Deodorizing effects of sugarcane extracts: deodorizing activity and active ingredients

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Abstract  In Okinawa, a non-centrifugal brown cane-sugar called Kokuto has been used for years to remove odors when cooking meat and fish. We focused on this deodorizing effect and developed a deodorizing substance from sugarcane mill byproducts. Trace constituents contained in volatile substances produced in the sugar-making process were absorbed on resins and were then eluted with ethanol - the extract is called 'Sugarcane extract (SCE2)'. Deodorizing activities of SCE2 were evaluated by organoleptic examinations (using cigarette odor, etc.) and by measuring the hydrogen-sulfide lowering rate using a gas-chromatography flame photometric detector (GC-FPD). SCE2 was also analyzed in open-column chromatography to isolate the primary components. Isolated components were tested to identify their deodorant activities, and a 1H-NMR analysis was conducted on the highly active components to determine their chemical structures. SCE2 showed higher deodorizing activity than commercially available deodorizing substances in the methyl mercaptan organoleptic deodorizing activity test. Superior deodorizing activity of SCE2 to typical deodorizing active components, such as β-cyclodextrin, was observed in the cigarette odor organoleptic examination, and SCE2 showed a higher hydrogen-sulfide lowering rate, measured by GC-FPD, compared to deodorizing products available in the market. Five components were isolated in the chromatography of SCE2, of which, three showed deodorizing activities. Structural analysis identified a stilbenoid structure in all three components, from which we concluded that the deodorizing-active component of the SCE2 is a sugarcane-derived polyphenol. This study proved that sugarcane has a deodorizing-active component and it can be extracted from the volatile substances produced in the raw-sugar process. That the deodorizing-active component has a polyphenolic structure supports the deodorizing mechanism, in which the polyphenol masks odor components by additive polymerization with them.

Key words  SCE2, deodorizing activity, sugarcane, MSX-245, polyphenol

INTRODUCTION

The deodorant/aromatic market in Japan is steadily increasing, growing from a value of under 100 billion yen in 2004 to 120 billion yen in 2014 (Fig.1). The largest market is for deodorant for use in spaces such as rooms or cars.

![Fig. 1. Sales in the deodorant/aromatic markets in Japan.](Image)
Similarly, the overseas deodorant/aromatic market is also steadily increasing, especially in Southeast Asia with the development of urban life. For example, the growth rate has increased by over 8% per year in the last 5 years in Thailand, and it is expected to increase by 8% or more per year in the next 5 years (Fig. 2). Therefore, the deodorant market is a fascinating field because it is a growing market throughout Japan and the world.

![Fig. 2. Deodorant/aromatic market and growth rate in Thailand.](image)

Deodorizing mechanisms are roughly divided into three types: chemical/physical deodorants, biological deodorants, and sensory deodorants. Chemical deodorants eliminate odors by breaking down odor substances, whereas physical deodorants eliminate odors by trapping them in pores. Physical deodorants include products such as cyclodextrin or activated charcoal. Biological deodorants eliminate odors by reducing the propagation of microorganisms, whereas sensory deodorants mask odors using other smells such as a perfume.

Active deodorant ingredients are divided into two categories: natural material products and chemical synthetics. Generally, the benefits of natural material products include a sense of security and reduced environmental impact. However, the deodorizing effect of natural products is often lower than that of chemical synthetics, and, due to the limited availability of raw materials, the cost of manufacture tends to be higher. Chemical synthetics are able to deodorize more and are superior to natural products in terms of mass production and the cost of manufacture; however, these products come with concerns of safety and persistence in environment. Hence, there has been increasing attention and a growing demand for natural deodorants materials that are both low in cost and high in deodorizing effect.

Dark molasses and brown sugar, both derived from sugarcane, have been used in cuisines in the Okinawa and Amami regions since ancient times. They add a rich taste to foods and reduce the smells of odoriferous ingredients. For this reason, sugarcane may have active deodorant or gustatory improvement ingredients. Mitsui Sugar Co., Ltd. has identified deodorant ingredients obtained from the byproducts of the sugar-manufacturing process and has commercialized these extracts for uses as deodorant (MSX-245) (Kawai et al. 2002). However, the active deodorant ingredients in MSX-245 have not been clearly defined and the mechanism has not been identified. In this study, we isolated the active deodorant ingredients from MSX-245, identified them and propose a mechanism for their activity.
MATERIALS AND METHODS

Isolation of active ingredients

We used the sugarcane extract MSX-245 from Mitsui Sugar Co., Ltd. MSX-245 is extracted by exposing vapor effluent from the sugar-manufacturing process onto acrylic ionic exchange resins at about 90°C, with Bed Volume (BV) = 10,000 and Space Volume (SV) = 100. After this step, absorbed ingredients are eluted by loading 80% (volume/volume [v/v]) ethanol with BV = 2 and SV = 2. After loading, 80% (v/v) ethanol, ion-exchanged water is added to wash out ethanol in the resin tower. After dilution with ion-exchanged water, the concentration of the eluted solution is approximately 60% (v/v) ethanol with BV = 2.5. The eluate is diluted to 45% (v/v) ethanol. After dilution, it is cooled to 10°C and the insoluble precipitates are filtrated with 0.5- and 0.45-μm filters to produce MSX-245.

