

# Effect of Citrus junos fermentation on skin improvement in vitro and in vivo

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## Introduction:

Human skin has been exposed to external stimuli such as cosmetics, UV radiation, fine dust and microbes. The skin's epidermis performs an essential barrier function to protect the body from external irritation. Increase in these factors can cause skin reactions such as inflammation, irritation, allergies and cancer. Recently, the demand for anti-pollution cosmetics has been increasing. Air pollution is recognized by the World Health Organization as the most important environmental issue in the world. When Skin cells are exposed to air pollution factors, changes in a number of skin properties can be observed, such as pH, sebum secretion rate, oxidative stress, inflammation markers, skin barrier, and collagen levels. Yuzu (*Citrus junos* Sieb ex TANAKA) is a citrus fruit and plant in the family Rutaceae of East Asian origin. Citron is known for containing abundant antioxidants such as vitamin C, flavonoids. In this study, we investigated the skin improvement effects of fermented Citrus junos (FCJ).

\*Keywords: Anti-pollution, Moisturizing, Wound healing, Citron junos fermentation, particulate matter.

## Materials & Methods:

**<Material and Manufacturing process of FCJ>**

Extraction → Fermentation → Efficacy evaluation

**<Scratch wound assay>**

Confluent monolayer → Scratch the monolayer surface carefully and wash (Time 0h) → Take pictures at timely intervals of the wounded area until its closure (Time 6h, 12h)

**<Anti-pollution assay>**

Standardized fine dust (PM<sub>10</sub>-like)

**<Moisturizing properties>**

- Transepidermal water loss (TEWL)
- Skin cell moisturizing effect (FLG)
- Skin Hydration

## Results & Discussion:

### Cell proliferation and wound healing.

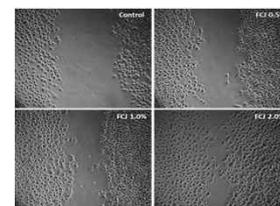
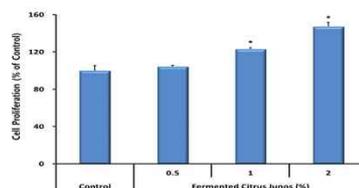


Fig. 1. Effect of FCJ on cell proliferation of human keratinocytes (HaCaT) at 24h. An increase of cell proliferation of HaCaT cells was produced by FCJ (0.5 ~ 2.0%) after 24h incubation, by WST-1 assay. The data represent the means ± SD of triplicate experiments. \*p<0.05 compared with Control group (culture medium only).

Fig.2. FCJ accelerated cell wound healing of HaCaT in the Scratch wound assay. A scratch was produced in a monolayer of HaCaT cells and photographs were taken after 24h of treatment with FCJ.

### Moisturizing Effect

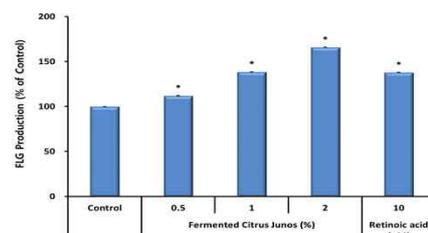


Fig. 3. Effect of FCJ on Filaggrin(FLG) production in HaCaT Cells. Cells were treated with the indicated concentrations of FCJ (0.5%~2.0%) for 24h. At the end of incubation, cell lysates were analyzed for FLG using an enzyme-linked immunosorbent assay (ELISA) kit. All values are means SD of 3 independent experiments. \*p<0.05 compared with Control group.

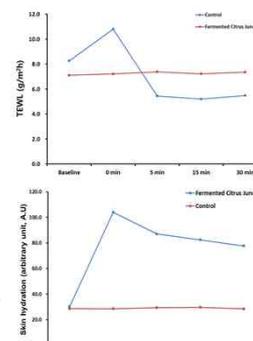


Fig. 4. Evolution of (A) transepidermal water loss (TEWL) and (B) skin hydration.

## Conclusions:

In Conclusion, these FCJ exhibit skin hydration activity, wound healing, and anti-pollution effects that are mediated by increasing FLG production, and increase in the growth of HaCaT cell. Based on the findings in this study, we concluded that the various effects of the FCJ can provide the scientific basis for the development of skin improvement based product.

## Aknowledgments:

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### Anti-pollution Effect

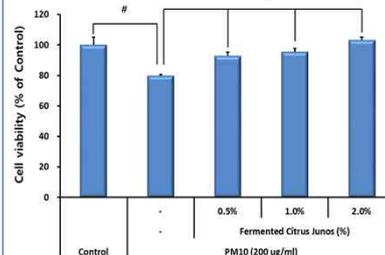


Fig. 5. Effects of FCJ on the viability of HaCaT cells exposed to PM10. The cells were exposed to PM10 (200ug/ml) for 48h in the absence or presence of FCJ at the indicated concentrations. All values are means SD of 3 independent experiments. #p<0.05 compared with PM10-untreated control group; \*p<0.05 compared with PM10 Control group.

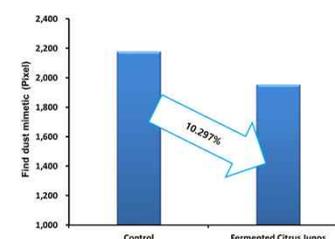


Fig. 6. Effect of the human fine dust adsorption prevention test.