Note

Appetite-Enhancing Effects of Curry Oil

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Inhalation of scent compounds with phenylpropanoidal structures, such as *trans*-cinnamaldehyde, is expected to increase the appetite. The scent of curry powder is well known for its appetite-enhancing effect on humans. In this work, we show that the appetite of mice after inhalation of curry powder essential oil or benzylacetone showed a similar increase. The components of curry oil, *trans*-cinnamaldehyde, *trans*-anethole, and eugenol, each showed appetite-enhancing effects; therefore, these three scent compounds may be the active compounds in curry powder oil.

Key words curry oil; inhalation; appetite-enhancing effect; spice

Inhalation of *trans*-cinnamaldehyde, which is abundant in cinnamon essential oil,[1] and its analogue benzylacetone, increases food intake in mice.[2] Both *trans*-cinnamaldehyde and benzylacetone contain the analogue moiety of phenylpropanoids, and our previous results indicate that phenylpropanoidal compounds possibly have appetite-enhancing effects.

Curry powder consists of various spices, such as cinnamon, cloves, and fennel, that contain phenylpropanoids as their main oil components.[3,4] The scent of curry powder is well known for its appetite-enhancing effects in humans, and in this work, we examine the appetite-enhancing effects in mice of inhaling the scent of curry powder, some of its essential oil components, and analogues of the components.

MATERIALS AND METHODS

Materials Compounds used in this study (Fig. 1) were obtained as follows. Benzylacetone (>95%), eugenol (>99%), *trans*-anethole (>98%), safrole (>96%) and β-caryophyllene (>90%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). *trans*-Cinnamaldehyde (>98%) was purchased from Nacalai Tesque (Kyoto, Japan). *p*-Allylanisole (estragole; >98%) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Triethyl citrate (>98/%) was purchased from Merck (Darmstadt, Germany). The materials for distillation of spices were curry powder (S&B Foods Inc., Tokyo, Japan) and fresh ginger (*Zingiber officinale* Roscoe, Zingiberaceae), and they were purchased from grocery stores. Authentic compounds for the retention indices on gas chromatography (GC) analyses were decane, dodecane, tetradecane, hexadecane, octadecane (Wako Pure Chemical Industries, Ltd., Osaka, Japan), docosane, tetracosane (Nacalai Tesque), and eicosane (Tokyo Chemical Industry). All other chemicals and reagents used in this study were of the highest grade available.

Animals The animal studies were designed according to the guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006) and the recommendations of the Animal Research Committee of Kyoto University, Kyoto, Japan (authorisation number: 2014–17). Four-week-old male ddY mice (approximately 16 g at the time of purchase) were from Japan SLC (Shizuoka, Japan). The mice were housed in colony cages (6 mice per cage) at an ambient temperature of 25±2°C under a 12-h light–dark cycle. The mice used in the feeding tests (approximately 19 g at the time of the experiments) were fasted for up to 24 h before starting the tests, but water was available *ad libitum*. Fasting time was determined according to the previous studies conducted by Tankam *et al.* to empty the stomach of a 4-week-old ddY mouse.[5] All studies were conducted from 08:00 to 17:00.

Distillation of Spices Curry powder (37 g) and fresh ginger (81.7 g) were hydrodistilled for 3 h using the Clevenger apparatus designated in the Japanese Pharmacopoeia 16th edition,[6] and the distilled oils were captured in hexane (Nacalai Tesque). The essential oils were dried over anhydrous sodium sulphate and stored at −20°C before analysis and animal experiments. The amount of essential oil obtained from curry powder was 0.2372 g and that from fresh ginger was 0.0546 g.

