Study of Fragrance Materials on Controlling Head-Odor Formation[a]

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In recent years, people have shown greater concern for body odors. Breath, axillary, foot and hair odor are examples of displeasing odors for humans. There are methods of controlling bad smells including hiding them by using stronger fragrance materials, and masking them by using materials that give a vague impression of the original smell in harmony with the actual smell. Although there are various reports on bad breath, axillary odor and foot odor[1,2], research into head odor is sparse. Therefore, we have performed the following studies aiming to develop fragrant hair cosmetics that control head odor by inhibiting the formation of head-odor-causing materials:

- Study of head-odor-causing materials
- Study of the process of odor formation
- Investigation of fragrance materials that can control odor formation
- Confirmation of odor formation control

Kubota et al. reported lower fatty acids and aldehydes[3] to be key components of head odors. We first performed a study on the materials which cause head odors, and then performed a study to confirm the process of fatty-acid formation. Materials that are able to control the formation of odor components were then investigated. Fragrance materials are known to have deodorizing and antibacterial effects and include many molecular structures. Therefore, using fatty-acid formation models, we carried out the evaluation of fragrance materials that control fatty-acid formation. The results indicate that there are many fragrance materials which control fatty-acid formation. We prepared a trial perfume for hair cosmetics from these fragrance materials that has a fragrance tone suitable for practical use. In a test of the practical use of perfumed shampoo and rinse, the effect on head-odor control was recognized.

We obtained findings on fragrance materials that control the formation of head-odor-causing fatty acids, and on fragrance materials that produce esters with fatty acids. The results of these studies are reported in this paper.

Experiments

The causes of head odor may be divided into two groups: external factors such as smoking, cooking or cosmetics, and internal factors derived from the epidermis, sweat glands and sebaceous glands, as shown in Figure 1.

The sensory test of head odor was performed on a total of 8 female subjects, two each in their 20s, 30s, 40s and 50s, immediately after hair washing with perfumed shampoo and rinse and 3 days later, during which time the use of hair cosmetics was not permitted (June 1995).

Analysis of Solvent-Extracted Compounds from the Hair

The analysis of acetone-obtained extracts from hair cut at the base (approximately 5 mm from the scalp) was carried out using the gas-mass (GC-MS).

Conditions for analysis:

Instrument: HP 5980 II GCD
Column: HP-5 15 m
Carrier gas: He 2 ml/min

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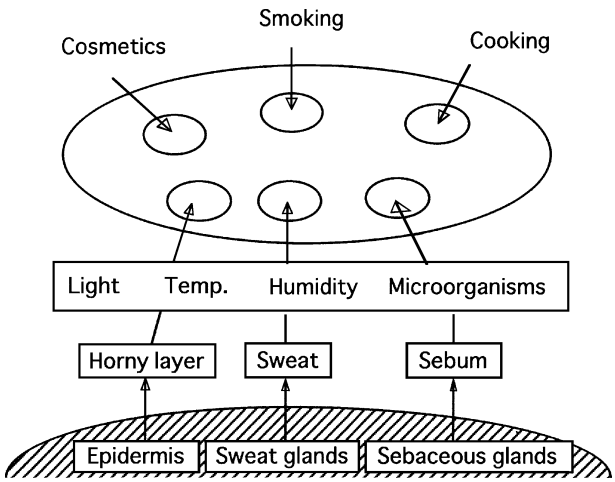

Figure 1. Elements of head odor

Injection temperature: 320°C
Detector temperature: 280°C
Oven temperature: 80-210°C, 4°C/min
Sample volume: 1 µl

Head-Space (HS) Analysis of Head Odors

Hair was covered with a cap and compressed air was sent through activated carbon and into the cap at 700 ml/min, while air in the cap was aspirated at 500 ml/min for 30 minutes using 0.05 g of Tenax TA (20-35 mesh) as an adsorbent. The aspirated air was used as a sample. The sample was analyzed using the GC-MS equipped with a thermal desorption cold trap injector (TCT).