We concentrated 4 L of MSX-245 using a rotary evaporator (pressure 90 mm Hg, temperature of vapor 30-40°C) to obtain 2.5 g of residues. Evaporation residues were then diluted with methanol and fractionated to six fractions (Fig. 3 I-VI) with an open column (filler: Sephadex LH-20, mobile phase: methanol). Of the six fractions, three showed high yield (Fig. 3 III-V). Fraction III was fractionated again by an 80% to 100% gradient using an ODS filler and a mobile phase of methanol. MSX-5 was obtained from recrystallization of III-1 (the product from re-fractionation of fraction III). Fraction IV was fractionated again using a Sephadex LH-20 filler and methanol. Fraction IV-2, obtained from fraction IV, was fractionated again under the gradient conditions using a Silica gel filler and a mobile phase, i.e., hexane:acetone = 3:1 to 1:1, to isolate three ingredients, MSX-2, MSX-3, and MSX-4. Fraction V was isolated by recrystallization; the resultant was MSX-1.

![Evaporation residue 2.5g](image)

**Fig. 3.** Schematic illustration of fractionation of the evaporation residues.

Deodorant tests of isolated ingredients

Concentrations of the isolated ingredients, MSX-1, MSX-2, MSX-3, MSX-4 and MSX-5, were adjusted to the same concentration as the percentage content of MSX-245 with 45% (v/v) ethanol. These samples were used in the following tests.
**Hydrogen sulfide lowering test**

Firstly, 0.1 mL of 720 mM hydrochloric acid was slowly added to 0.1 mL of 360 mM sodium sulfide solution in a 20 mL headspace vial and mixed. Then, it was put in an incubator at 30°C for 30 min to generate hydrogen sulfide gas. The headspace gas was used as hydrogen sulfide gas for each test. The hydrogen sulfide gas for a test (0.04 mL) was injected with an air syringe to a sealed headspace vial in which 1 mL of MSX-1, MSX-2, MSX-3, MSX-4, MSX-5, MSX-245 (positive control), or 45% (volume/volume) ethanol (Blank) was individually dispensed. After injection, vials were incubated at 30°C for 30 min and 0.1 mL of the collected headspace gas was analyzed by gas chromatography-flame photometric detector (GC-FPD) under the conditions outlined in Table 1. The results were evaluated by the hydrogen-sulfide lowering rate calculated with the following formula:

\[
\text{Hydrogen sulfide lowering rate (\%)} = \frac{\text{Area of hydrogen sulfide in blank} - \text{Area of hydrogen sulfide in a sample}}{\text{Area of hydrogen sulfide in blank}} \times 100
\]

**Table 1. Analysis conditions using GC-FPD.**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>G-3900 (Hitachi, Ltd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CHROMPACK CP-Silica Plot (φ0.32 mm × 15 m)</td>
</tr>
<tr>
<td>Detector</td>
<td>Flame Photometric Detector (FPD)</td>
</tr>
<tr>
<td>Temperature of the column</td>
<td>220°C</td>
</tr>
<tr>
<td>Detection temperature</td>
<td>245°C</td>
</tr>
<tr>
<td>Temperature of a sample inlet</td>
<td>200°C</td>
</tr>
<tr>
<td>Analysis time</td>
<td>0.8 min</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>N2 200 kPa</td>
</tr>
</tbody>
</table>

**Sensory test against a single odor**

Measurement samples were prepared by adding either 1 ppm (weight/volume) of methyl mercaptan solution or 0.5 mL of trimethylamine (TMA) solution to a 1.5 mL Eppendorf tube with 0.05 mL of MSX-1, MSX-2, MSX-3, MSX-4, MSX-5 or MSX-245 each. Tubes were then shaken vigorously. An Eppendorf tube with methyl mercaptan solution or TMA solution only was used as a blank control. Panelists were asked to sniff the test samples to evaluate the efficacy of each deodorant and odor offensiveness. Deodorant effectiveness was evaluated based on the six-step odor intensity scale and the mean value was determined. The six-step scale was: 0: no odor; 1: very faint odor (detection threshold); 2: faint odor that can be discerned (recognition threshold); 3: distinct odor that can be detected easily; 4: strong odor; 5: very strong odor.

