ureases or other enzymes [cf. Amos et al. (1980)]. Finally, our observations that DMU is a selective reagent for tyrosine residues in proteins suggests that it may have special value in studying the role of tyrosine in structural proteins and enzymes.

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Physicochemical Aspects of Sweetness in Cocoa Drinks

Olugbenga A. Ogunmoyela and Gordon G. Birch*

The role of lecithin and glycerol monosterate in modifying the sweetness of cocoa drinks was investigated. While both surfactants enhanced the sweetness of the drinks and both reduced their surface tension, only glycerol monostearate increased their viscosity. Statistical analysis of panellists' responses showed that a significant inverse relationship exists between surface tension and sweetness response, the latter being measured in terms of both intensity and persistence. These results help to resolve conflicting reports of the effects of hydrocolloids on basic taste. They are discussed in terms of possible mechanisms of taste chemoreception.

Many surfactants or emulsifying agents find frequent use in food systems, being generally chemically similar to stabilizers, solubilizers, and wetting agents (Nash and Brickman, 1972). However, most studies of the gustatory effects of hydrocolloids in foods have been concerned mainly with the investigation of viscosity [e.g., Moskowitz and Arabie (1970), Vaisey et al. (1969), and Pangborn et al. (1978)], and relatively little work has shown quantitative relationships between taste intensity and other physical properties of the stimulant. In their attempts to elucidate relationships between relative sweetness of molecules and various physicochemical properties, Ferguson and Lawrence (1958) suggested that surface tensions of solutions of sapid molecules could affect their penetration into taste bud pores or alter the permeability characteristics of taste cells and thereby affect taste response. More recently, DeSimone (1980) has suggested that a lowering of surface tension may cause taste intensity to be lowered. Harkins (1954) has stated that the importance of surface effects is best illustrated by highly disperse or colloidal systems, since colloidal particles generally exhibit a relatively high activity of so-called surface forces. It is not known whether DeSimone's conclusions are applicable to such colloidal systems.

Thermodynamically, a physicochemical system tends to assume the condition in which its free energy is lowest. Since surface molecules attract each other, forming a film of greater or less strength, there is a resulting surface tension which may in turn affect molecular volume. Thus, modification of the effects of stimulus molecules in the dynamics of taste chemoreception by modifying surface tension should be very significant. West (1963) noted that the degree of molecular association and the strength of intermolecular forces of attraction in colloidal solutions are governed by surface tension, and since according to Shallenberger's AH–B concept (Shallenberger and Acree, 1967) these considerations underlie the stereochemistry of taste, it is conceivable that surface tension effects may be more important than viscosity effects in gustation.

Although sucrose like all sugars is surface inactive (Browne and Zerban, 1941), it may form complexes with surfactants (Birch and Ogunmoyela, 1980a) which enhance sweetness response. In the work reported in this paper, the viscosities and surface tension values of solutions of sucrose-sweetened cocoa drinks to which increasing con-

National College of Food Technology, University of Reading, St. George's Avenue, Weybridge, Surrey KT13 0DE, United Kingdom.

centrations of surfactants glycerol monostearate (GMS) and lecithin (LEC) have been added are compared with corresponding magnitudes of elicited sweetness response (in terms of both subjective intensity of sweetness and persistence time). Attempts are made to quantify the relative importance of viscosity and surface tension in the modification of sweetness response and to explain the role of surfactants in the dynamics of taste chemoreception.

MATERIALS AND METHODS

Preparation of Test Solutions. Solutions for tasting were made up by using cocoa powder (Cadbury's Bournville) and 5% (w/v) sucrose (AR grade from BDH, Poole, England) at 10, 20, and 30% (w/v). Surfactants GMS (also obtained from BDH, Poole, England) and lecithin (LEC) Wylfo "A" (obtained from Wynmouth Lehr and Fatoils, Ltd., London) were added at the three concentrations of 0, 0.5, and 1.0% (w/v), respectively. A solution of 5% (w/v) cocoa powder alone was made up and presented to panellists as the standard.