Conditions for analysis:

Precool temperature: –100°C
Desorption temperature: 210°C
Desorption time: 5 min
Instrument: HP 5980 II GCD WITH TCT
Column: HP-INNOWAX 30 m × 0.25 mm × .25 µm
Carrier gas: He 1 ml/min
Injection temperature: 250°C
Detector temperature: 280°C
Oven temperature: 80 - 210°C, 4°C/min

Malassezia furfur (IFO 0656) and staphylococcus epidermidis (IFO 12993), bacteria resident in human skin, were supplied from Institute for Fermentation, Osaka, Japan (IFO). The test to confirm fatty-acid formation was conducted using these bacteria.

For malassezia furfur, a bacterial solution was added at 10^4cfu/cm^2 to IFO-designated 103 culture medium (containing olive oil, the main component of which is triolein), the medium was incubated at 35°C for 3 days, and the extracts with ether were analyzed using GC.

For staphylococcus epidermidis, a bacterial solution

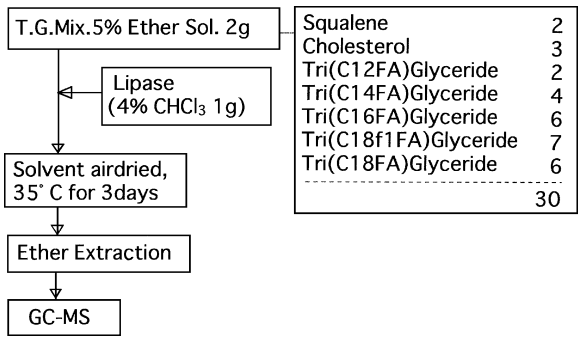

Figure 2. Fatty-acids formation test by lipase

was added at 10^4cfu/cm^2 and triolein (as a triglyceride) was added at 0.005 cc/cm^2 to triptosoy agar culture medium (Eiken Chemical Co. Ltd.), the medium was incubated at 35°C for 3 days, and the extracts with ether were analyzed using GC.

A compound similar to the sebum of the hair was prepared using squalene, cholesterol, and triglycerides (Figure 2). One g of 4% chloroform suspension of commercial lipase (reagent) (Wako Pure Chemical Industries, Ltd.) was added to 2 g of the 5% ether solution of this compound (T.G.Mix. 5% Ether Sol. 2 g), and the solvent was air dried in a dish. After standing at 35°C for 3 days, extraction with ether was performed, and the extracts were analyzed by GC-MS.

Products containing 0.2 g of a fragrance material were added to T.G.Mix. Five percent ether solution (2 g), and the evaluation of materials controlling fatty acid formation were carried out in a similar method to the experiment for lipase fatty acid formation shown in Figure 2.

Using a method similar to that for the experiment for the formation of fatty acid by malassezia furfur and staphylococcus epidermidis described above, a sample of fragrance material at a volume of 0.01 cc/cm^2 was applied to the medium after bacterial solution application, the medium was incubated at 35°C for 3 days, and a macroscopic examination on bacterial growth was conducted and analysis of ether extracts using GC was performed.

Confirmation of Odor Control and the Sensory Test in Practical Use

The effect on head-odor control was evaluated in a practical-use test of shampoo (perfume ratio: 0.45%) and rinse (perfume ratio: 0.3%) with a prepared fragrance. The effect was evaluated based on the data obtained in a questionnaire (surveyed in February 1996). Questionnaire items are as follows:

During everyday living, do you feel your head odors are

(a) Disagreeable.
(b) Slightly disagreeable.
(c) Not disagreeable.