**Sensory test against complex odors**

Cigarette smoke was collected in a 5 L upside-down Erlenmeyer flask for 1 min. A piece of 100% cotton cloth (10 cm × 10 cm) was placed in a smoke-filled Erlenmeyer flask and sealed. The flask was shaken for 5 min, the cloth removed and used as a deodorant object. A solution of each sample, diluted by 200 times with water, was spread on the deodorant object by spraying 0.15 g five times (total = 0.75 g). The object was then left standing for 3 h and then used for the sensory test. We used 45% (volume/volume) ethanol as a blank control and MSX-245 as a positive control.

The effect of each deodorant on sensory perception was evaluated using four scales: 0: odor was completely eliminated; 1: odor was almost eliminated; 2: odor was slightly eliminated; 3: odor was not eliminated.

**RESULTS AND DISCUSSION**

**Isolation of active ingredients**

As a result of the fractionation of evaporation residues of MSX-245, 310 mg of MSX-1, 40 mg of MSX-2, 3 mg of MSX-3, 12 mg of MSX-4, and 11 mg of MSX-5 were isolated. The yield against evaporation residues was 12.4% for MSX-1, 1.6% for MSX-2, and under 1% for other isolated ingredients.
**Hydrogen sulfide lowering test**

The hydrogen sulfide lowering rate was 60% for MSX-245, 25% for MSX-1, 46% for MSX-2, 51% for MSX-3, 42% for MSX-4, and 24% for MSX-5 (Fig. 4). Because strong hydrogen sulfide lowering rates were observed in MSX-2, MSX-3, and MSX-4, we concluded that the hydrogen sulfide lowering activity was derived from these three ingredients.

![Graph showing hydrogen sulfide lowering rates for different fractions](image1.png)

*Fig. 4.* Lowering rates of hydrogen sulfide by the five fractions.

**Sensory test against a single odor**

Results of the sensory test using isovaleric acid and TMA are shown in Figure 5. In contrast with the evaluation of strong odor to very strong odor for the blank, the odor for positive control of MSX-245 as well as MSX-2, MSX-3, and MSX-4 was very faint. Although odor intensity was lower in MSX-1 and MSX-5 compared to that of the blank, the deodorant effect in these compounds was lower than that in MSX-2, MSX-3 and MSX-4. Therefore, we concluded that the deodorant activity of MSX-245 against isovaleric acid or TMA is mainly derived from MSX-2, MSX-3 and MSX-4.

![Graph showing sensory test results for different fractions](image2.png)

*Fig. 5.* Deodorant activity of the five fractions in isovaleric acid and TMA by a sensory test.
Sensory test against complex odors

MSX-2, MSX-3, and MSX-4 completely eliminated complex odors, as did the positive control MXS-245. MSX-1 (slightly eliminating) and MSX-5 (almost eliminating) had deodorant properties weaker than that of the positive control. The blank control, as expected, did not eliminate odors.

Identification of active ingredients

The instrumental analysis by GC-FPD and the sensory test with isovaleric acid and TMA showed that MSX-2, MSX-3 and MSX-4 had deodorant activity. Therefore, we identified these three ingredients using 1H-NMR. All three were confirmed as novel polyphenols with a stilbenoid structure (Fig. 6) as:

- **MSX-2**: 4-hydroxy-3-(2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl)-5-methoxystyrene.
- **MSX-3**: 5-(2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl)-vanillin.
- **MSX-4**: 4-hydroxy-3-(2-hydroxy-2-(4-(2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethoxy)-3,5-dimethoxyphenyl)ethyl)-5-methoxystyrene.

![Fig. 6. Active deodorant ingredients in MSX-245.](image)

Because MSX-2 was more abundant than the other compounds and its deodorant activity is strong, we conclude that MSX-2 is the main deodorant ingredient in MSX-245.

CONCLUSIONS

Active deodorant ingredients in MSX-245 were identified as novel polyphenols and sugarcane polyphenols. Many polyphenol materials have been shown to be effective as deodorants; for example, the deodorant mechanism of green tea extract of catechin (Yasuda and Arakawa 1996).
We consider that the deodorant mechanism of sugarcane polyphenols is two-fold: they include odoriferous ingredients by repeating additive polymerization and making human sensory difficult; they prevent the release of odors by preventing changes and decomposition of substances due to antioxidation.

