Mechanism and regulation of body malodor generation (1)

–Effect of iron in axillary malodor and using an antioxidant as a deodorant–

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SUMMARY

Using GC/MS and GC/Olfactmetry analysis, we identified two vinyl ketones such as 1-Octen-3-one (OEO) and cis-1,5-Octadien-3-one (ODO) as key materials in axillary odor. OEO and ODO showed a strong metallic odor and low odor threshold. These two materials were occurred from the reaction of unsaturated long fatty acids in lipids and the iron ion in our body's metabolism. Then, it was recognized that *Morus alba* (Japanese name, Kuwa) extract, one of the plant extract, showed a very good effect to control the generation of these vinyl ketones due to its remarkable anti-oxidization effects.

INTRODUCTION

The environment of our life is making sweating increasingly common. The changes in the living environment due to global warming, heat island phenomena, etc., are resulting in more and more people complaining about discomfort, embarrassment and malodor due to sweat.

Sweat is secreted by eccrine glands distributed over most of the body's surface and by apocrine glands distributed over specific areas. The sweat is decomposed by resident skin flora along with wastes and skin lipids, and this process generates malodor. As a result of sensory evaluation and instrumental analysis, we discovered that the vinyl ketones (1-Octen-3-one and cis-1,5-Octadien-3-one) are the key

components as human axillary malodor. These vinyl ketones showed a very strong metallic odor and a low odor threshold. So, we examined the hypothesis that they contributed greatly to axillary malodor.

Examination of the mechanisms for generation of vinyl ketones revealed that unsaturated fatty acids and iron in the body metabolism were the key factors. In vitro study on the antioxidant effects using several plant extracts, *Morus alba* extract showed the most effective result to control the generation of vinyl ketones.

EXPERIMENTAL

Collection and sensory evaluation of axillary malodors

After washing the body by themselves using the fragrance-free body soap, 66 adult male subjects wore T-shirts with odorless gauze sewn into the axilla region for 24 hours. The axillary malodors were collected from the gauze and subjected to sensory evaluation. These samples were classified according to the odor character and intensity of the axillary malodor.

Analysis of axillary malodor GC /MS /Olfactometry)

The collected axillary malodor was analyzed using dynamic headspace analysis and solvent extraction under the condition described below.

[Headspace analysis]

Headspace trap of axillary malodor in the gauze

☐Adsorption : Tenax TA 20 mg ☐Air : 100 mL/min. ☐Duration : 30min.

Thermal cold trap injection

☐Apparatus : Chrompack CP-4020 ☐Heating & Trapping Temp. : 200℃ & -100℃

☐Heating & Injection Temp. : 200℃

[Solvent extraction]

Axillary malodor was extracted from the gauze, dipped in diethyl ether 500 mL for 2 hours and filtered. The ether layer was concentrated and the residue (the axillary malodor) was injected into GC/MS/Olfactometry and analyzed. (Diethyl ether used in this extraction was distillated under the nitrogen atmospheres before use.)

[GC/MS/Olfactometry conditions]

☐ Gas Chromatograph : HP6890Plus(Agilent) ☐Column : DB-WAX(30 m x 0.25 mm)

☐Carrier gas : He

☐Oven Temp : initial temp, 50℃ hold 5min.→ rate, 4.5℃/min.→ final temp, 220℃ hold 20 min.

☐Mass : Agilent 5973 N at 70 eV

□Olfactometry : Gerstel ODP2

Iron content in sweat

Three male subjects sweated in a mini-sauna an 80℃ for 1 hour and about 1000mL of sweat was collected from the each subjects. Each of these three samples was then condensed and quantitative analysis of the iron content in human sweat was carried out using Inductively Coupled Plasma Atomic Emission Spectrometry (Seiko Instruments SPS 1500).

Generation and control of vinyl ketones (OEO and ODO)

Generation of vinyl ketones

Unsaturated long fatty acids such as linoleic acid or linolenic acid with tetradecane added at 0.01% to provide an internal quality standard was used as the reaction substrate. To the 1.0g of substrate, was added 0.005eq of $FeSO_4 \square 7H_2O$aq or $Fe_2(SO_4)_3 \square xH_2O$aq and the mixture was agitated at 40℃ for 1 hour. To this mixture, 10 mL of 0.2N EDTA2Na was added to stop iron activity. The reaction mixture was extracted using n-hexane (1 mL) and the hexane solution was analyzed using GC/MS/Olfactometry.