Both surfactants had no interfering odor or taste, and complete mixing was achieved by using the Ultra-turrax tissue macerator. The solutions were then placed in a water bath at a controlled temperature (50 °C) for 2–3 h before presentation to panellists.

Aqueous solutions of both surfactants were also made up (0.15–0.55% w/v) for viscosity determinations while solutions of 5% (w/v) sucrose and surfactants GMS and LEC (0–0.8% w/v) were prepared for surface tension measurements.

Cocoa solutions used for tasting were also subjected to viscosity and surface tension measurements.

Apparent Viscosity Measurements. Apparent viscosities were measured on the Brookfield Synchro-Lectric viscometer (UL adapter model) because of the very low viscosities of the solutions. For the concentrations of surfactants used, measurements were made at 12 rpm and 20 °C for aqueous solutions and 50 °C for test cocoa drinks for consistency, and readings were converted to centipoise (cP) by using conversion factors supplied by the instrument manufacturer.

Surface Tension Measurements. Both the cocoa solutions described above and the aqueous sucrose–surfactant solutions were subjected to surface tension measurements by using the Du Noüy tensiometer (Cambridge Instrument Co., Ltd., London) which is a torsion balance method in which a platinum loop is dipped into the liquid and the force (in millinewtons per meter) required to separate the loop from the surface is measured directly on a calibrated scale. Since surface tension decreases with increasing temperature, like viscosity, readings were made on solutions at 20 °C for aqueous solutions and 50 °C for test cocoa drinks under equilibrium conditions, but exactly similar trends were observed. The three concentrations of surfactant tested allowed the trends to be established.

Time–Intensity Measurements. Ten-milliliter aliquots of the solutions of cocoa drinks for tasting were maintained in the thermostat at 50 °C and were only presented to each of 10 panellists immediately prior to tasting to ensure fairly uniform solution temperature (50 °C) at the time of tasting.

Panellist evaluations were carried out after appropriate training using the moving chart recorder ["SMURF" = Sensory Measuring Unit for Recording Flux (Birch and Munton, 1981)] linked to a potentiometer "dial box", the dial of which could be moved from 0–10 arbitrary units and back again according to the intensity of basic taste experienced and its duration in the mouth. Details of this procedure have been described earlier (Birch and Ogun-

Table I. Apparent Viscosities of Sucrose-Sweetened Cocoa Drinks Containing Varying Levels of Added Surfactants GMS or LEC

concn of added sucrose, % (w/v)	apparent Brookfield viscosity, cP					
	GMS concn, % (w/v)			LEC concn, % (w/v)		
	0	0.50	1.00	0	0.50	1.00
10	15.0	35.0	77.5	15.0	15.0	17.5
20	20.0	85.0	97.5	20.0	22.5	25.0
30	22.5	90.0	112.5	22.5	25.0	27.5

Table II. Static Surface Tension Values of Sucrose-Sweetened Cocoa Drinks Containing Varying Levels of Added Surfactants GMS or LEC

concn of added sucrose, % (w/v)	static surface tension, mN/m					
	GMS concn, % (w/v)			LEC concn, % (w/v)		
	0	0.50	1.00	0	0.50	1.00
10	59.5	51.2	50.7	59.5	45.4	44.3
20	59.6	51.7	50.9	59.6	45.4	44.1
30	59.8	52.3	51.1	59.8	45.1	43.8

moyela, 1980b). Four solutions were presented to panellists at each sitting, one of which was the standard, given an arbitrary score of 3.0 on the SMURF dial. The standard solution of 5% (w/v) cocoa powder gave a mean apparent viscosity of 13.0 cP and surface tension of 59.2 mN/m. Having established this level for the standard after instructions, panellists then judged the other test solutions on the SMURF in comparison to the standard. The high value of 3.0 for the standard (zero-added sucrose) tended to cause compression of subsequently higher sweetness values. The SMURF measurements are therefore basically category scaling values rather than magnitude estimation values. The same panellists were used throughout in order to minimize panellist variations. All samples were tasted and swallowed. All panellists tasted each of the concentrations of added sucrose in cocoa, containing each of three concentrations of added surfactant (i.e., nine solutions for each surfactant). Solutions were presented in random order.