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REFERENCES


Effets désodorisants d’extraits de canne à sucre: degré de désodorisation et ingrédients actifs

Résumé. A Okinawa, un sucre brun de canne non centrifugé, appelé Kokuto a été utilisé depuis des années pour éliminer les odeurs lors de la cuisson des viandes et poissons. Nous avons étudié cet effet désodorisant et développé un coproduit désodorisant. Des constituants a faibles concentrations contenus dans les substances volatiles produits pendant la fabrication ont été absorbés sur les résines et sont ensuite élués avec de l'éthanol : l'extrait s'appelle « Extract de la canne à sucre (SCE2) ». Les activités de désodorisation du SCE2 ont été évaluées par des examens organoïliques (à l'aide de l'odeur de cigarette, etc.) et en mesurant la vitesse de la diminution de l'odeur du sulfure d'hydrogène à l'aide de chromatographie en phase gazeuse avec un détecteur photométrie de flamme (GC-FPD). La SCE2 a été également analysée en chromatographie colonne ouverte pour isoler les composants primaires. Les composants isolés ont été testés afin d'identifier leurs activités de désodorisation, et on a réalisé une analyse de la RMN du 1H sur les composants hautement actifs pour déterminer leurs structures chimiques. La SCE2 a montré une activité désodorisante supérieure en comparaison avec des produits de désodorisation commerciaux, en se servant de tests organoïliques basés sur le méthyl mercaptan. La SCE2 est aussi supérieure à d'autres désodorisants tels que les β-cyclodextrine, à partir d'examens organoïliques avec l'odeur de cigarette; la SCE2 a montré un taux plus élevé de diminution du sulfure d'hydrogène, mesuré par GC-FPD, par rapport aux produits désodorisants disponibles sur le marché. Cinq composants ont été isolés dans la chromatographie de la SCE2, dont trois ont montrés des activités désodorisantes. L'analyse structurale a identifié une structure stilbénoïdes dans chacun des trois composants; nous avons conclu que le composant actif de désodorisation de la SCE2 est un polyphénol de la canne à sucre. Cette étude a prouvé que la canne à sucre a un composant actif pour désodoriser; il peut être extrait des substances volatiles produits pendant la fabrication de sucre brut. Sa structure polyphénolique permet au composant de masquer les odeurs par polymérisation additive avec elles.

Mots-clés: SCE2, activité de désodorisation, canne à sucre, MSX-245, polyphénol

Efecto deodorizador de un extracto de caña de azúcar: actividad deodorizadora e ingredientes activos

Resumen. En Okinawa un azúcar pardo no centrifugado, denominada Kokuto, se ha empleado desde años para remover los olores durante la cocción de las carnes y pescados. Nos enfocamos en el efecto deodorizador y desarrollamos un sustancia deodorizadora de los sub productos del azúcar cençurado. Constituyentes traza, contenidos en las sustancias volátiles producidas en el proceso de fabricación de azúcar, se absorvieron en resinas y se eluaron con etanol, el extracto se denomina “Extracto de Caña de Azúcar (SCE2). Las actividades deodorizantes del SCE2 fueron evaluadas mediante exámenes organoílicos (empleando olor de cigarrillos, etc.) y mediante mediciones de índice de reducción del sulfito de hidrógeno, empleando cromatografía de gas y un detector de fotometria de llama (GC-FPD). El SCE2 se analizó también con cromatografía de columna abierta para aislar sus componentes primarios. Los componentes aislados se utilizaron para identificar su actividad deodorizante y un análisis 1H-NMR se hizo de los componentes altamente activos para determinar su estructura química. El SCE2 mostró una actividad deodorizadora superior a la de las sustancias deodorizadoras comerciales disponibles, cuando se evaluaron en las pruebas organoílicas de actividad deodorizante al metil mercaptano. Se observó una actividad deodorizadora superior del SCE2 respecto a la actividad deodorizantes como la β-ciclodextrina, en las pruebas organoílicas al olor del cigarrillo, así mismo el SCE2 mostró una superior reducción del nivel de sulfito de hidrógeno, medido por GC-FPD, comparado con productos existentes en el mercado. Se aislaron cinco componentes en la cromatografía del SCE2, tres de los cuales mostraron actividad deodorizante. Los análisis estructurales identificaron estructuras estilboides en los tres, de lo que se concluyó que el componente con actividad deodorizante en el SCE2 es un fenol derivado de la caña de azúcar. El presente estudio demostró que la caña de azúcar posee un componente de actividad deodorizante y que este puede ser extraído de las sustancias volátiles producidas durante el proceso de fabricación de azúcar; que el componente tiene una estructura polifenólica apoya el mecanismo deodorizador, en el cual los polifenoles enmascaran los componentes odoríferos mediante una polimerización aditiva con ellos.

Palabras clave: SCE2, actividad deodorizadora, caña de azúcar, MSX 245, polifenol