Gas Chromatography and Gas Chromatography-Mass Spectrometry Analysis Curry and ginger oils were diluted in hexane and analysed on a GC-MS system (6850GC/5975MSD, Agilent Technologies; Santa Clara, CA, U.S.A.) equipped with a DB-WAX column (60 m ×0.25 mm, 0.25 μm film thickness; Agilent Technologies). The operation conditions were as follows[7]: injector temperature, 180°C; oven program starting at 60°C and holding at this temperature for 4 min; increasing by 2°C/min to 180°C; increasing by 10°C/min to 200°C and holding at this temperature for 30 min. The operation conditions were as follows: carrier gas, helium; column flow, 1.0 mL/min; split ratio, 99 : 1; injection volume, 1 μL. The components of curry essential oil and of ginger essential oil were identified by comparing their mass spectra with those in an MS data library (NIST11, National Institute of Standards and Technology) and/or those in the literature. The content ratios of the oil components were calculated by using the charts obtained with a GC system (GC-FID G-5000, Hitachi, Tokyo, Japan) equipped with an Inertcap WAX column (60 m×0.25 mm, 0.25 μm film thickness; GL Sciences; Tokyo, Japan). The operation conditions were as follows: injector temperature, 180°C; detector temperature, 200°C (flame ionisation detector; FID); carrier gas, helium; column flow, 1.0 mL/min; split ratio, 99 : 1; injection volume, 1 μL. The oven program was the same as that for GC-MS. Quantitative analysis was achieved with an FID.

Feeding Test Feeding tests were performed according

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to our previous study.[2] The control group inhaled air after triethyl citrate was placed in the cage. The positive control was 4.5×10^{-4} mg/cage benzylacetone.[2] The oils were used for experiments after checking that they contained no hexane by using solid-phase microextraction-GC-MS.

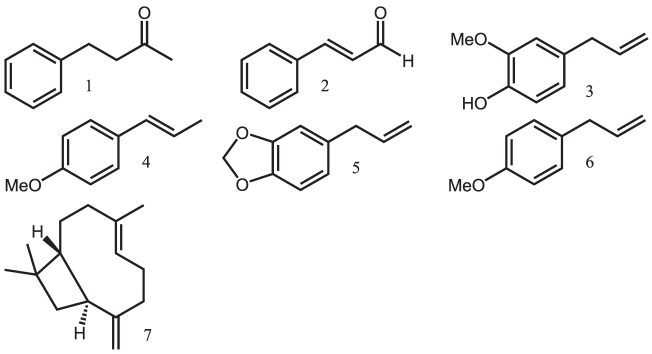

Fig. 1. Compounds Tested in This Study

1, Benzylacetone; 2, *trans*-cinnamaldehyde; 3, eugenol; 4, *trans*-anethole; 5, safrole; 6, estragole; 7, *β*-caryophyllene.

Statistical Analysis Results are presented as the mean±standard error of the mean (S.E.M.). Statistical analyses were performed by Dunnett's test[2] using GraphPad Instat (GraphPad Software; San Diego, CA, U.S.A.). A probability level of $p<0.05$ was considered statistically significant.[2]

RESULTS

trans-Cinnamaldehyde, eugenol, and *trans*-anethole showed appetite-enhancing effects. The oil composition analysis of curry powder essential oil showed these three compounds accounted for 24.85% of the oil composition (Table 1), and the oil showed a strong appetite-enhancing effect (Fig. 2). *trans*-Cinnamaldehyde, eugenol, and *trans*-anethole increased food intake by 0.95, 0.92, 0.91 times the increase induced by curry oil, respectively (Fig. 2).

