twice daily (morning and night) for 8 weeks in these subjects. Subjects were equilibrated for more than 15 minutes in a closed environment with 20 °C and 50 % relative humidity conditions. The water content in SC was measured using a corneometer (Model CM825; Courage and Khazaka, Germany). TEWL was measured using a vapometer (Model SWL5001JT; Delfin Technologies, Finland). Skin elasticity was measured using a cutometer (Model MPA580; Courage and Khazaka). Facial analysis was also performed using VISIA-Evolution (Integral, Japan). Each measurement was conducted at baseline, 4 and 8 weeks post-application and percentage change from baseline in skin parameters at each time point was used for evaluation. Statistical analyses were conducted using SPSS Statistics (IBM, USA). A statistical significance level of  $p < 0.05$  as determined by two-tailed t-test was applied to all analyses.

## Results & Discussion:

### Effects of rice fermented liquid lotion on skin hydration and elasticity

Application of rice fermented liquid lotion for 8 weeks significantly increased the water content in SC as compared with the blank lotion (Fig.1) and tended to decrease TEWL (Fig.2). Skin elasticity following the application of rice fermented liquid lotion showed no significant effect (Fig.3). The number of skin textures was significantly increased by the application of rice fermented liquid lotion at 8 weeks (Fig.4). In an age-stratified analysis concerning skin elasticity, an improvement tendency was found by the application of rice fermented liquid lotion in subjects aged under 50 years (Fig.5).



In the process of fermentation, the enzymes from microorganisms are known to break down proteins in raw materials as a substrate and turn them into amino acids. Free amino acids account for the largest component of the natural moisturizing factor (NMF) which is indispensable for retaining moisture [4] [5] [6]. Lactates are also one of the main components of NMF [6]. Therefore, we thought that supply of a fermented liquid containing amino acids and lactates to the skin as a substitute for water used as a base in the formulation of cosmetics has the potential to increase skin hydration. We focused on microbial fermentation by the combination of rice and yeast and developed a rice fermented liquid as a cosmetic ingredient with higher compatible with the skin. So far, we have reported that one of the components in the rice fermented liquid, 2-KG, has promoting effects on the moisturizing and barrier function in *in vitro* tests. As rice fermented liquid is expected to be effective on the skin, we conducted a human clinical trial. Application of rice fermented liquid lotion resulted in a significant increase in SC water content and the number of skin textures and showed a tendency to decrease TEWL as compared with the blank. These results suggested that rice fermented liquid might be more functional than water as a humectant. On the other hand, there was no difference between rice fermented liquid lotion and the blank on skin elasticity. However, in an age-stratified analysis, an improvement tendency of skin elasticity by the application of rice fermented liquid lotion was found in subjects aged under 50 years, which means that rice fermented liquid might be more effective for users under the age of 50.

## Conclusions:

In this study, we found that the rice fermented liquid has promoting effects on the moisturizing and barrier function in a human clinical trial. These results suggest that the rice fermented liquid is a promising cosmetic ingredient as functional water boosting the ability to retain skin moisture and elasticity.

## References:

- Domżał-Kędzia M, et al (2019) Fermentation parameters and conditions affecting levan production and its potential applications in cosmetics. *Bioorg Chem* 93:102787.
- Wang Z, Sun Q, et al (2022) The anti-aging activity of Lycium barbarum polysaccharide extracted by yeast fermentation: In vivo and in vitro studies. *Int J Biol Macromol* 209:2032–2041.
- Huang H-C, et al (2022) Phytochemicals levels and biological activities in Hibiscus sabdariffa L. were enhanced using microbial fermentation. *Ind Crops Prod* 176:114408.
- Horii I, et al (1989) Stratum corneum hydration and amino acid content in xerotic skin. *Br J Dermatol* 121:587-592.
- Solano F (2020) Metabolism and Functions of Amino Acids in the Skin. *Adv Exp Med Biol* 1265:187–199.
- Fowler J (2012) Understanding the role of natural moisturizing factor in skin hydration. *Pract Dermatol* July:36–40.