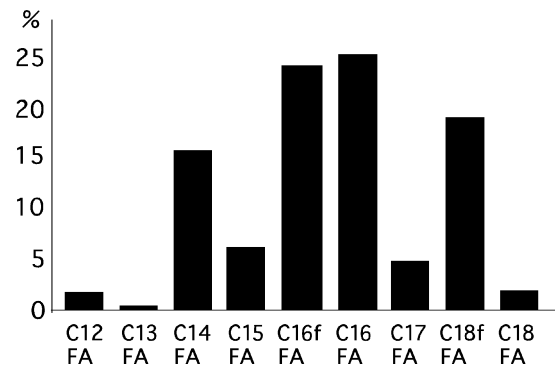

Figure 3. Fatty-acid ratio of human hair

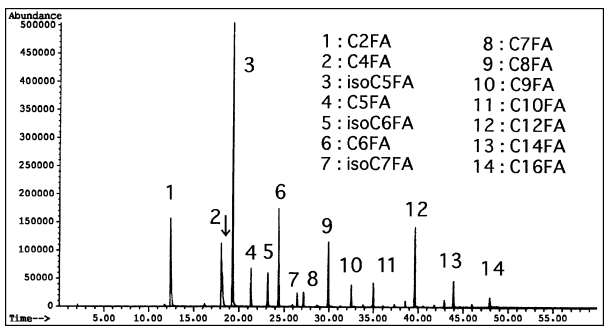

Figure 4. Fatty acids of HS analysis (60+, one day after non-perfumed shampoo)

When you used this product, did you notice any effect on head odor control?

(a) Yes.
(b) A little.
(c) Did not notice.

The hair over half of the head was washed with perfumed shampoo and rinse, while the hair over the other half was washed with non-perfumed shampoo and rinse. One day later, the amount of fatty acid present was analyzed and compared. Collection and analysis of samples was conducted under the same conditions as those for the HS analysis of head odor.

The results of the sensory evaluation of head odor showed that:

- Almost no bad odor was experienced immediately after washing with shampoo.
- At 3 days after washing, specific oily and fatty head odors were felt in all subjects, although there were individual differences in intensity and quality.

It has been reported that the sebum component of hair is recovered by 24 h after washing, and increases thereafter[4,5]. It is also reported that the ratio of fatty acids increases with time. Head odors seemed to be related to fatty acids when considered in relation to the quality of odors in the sensory evaluation. For this reason, the experiments were conducted focusing on the pathway of sebum microorganism fatty acid.[9]

First, the composition of hair collected from subjects was examined. In Figure 3, the ratio of fatty acids in the hair extracts of the sensory test subjects (8 persons in average) 3 days after shampoo is shown. Main fatty acids range from lauric acid (C12 FA) to stearic acid (C18 FA). In particular, the ratio of myristic acid (C14 FA), palmitoleic acid (C16 f FA), palmitic acid (C16 FA) and oleic acid (C18 f FA) was high.

Furthermore, fatty-acid composition was analyzed using the HS method to examine the composition of odors similar to those felt by people. A chart on the analysis of fatty acids is shown in Figure 4. In a 30 min sample, higher as well as lower fatty acids were detected; that is, those ranging from acetic acid (peak 1: C2 FA) to palmitic acid (peak 14: C16 FA).

Table 1. Example formula for head odor

Stearic acid (C18 FA)	20000
Oleic acid (C18 f 1FA)	50000
Palmitic acid (C16 FA)	80000
Palmitoleic acid (C16 f 1 FA)	30000
Myristic acid (C14 FA)	50000
Lauric acid (C12 FA)	30000
Capric acid (C10 FA)	25000
Pelargonic acid (C9 FA)	15000
Caprylic acid (C8 FA)	15000
Caproic acid (C6 FA)	6
Valerianic acid (C5 FA)	6
Isovalerianic acid (isoC5 FA)	6
Butyric acid (C4 FA)	1

Based on the data on the quality of odors in the sensory test, the data on higher fatty acids in the analysis of hair extracts, and the data on the low to high class fatty acid range in the HS analysis, we attempted to prepare head odors. An example of the formula for head odor is indicated in Table 1.