Table 1. Main Components of Curry Powder and Ginger Essential Oil

Components	Curry powder essential oil		Ginger essential oil	
	Peak area (%)	RI	Peak area (%)	RI
β-Phellandrene	—	—	5.46	1212
γ-Terpinene	1.16	1246	—	—
δ-Elemene	1.02	1473	—	—
α-Copaene	1.71	1497	—	—
Linalool	3.13	1550	1.17	1550
Bornyl acetate	—	—	1.70	1584
2-Undecanone	—	—	1.31	1600
β-Caryophyllene	4.64	1600	—	—
Terpinen-4-ol	—	—	1.41	1606
Myrtenal	—	—	1.13	1631
Neral	—	—	10.81	1684
α-Terpineol	—	—	1.83	1702
endo-Borneol	—	—	3.28	1708
Zingiberene	1.25	1724	8.59	1724
β-Bisabolene	—	—	1.61	1730
Geranial	—	—	22.29	1734
α-Farnesene	—	—	1.92	1750
δ-Cadinene	1.87	1761	—	—
β-Sesquiphellandrene	2.57	1772	3.04	1772
α-Curcumene	1.01	1775	1.41	1775
2-Methyl-3-phenylpropanal	11.46	1781	—	—
Methylenebornane	2.45	1793	—	—
trans-Anethole	15.18	1827	—	—
Geraniol	—	—	2.10	1852
7-*epi*-*cis*-Sesquisabinene hydrate	—	—	1.07	2004
trans-Cinnamaldehyde	2.70	2045	—	—
cis-Nerolidol	—	—	1.40	2051
p-Cymen-7-ol	1.82	2118	—	—
7-*epi*-*trans*-Sesquisabinene hydrate	—	—	1.95	2119
Zingiberenol	—	—	2.55	2129
Eugenol	6.97	2172	—	—
Turmerone	7.81	2185	—	—
β-Eudesmol	—	—	1.00	2232
Curlone	6.31	2240	—	—
ar-Turmerone	10.32	2251	—	—
Unknowns	—	—	3.70	—
Main Component total	83.38	(18 compounds)	80.74	(24 compounds)
Other compounds	16.62	(36 compounds)	19.26	(41 compounds)

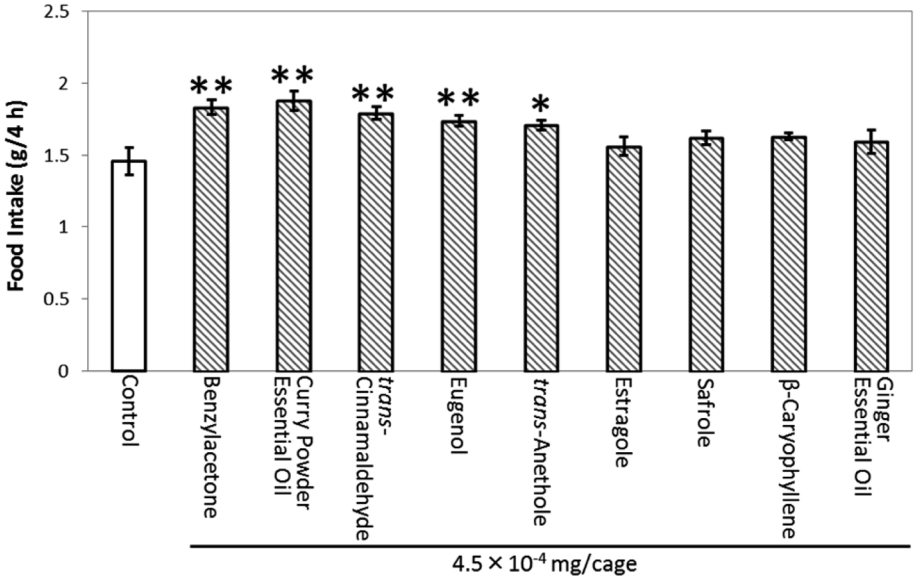

Fig. 2. Appetite Enhancement in Mice Treated with Fragrances

Control: triethyl citrate inhalation. Positive control: benzylacetone inhalation. Data are expressed as the mean±S.E.M. for 8 mice. The statistical analysis was performed using one-way ANOVA followed by Dunnett's test. *$p<0.05$, **$p<0.01$ *vs.* the control group.

