# Identification of a mechanism by Mandom to perceive cold temperature as cold change

- Aiming for comfortable refreshing cosmetics utilizing TRP channels -

Mandom Corporation (Head Office: Osaka, President Executive Officer: Motonobu Nishimura, hereafter "Mandom"), through collaboration with Professor Makoto Tominaga of the Okazaki Institute for Integrative Bioscience, has developed a cosmetics evaluation method focused on using TRP (Transient Receptor Potential) channels (see "Reference material: Working with TRP channels") as sensors of skin sensations, and applying this method to product development.

We discovered that TRPM8 (a cooling sensor), one of the TRP channels, responds significantly to external temperature fluctuations, and successfully identified the mechanism by which temperatures perceived as cold are affected by external temperatures. In addition, we identified the role of a specific phospholipid that is involved in the TRPM8 reaction. Furthermore, we discovered that the eucalyptus-derived ingredient eucalyptol, is a freshening ingredient effective at hot temperature, utilizing the changes in TRPM8 reaction temperature based on external temperature variations.

Furthermore, we presented the results of this study at the "27th Congress of the International Federation of Societies of Cosmetic Chemists (IFSCC) in Johannesburg" held from October 15 to 18, 2012 in South Africa.

**1. Human temperature perception governed by external temperature**

The mechanism of perceiving temperature is becoming clearer since the discovery of temperature-sensitive "TRP channels" about 15 years ago. Humans have nine temperature sensors each differing in reaction temperature, that enable accurate temperature perception. While it is well-known that humans are easily affected by external temperature fluctuations, such as "perceiving "warm feeling" when immersed in lukewarm water after immersion in cold water, and yet perceive "cold" when immersed in the same lukewarm water after being soaked in warm water. It is possible that the mechanism by which cold and hot is felt probably the same, as these findings remains unclear. So, we focused on the cool temperature, and investigated whether the TRPM8 reaction temperature is affected by external temperature.

**2. Identifying the mechanism of cold temperature perception**

The reaction temperature of TRPM8 is approximately 27°C, so it is believed that humans are able to perceive cold when the skin temperature (approximately 32°C) drops by 5°C. Although, why we experience cold even at higher temperatures remain a mystery. Therefore, using cells, we investigated whether the TRPM8 reaction temperature remains the same even when the external temperature changes. Based on the results, we found that TRPM8 is able to function at a temperature of 27°C or higher. This indicates that the reaction temperature adjusts to the external temperature (Fig. 2). In other words, it is clear that the differences in cold perception may be a function of external temperature rather than a constant temperature, governed by changes in the reaction temperature of TRPM8.

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To examine the mechanisms controlling the changes in TRPM8 reaction temperature, we focused on the binding of a specific phospholipid (phosphatidylinositol 4,5-diphosphate) to TRPM8 in the cell membrane. We found that reduction of the phospholipid did not result in changes in the TRPM8 reaction temperature due to external temperature changes. We confirmed that the presence of this phospholipid in the cell membrane influences changes to the cooling sensor reaction temperature in response to changes in external temperature.

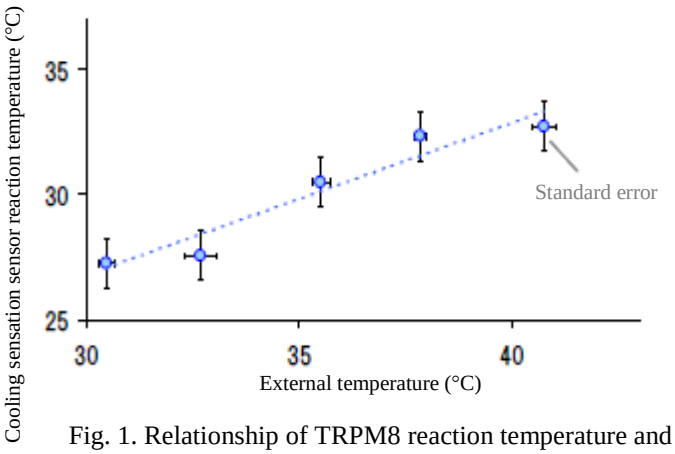



Fig. 1. Relationship of TRPM8 reaction temperature and external temperature

### 3. Freshening ingredient "eucalyptol" provides comfortable coolness even during rapid changes in external temperature

Using our preliminary results, we further investigated how freshening ingredients could provide comfortable coolness even when external temperature changes occur.

TRPM8 reaction temperature rises depending on the freshening ingredient. As a result, people may perceive cold even when it is actually warm. An excess of any freshening ingredient may provide coolness even when skin temperature is dropping, which may lead to an unpleasant sensation depending on the individual. Therefore, in order to determine which freshening ingredients are effective during warmth but has no effect when cool, we used the results of this study, and examined the effects of freshening ingredients on changes to TPM8 reaction temperature due to changing external temperature. Based on the study, we discovered that eucalyptol, the main ingredient in eucalyptus oil, works effectively when the external temperature is high (Fig. 2). Furthermore, the cooling sensation from eucalyptol when the skin temperature rises, for instance during exercise or bathing was found to be about the same as when the skin temperature is normal (Fig. 3). In contrast, the cooling sensation of menthol, the main freshening ingredient, at normal skin temperatures tended to be higher than at high temperature. From these results, it is clear that eucalyptol is a freshening ingredient capable of effectively providing comfortable coolness irrespective of the skin temperature, be it normal or elevated.