The odor of this mixture, composed of higher fatty acids and a trace amount of lower fatty acids, was recognized to be similar to head odors in the sensory test, not only by perfumers but also by lay people.

To develop a method of evaluation for materials controlling the formation of head-odor-causing fatty acids, experiments on the confirmation of fatty acid formation were performed. It is known that triglycerides in the lipids

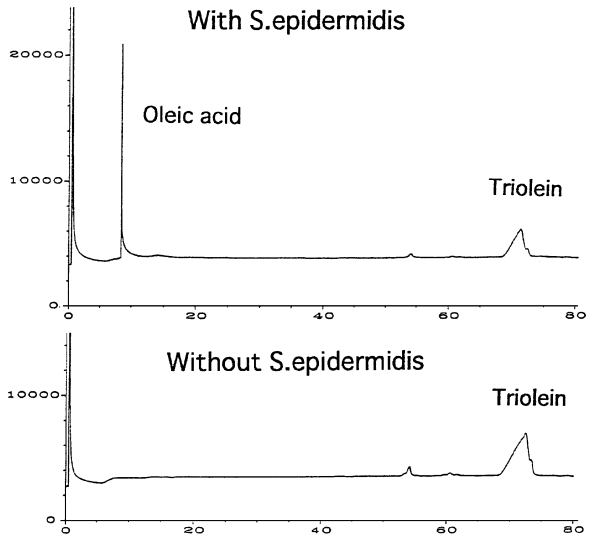

Figure 5. Oleic-acid formation from triolein by staphylococcus epidermidis

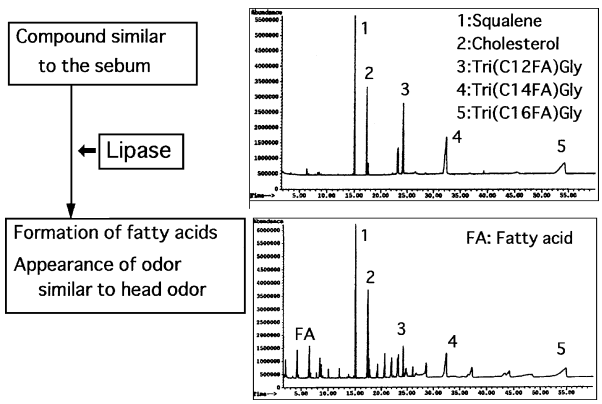

Figure 6. Fatty acids from triglycerides by lipase

secreted from sebaceous glands are hydrolyzed by a lipolytic enzyme, lipase, produced by bacteria resident in the scalp, thus resulting in the formation of fatty acids[4]. Therefore, experiments to confirm fatty-acid formation by bacteria resident in human skin and by lipase were performed.

Fatty acids produced from the hydrolysis of triglycerides by malassezia furfur and staphylococcus epidermidis were confirmed by GC analysis. A sample of the staphylococcus epidermidis case is shown in Figure 5.

Charts obtained before and after adding lipase are shown in Figure 6. As seen in the lower chart in Figure 6, the concentration of triglyderides (peaks 3, 4, 5) decreased, and fatty acids (FA) constituting each triglyceride are formed after the addition of lipase (reagent of Wako Pure Chemical Industries Ltd.). Simultaneously, odors specific to hair appeared. There were no significant changes in the amount of squalene (peak 1) or cholesterol (peak 2).

Judging from the data obtained from the above-described experiments, it was confirmed that triglycerides are hydrolyzed by lipase and microorganisms to form fatty acids that cause head odors. Therefore, using this model of experiment, evaluation of fragrance materials which control fatty acid formation was investigated. Approximately 70 synthetic and natural fragrance materials were tested for lipase, and approximately 20 typical fragrance materials were tested for microorganisms. Fragrance materials for which fatty-acid formation was found to be low in the lipase model are indicated in Figure 7.