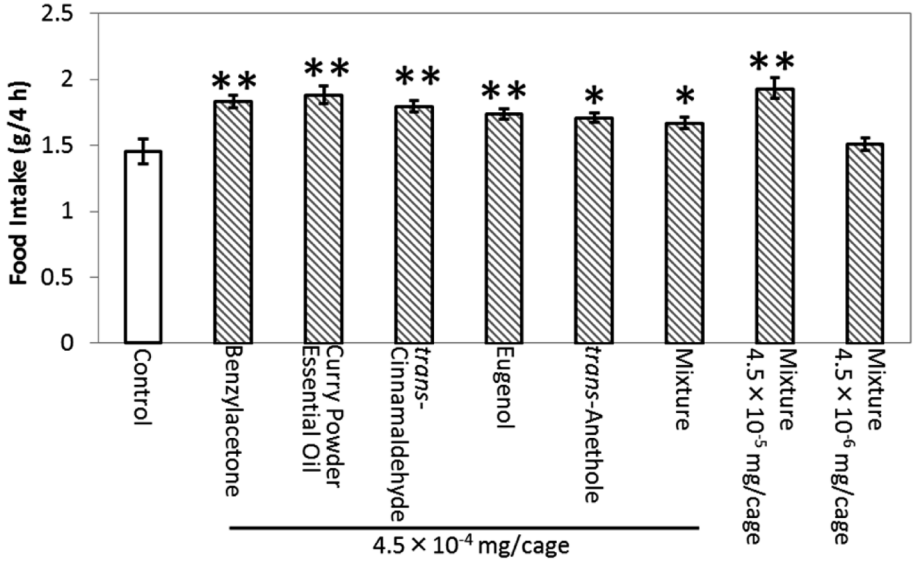

Fig. 3. Effect on Appetite in Mice of Inhaled Mixtures of Active Compounds in Curry Essential Oil

Control: triethyl citrate inhalation. Positive control: benzylacetone inhalation. Mixture: mixture of *trans*-cinnamaldehyde/eugenol/*trans*-anethole (1.00 : 2.58 : 5.62). Data are expressed as the mean±S.E.M. for 8 mice. The statistical analysis was performed using one-way ANOVA followed by Dunnett's test. *$p<0.05$, **$p<0.01$ *vs.* the control group.

β-Caryophyllene, ginger essential oil containing zingiberene, estragole, and safrole did not show appetite-enhancing effects.

A mixture of *trans*-cinnamaldehyde, eugenol, and *trans*-anethole in a ratio of 1 : 2.6 : 5.6 (Table 1) as they are in curry oil at a dose of 4.5×10^{-4} mg/cage was administered to mice, and its appetite-enhancing effect was weaker than that of each component alone (Fig. 3). However, at a dose of 4.5×10^{-5} mg/cage of the same mixture showed appetite-enhancing effects equivalent to that of curry oil.

DISCUSSION

Curry powder essential oil showed an appetite-enhancing effect that was equivalent to that of benzylacetone. The phenylpropanoids contained in curry powder essential oil, *trans*-cinnamaldehyde, eugenol, and *trans*-anethole, also showed appetite-enhancing effects. Ginger is often contained in curry powder,[3,4] and is used medicinally in acrid as well as aromatic stomachics.[8] For this reason, the appetite-enhancing effects of inhalation of ginger essential oil in mice were investigated. However, *β*-caryophyllene and ginger essential oil containing zingiberene, which is a sesquiterpene with a benzylacetone-like structure, did not show appetite-enhancing effects (Fig. 2). In our previous study,[2] phenylpropanoids showed appetite-enhancing effects, whereas terpenoids and geranium essential oil, which are mainly composed of terpenoids, did not show such effects. These results suggest that phenylpropanoids may

have appetite-enhancing effects.

However, some phenylpropanoids, like safrole and estragole, did not show appetite-enhancing effects. Some phenylpropanoids, including safrole, have low affinity for the olfactory receptor to which eugenol binds.[9] Furthermore, Furudono *et al.*[10] reported that eugenol and vanillin[7] stimulated a different type of olfactory receptor from the receptors that have high affinities with *trans*-cinnamaldehyde, *trans*-anethole, and estragole. These results suggest that the appetite-enhancing effects of inhaling curry powder essential oil may be caused by stimulation of several receptors.