Anti-oxidization test Oven test)

One hundred μL of test solution was added to 3 g of linoleic acid. Methanol was added as a control. This oil mixture was spread on a petri dish, which was 60 mm in diameter, and incubated at 50℃. Conjugated diene, which was induced by oxidation, was measured by absorbance at 234 nm in the constant interval, and the molarity of the conjugated diene was calculated using the absorption coefficient. We then timed the oxidation period that the conjugated diene took to reach 100 mM. The antioxidant effect of the samples was evaluated by comparing the oxidation period with the control period.

Control of OEO generation

The same procedure was followed as in the OEO and ODO generation experiment except for adding 3.0g of 0.5% plant extract to the substrate. (to positive control, added 3.0 g of 0.5% tocopheryl acetate)

Rate of OEO generation(%) = (Amount of OEO generation(with plant extract) / Amount of OEO generation(without plant extract) × 100

RESULTS AND DISCUSSION

Sensory evaluation and GC, GC/MS analysis of axillary malodors

The axillary malodor was classified into five groups by sensory evaluation of the gauze, which were milk like, green, acidic, moldy and cumin-like as shown in Fig.1. The differences of the odor character from the axillary malodor among the five subjects were investigated by Gas Chromatograph using headspace analysis and solvent extraction, but GC peaks of all the samples showed the very similar patterns. It therefore appeared that the differences in the axillary malodor were based on very small quantities of specific ingredients. On GC/MS analysis of the axillary malodor, about 60 components were identified from the all the samples.

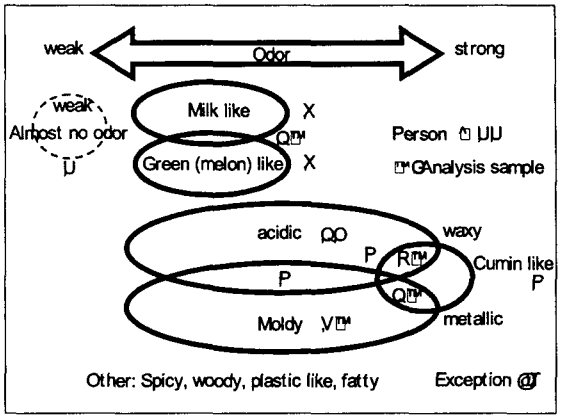


Fig. 1 Classification of human axillary malodor

Analysis of axillary malodor GC /Olfactometry)

Four types of typical axillary malodor such as milk like, green, acidic and moldy odor from each group classified in Fig.1 were analyzed by GC/O using headspace analysis. The very strong metallic odors with mushroom overtones were detected in the axillary malodor samples of all four subjects characterized by two different places on GC retention time. We estimated these odors would be the key compounds in axillary malodor.

Kamimura et al. reported that iron exists mostly in an axillary malodor patient's apocrine gland [1, 2], and Swoboda et al. commented on off-flavors generated by the butter fat and transition metal [3, 4]. These reports suggested that the metallic odor in axillary malodor is caused by vinyl ketones generated by a combination of unsaturated fatty acid and iron. As a result of GC/MS analysis shown in Fig.2, we identified that one compound with the mushroom-like metallic odor was 1-Octen-3-one (OEO). Although theo ther compound has not been identified by GC/MS analysis, it was estimated as cis-1,5-Octadien-3-one (ODO) by the comparison with GC/O analysis data of its reference material. These components were detected from the all of the four characteristic subjects as the axillary malodor. The odor threshold level for both of OEO and ODO was very low and these vinyl ketones have an intense

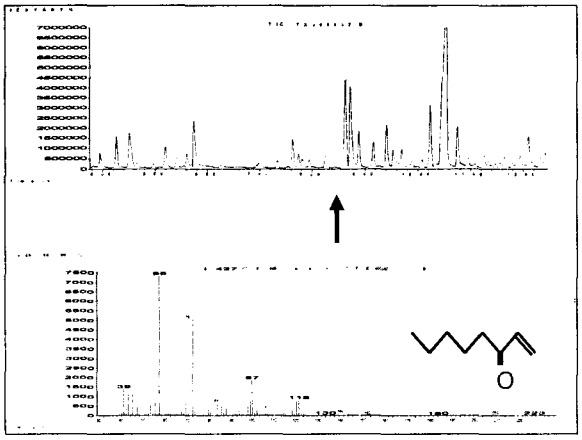


Fig. 2 GC / MS spectrum of OEO

metallic malodor. It is interesting that OEO and ODO were detected in human axillary malodor. The structure, a description of the odor, the reference odor threshold [5] and measured odor threshold of these vinyl ketones (OEO & ODO) using GC/O are shown in Table 1.