The amount of fatty acids produced when a fragrance material such as benzyl alcohol or geraniol were added, shown in Figure 7, was approximately 5-20% of that formed when the fragrance material was not added.

In Table 2, typical fragrance materials controlling fatty-acid formation are listed, determined by evaluation from the lipase and microorganism models. Antibacterial refers to bacterial growth recognized to be small in macroscopic examination. Growth of staphylococcus epidermidis was similar to the minimal inhibitory concentration[2] of fragrance materials reported by Sawano et al. In the test using microorganisms, evaluation of fatty-acid formation control is affected by the antibacterial activity of the fragrance materials. An inhibitory effect on the hydrolysis of triglycerides was detected in the cases of aldehydes and alcohols that have high antibacterial activity. Also, in some cases of fragrance materials that have low antibacterial activity, formation of fatty acids was found to be low. It is thought that the fragrance materials of the latter cases may inhibit the activity of lipase.

'ES' in Table 2 refers to the ester formed from fatty acid and fragrance material being detected. 'ES' for staphylo-

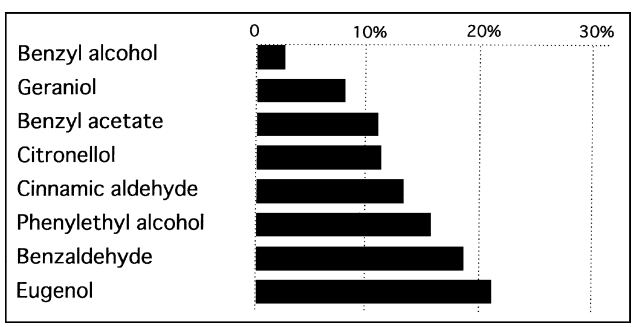

Figure 7. Fragrance materials controlling fatty-acid formation by lipase (GC areas of fatty acids without lipase : 100)

Table 2. Fragrance materials controlling fatty acid formation

Fragrance material	Lipase	S. epidermidis	M. furfur
Cinnamic aldehyde	Controlled	Controlled (antibacterial)	Controlled (antibacterial)
Eugenol	Controlled	Controlled (antibacterial)	Controlled (antibacterial)
Benzaldehyde	Controlled	Controlled (antibacterial)	Controlled(antibacterial), ES
Citronellol	Controlled, ES	Controlled (antibacterial)	Controlled(antibacterial), ES
Geraniol	Controlled, ES	Controlled (antibacterial)	Controlled(antibacterial), ES
Benzyl alcohol	Controlled, ES	Controlled (antibacterial), (ES)	Controlled(antibacterial), ES
Benzyl acetate	Controlled, ES	Controlled	Controlled(antibacterial), ES
Benzyl benzoate	Controlled, ES	Controlled	Controlled(antibacterial), ES
Citronellyl acetate	Controlled, ES	Controlled	Controlled, ES
Phenylethyl alcohol	Controlled, ES	Controlled, (ES)	Controlled, ES