The rhizome of *Z. officinale* is used as a natural medicine, such as ginger (*Shōkyō*, 生薑) and processed ginger (*Kankyō*, 乾薑), and the pungent-tasted compounds [6]-gingerol[11] and [6]-shogaol[12] are the marker compounds for these natural medicines in Japanese Pharmacopoeia 16th edition (JPXVI). However, ginger essential oil in this study did not contain these compounds. There are less of these marker compounds in the methanol extract of fresh ginger than in dried or steamed extracts.[13] Others studies reported that the essential oil of dried ginger did not contained [6]-gingerol.[14] Our finding that steam-distilled ginger oil contained only monoterpenes and sesquiterpenes confirmed these previous results.

The mixture of the active compounds of curry powder essential oil, *trans*-cinnamaldehyde, eugenol, and *trans*-anethole, was administered to mice *via* inhalation, and it enhanced the appetite of mice at a dose of 4.5×10^{-5} mg/cage. The effect was more potent than that at a dose of 4.5×10^{-4} mg/cage (Fig. 3). Fujiwara and Ito reported that inhalation of a mixture of five essential oils decreased the locomotor activities of mice more than the inhalation of the five essential oils individually.[15] Our previous study indicated that the appetite-enhancing effects diminished when the compounds were administered above the concentration at which the strongest effects were observed.[16] These results suggest that appetite-enhancing effects of a mixture of active compounds were greater than those of the individual compounds, and that the strongest effect of the mixture would be observed at a lower concentration (4.5×10^{-5} mg/cage) compared with those of the individual active compounds (4.5×10^{-4} mg/cage). Therefore, the mixture of active compounds showed weaker effects at 4.5×10^{-4} mg/cage. The three active compounds accounted for 24.85% of the curry oil, and the rest of the oil consisted of terpenoids, such as β-caryophyllene and linalool, that did not show appetite-enhancing effects. The terpenoids might function as an inactive organic solvent in curry oil. Therefore, the active compounds can be diluted with inactive compounds.

The curry powder used in this study contained spices such as cumin seed (*Cuminum cyminum* L., Umbelliferae), cardamom (*Elettaria cardamomum* (L.) Maton, Zingiberaceae), cinnamon (*Cinnamomum zeylanicum* J. Presl, Lauraceae), clove (*Syzygium aromaticum* (L.) Merrill & Perry, Myrtaceae), coriander (*Coriandrum sativum* L., Umbelliferae), bay leaf (laurel; *Laurus nobilis* L., Lauraceae), allspice (*Pimenta dioica* (L.) Merr., Myrtaceae), garlic (*Allium sativum* L., Liliaceae), turmeric (*Curcuma longa* L., Zingiberaceae), chilli (*Capsicum annuum* L., Solanaceae), ginger, and black pepper (*Piper nigrum*, Piperaceae).[3] The curry powder was ready-made; thus, the proportions of spices in the curry powder were not readily available. The components of the curry oil (Table 1) suggested that in addition to cinnamon and clove, the curry powder contained fennel (*Foeniculum vulgare* Mill., Umbelliferae) or aniseed (*Pimpinella anisum* L., Umbelliferae).[1,17,18] The curry powder essential oil showed an appetite-enhancing effect that was equivalent to the effect of benzylacetone, and *trans*-cinnamaldehyde, eugenol, and *trans*-anethole were the active compounds of the essential oil. This suggests that essential oils of spices such as fennel, cinnamon, and clove[2,3] would show appetite-enhancing effects, because they contain the active compounds of curry oil as their main components. Kariyone[8] described the fragrant compounds of herbal medicines, such as cinnamon bark, clove, and aniseed, contained in stomachics sold as over-the-counter or prescription drugs as possible active compounds in these products, and results of our study indicate that the active compounds of aromatic stomachics might be *trans*-cinnamaldehyde, eugenol, and *trans*-anethole.[1,17,18]

Volatile compounds with a phenylpropanoidal structure have often showed appetite-enhancing effects in our previous studies. It may be interesting to investigate scent compounds in foods, spices, and herbal medicines that show biological activities similar to curry powder. Collecting evidence about scent compounds with biological activities will help establish a pharmacological approach for flavours and fragrances.

Conflict of Interest The authors declare no conflict of interest.

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