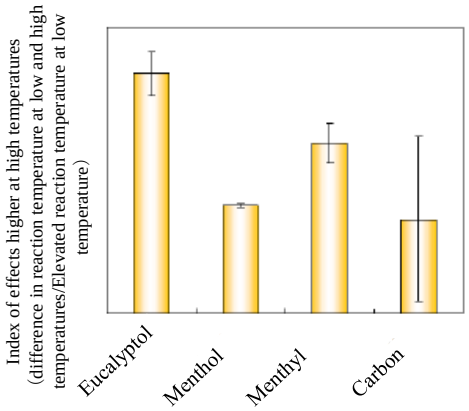



Fig. 2. Results of evaluating ingredients higher in effects at high temperatures

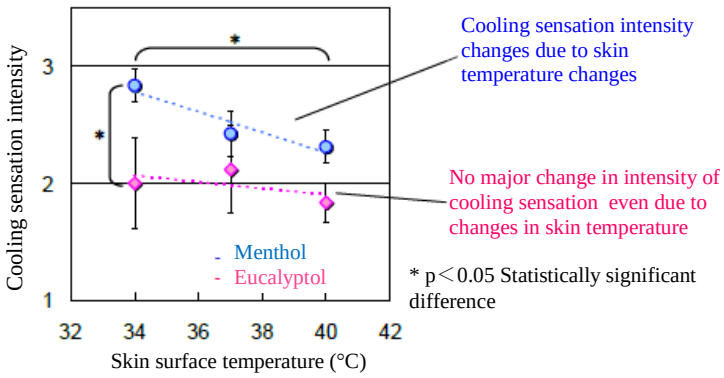


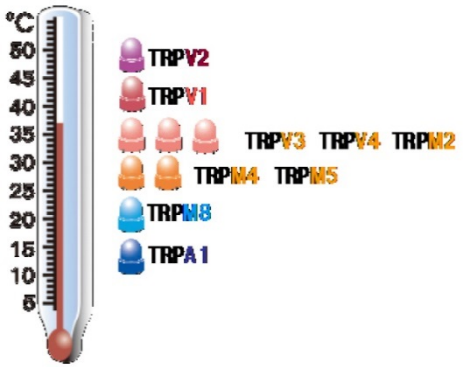

Fig. 3. Cooling sensation due to freshening ingredients at different skin temperatures

Furthermore, eucalyptol is being systematically applied to products offering a comfortable cooling sensation.
Mandom is continuing to explore this ingredient and apply the cooling sensations in its future products for consumer use.

Reference material: Working with TRP channels

<Sensory irritation mechanism>

"Sensors" referred to as temperature sensitive "TRP channels" that perceive chemical substances and temperature, and convert them into electrical signals, are present in the skin nerve cells. These are involved in sensing temperature and irritants. Mandom in collaboration with Professor Makoto Tominaga of the Okazaki institute for Integrative Bioscience previously concluded that TRP channels are involved in the unpleasant "tingling" and "stinging" sensations when using cosmetics.




Temperature sensitive "TRP channels"

<Past Mandom press releases>

1. Application of TRP channels as sensory irritation sensors in the evaluation of cosmetics (released October 9, 2007)

Parabens are very safe preservatives used in cosmetics, however, they cause sensory irritation. The unpleasant "tingling" sensations caused in rare instances is well-known, but the mechanism is unclear. Therefore, when we examined their connection to TRP channels, we found that TRPA1, a receptor for the spicy ingredient in wasabi, is also activated by parabens that cause irritation. Based on this discovery, we became the first cosmetics company to utilize them in evaluating cosmetics.

2. Correlation between skin sensation and TRP channel activation (released September 22, 2010)

Using preservatives and polyalcohols that are highly safe but cause "stinging" and "tingling" irritations, we investigated the correlation between the irritating skin sensations and TRP channels. Based on the results, we discovered that sensory irritations due to preservatives correlate to TRPA1, and the sensory irritations due to polyalcohols correlate to TRPV1, the receptor for the spicy ingredient in red pepper.

3. Identification of the mechanism of irritation in hair coloring and the discovery of beneficial effects of carbonate ions (released December 6, 2010)

Hair colorings (oxidative hair dyes) contain high concentrations of alkaline agents. We have identified the molecular mechanism by which TRPA1 is activated by alkaline agents. Based on this finding, we discovered "carbonate ions" block TRPA1 activation, and developed an extension that they are effective in reducing sensory irritation from hair coloring. This discovery has been adopted by Mandom in hair coloring products.

4. Discovery of eucalyptol to reduce unpleasant sensory irritation from menthol (released March 8, 2012)

Menthol is an ingredient that is extremely effective as a freshening ingredient. Its cooling sensation is known to be caused by the activation of TRPM8, which is a cooling sensor. However, it also causes an unpleasant "stinging" sensation at high concentrations. Since this unpleasant sensation is caused by TRPA1 activation, we discovered "eucalyptol" (the main ingredient in eucalyptus oil), a freshening ingredient that activates the cooling sensor TRPM8, but does not activate TRPA1. Eucalyptol, thus has a TRPA1-inhibiting effect, making it possible to realize cooling with milder "stinging" sensory irritation by simultaneously mixing it with menthol. This discovery by Mandom has been applied to freshening ingredients.