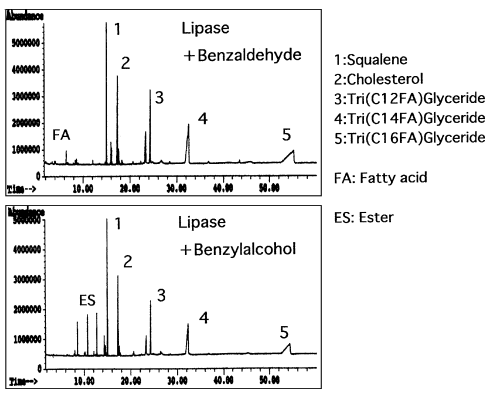

Figure 8. GC chart of benzaldehyde and benzyl acetate with lipase

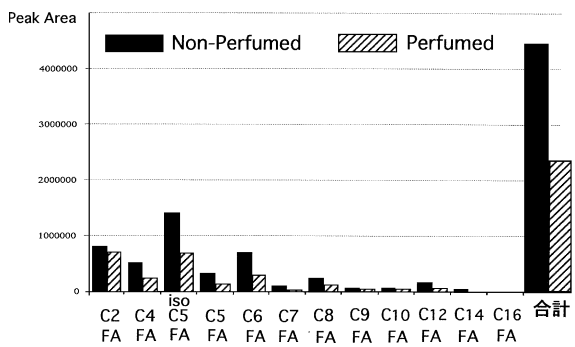

Figure 9. Fatty acids of each half head by HS analysis

coccus epidermidis indicates that the formation of esters with fatty acid was detected in other culture media. Fragrance materials that form esters with fatty acid using lipase were discovered, and these materials formed esters in the presence of M. furfur as well. In Figure 8, ester formation by lipase in the case of benzyl alcohol is compared with that in the case of benzaldehyde. Using benzaldehyde, there were no marked changes in squalene (peak 1), cholesterol (peak 2) or triglycerides (peaks 3, 4, 5), and only trace amounts of fatty acid detected. Using benzyl alcohol, a decrease in triglycerides was not significant, and the amount of fatty acid was negligible. However, esters corresponding to each fatty acid, identified by retention time and mass spectrometry, that is, esters such as benzyl laurate, benzyl myristate or benzyl palmitate, were detected. In other experiments using benzyl acetate, similar changes were observed.

Among the samples tested in this study, formation of esters from fatty acid was detected mainly in primary alcohols of aromatic and terpenes, and their esters. Many esters derived from fatty acid have a fruity or floral note in general, and are used also as perfumes.

In the experiment in humans, benzyl palmitate was detected in a trace amount in the GC-MS analysis of extracts obtained one day after application of benzyl alcohol solution.

Based on the results obtained from the experiment in which fatty-acid formation control was tested, a perfume with a bouquet note was prepared, using fragrance materials controlling fatty-acid formation and those producing esters with fatty acid. After confirmation by GC that this perfume decreases fatty-acid formation, evaluation was conducted using the sensory test and the half-head HS analysis in order to confirm the effect on odor control.

It was recognized in the sensory evaluation that the odor level was lowered when shampoo and rinse containing the above-described perfume were used, and evaluation was conducted based on the data from practical use. In 75% of persons who experienced disagreeable or slightly disagreeable head odors in ordinary living (36 persons: 46%), the effect on head-odor control was recognized.

To objectively assess the results of the sensory evaluation or the evaluation in practical use, hair over half of the head was washed with perfumed shampoo and rinse, and

hair over the other half was washed with non-perfumed shampoo and rinse. After drying in natural air, the HS analysis was conducted at 24 h later and data from each half-head was compared. The results showed that the amount of fatty acid in the part where perfumed shampoo and rinse were used was lower than that where non-perfumed shampoo and rinse were used (Figure 9).

We believe that this resulted from the antibacterial activity of the fragrance material added to the shampoo and rinse and from the control of fatty-acid formation due to the inhibition of lipase activity.

Conclusion

The results of our tests found, in summary:

- Head odors of ordinary persons are thought to be composed of fatty acids ranging from the lower to higher classes.
- We developed a method for the evaluation of fatty-acid formation resulting from the hydrolysis of triglycerides by the action of lipase or microorganisms, and found many fragrance materials that control fatty acid formation.
- We discovered a new method by which odors derived from fatty acid are controlled by esterification to become fragrant products, making use of a feature of lipase.
- We found a new method of controlling odors by using of an enzyme or bacteria resident in the skin without significantly changing the normal bacterial flora of human skin, not using antibacterial agents, covering odors by stronger fragrance materials, or masking odors by fragrance materials. We further believe that this method may be put to practical use for odors other than head odors in the future.

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