Generally, we experience an iron metallic malodor when grasping an iron bar or when blood adheres to the skin, etc. However, neither FeO nor FeSO4 gives off a metallic malodor, but a metallic malodor does occur when these compounds are touched. Similarly, when iron comes into contact with linoleic acid or linolenic acid, the generation of a metallic malodor has been noted.

Iron content in human sweat

The iron in sweat from all areas of the body was detected by Inductively Coupled Plasma Atomic Emission Spectrometry. When the quantity of iron in three male subjects' sweat was investigated, the sweat contained about 10 ppb of iron. Iron is an important metal of the living body, and it is indispensable component of various enzymes as Hemoglobin, NADH dehydrogenase, etc. And iron is stored in living cell in the form of ferritin.

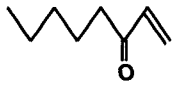
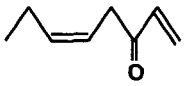
	P□ Octen-3-one (OEO)	Cis-P05-□f",□dien-3-one (ODO)
Structure		
Odor	MetallicC Mushroom like	MetallicCMoldy
Threshold tGC/ O□	P0P □□,	P0 □□,
Threshold fReferencej	0D0T ₣ ‡□ ɾ ‡	0D00P0₣ ‡□ ɾ ‡

Table 1 New key compounds in axillary malodor

Generation of OEO and ODO

It has been hypothesized that unsaturated long fatty acids are oxidized with iron and generate vinyl ketones. The hypothesis was tested in an experiment using linoleic or linolenic acid, and iron. GC/MS and GC/O analysis showed that OEO was generated from linoleic acid and ODO was generated from linolenic acid. Moreover, these reactions using Fe^{2+} proceeded more easily than using Fe^{3+}.

OEO, which exists in plants and food, is generated from fatty acids having the structure of ω -6, 1, 4-dien likeli noleic acid or arachidonic acid. ODO is similarly generated from fatty acid with a structure of ω -3, 1, 4, 7-trien, such as linolenic acid. It is reported that each of these compounds is generated by an enzyme reaction [5].

From these knowledge, we assumed that unsaturated fatty acids with a similar structure were oxidized with iron and then vinyl ketones such as OEO and ODO were generated. The estimated generation flow is shown in Fig. 3.

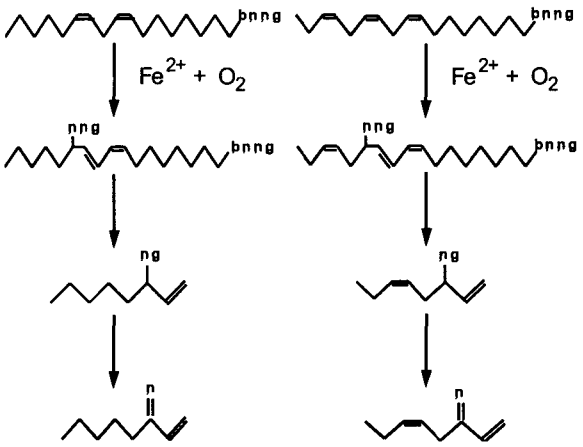


Fig. 3 Generation of vinyl ketones

Control of OEO Generation

To control the generation of vinyl ketones, the anti-oxidization effects of various plant extracts were screened by the anti-oxidization test. Among them, three kinds of plant extracts showed the excellent anti-oxidization effects. Furthermore, investigating their ability to control OEO generation, *Morus alba* extract showed the remarkable effects in linolenic acid and Fe^{2+} iron system shown in Fig.4.

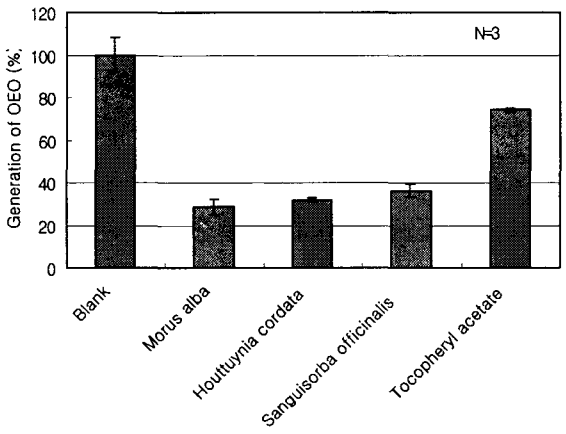


Fig. 4 Control of OEO generation by plant extracts

At the present study, it is not clear which step of the processes flow in Fig. 3 is controlled by antioxidant of the plant extracts. However, according to a result that the generation of allyl alcohol was also controlled, we think that *Morus alba* extract is effective against first or second step of Fig. 3.

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