RESULTS AND DISCUSSION

Viscosity and Sweetness Response. The effect of surfactant concentration on apparent viscosity for aqueous solutions of both GMS and LEC and the modified cocoa drinks to which they are added is shown in Figure 1. While LEC does not appreciably affect the viscosity of the solutions, GMS caused a steady increase of viscosity with increasing concentration of surfactant. This is clearly evident also from Table I which lists the apparent viscosity changes for test cocoa solutions containing added surfactants and increasing concentrations of sucrose. Pangborn et al. (1973) have shown (using aqueous solutions of various sapid compounds containing low concentrations of selected hydrocolloids) that modification of taste intensity was independent of viscosity and appeared to be related more to other physicochemical properties of the hydrocolloid and the sapid compound. Moskowitz and Arabie (1970), however, using selected hydrocolloids at higher viscosities, showed that an increase in viscosity decreases taste intensity. Table II lists the surface tension values of the test cocoa drinks while Tables III lists the sweetness intensities and persistence times of the solutions as judged by panellists for both types of surfactant. When a regression model (Bennett and Franklin, 1954) was tested to consider whether viscosity effect is important after allowing for surface tension, using the F distribution, it was found that $F_{3,81} = 1.1$ or 0.95, respectively, for GMS or LEC, which is not significant. This model was based on combined

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Table III. Sweetness Intensities and Persistence Times of Sucrose-Sweetened Cocoa Drinks Containing Varying Levels of Added Surfactants GMS or LEC[a]

concn of added sucrose, % (w/v)	sweetness intensity[b,d] 0-10 scale of arbitrary units					
	GMS concn, % (w/v)			LEC concn, % (w/v)		
	0	0.50	1.0	0	0.50	1.00
10	6.45 (0.85)[e]	7.30 (1.11)	7.90 (0.85)	6.45 (0.85)	7.19 (1.37)	7.80 (1.12)
20	7.50 (0.59)	8.26 (0.79)	8.51 (0.98)	7.50 (0.59)	7.72 (1.06)	8.15 (1.00)
30	7.95 (0.98)	8.40 (0.96)	8.92 (0.73)	7.95 (0.98)	8.14 (1.01)	8.60 (0.92)

concn of added sucrose, % (w/v)	persistence time,[c,d] s					
	GMS concn, % (w/v)			LEC concn, % (w/v)		
	0	0.50	1.00	0	0.50	1.00
10	24.35 (14.30)[e]	26.75 (15.42)	28.45 (17.78)	24.35 (14.30)	25.20 (14.64)	27.75 (16.73)
20	24.55 (15.23)	35.95 (12.78)	36.50 (12.75)	24.55 (15.23)	31.55 (13.03)	31.95 (13.32)
30	26.60 (14.40)	36.65 (13.69)	39.80 (14.55)	26.60 (14.40)	34.75 (13.95)	39.50 (14.49)

[a] Mean values of 10 replicated judgments. [b] Sweetness intensities were significantly different at different concentrations of sucrose addition ($p < 0.001$). Sweetness intensities were significantly different from different concentrations of surfactant addition ($p < 0.01$) but not significantly different between surfactants. [c] Persistence times were significantly different from different concentrations of sucrose ($p < 0.01$) and at different levels of surfactant addition ($p < 0.001$) but not significantly different between surfactants. [d] Standard solution = 5% (w/v) cocoa powder only, given an arbitrary score of 3.0 on the SMURF dial scale for sweetness intensity but the mean persistence time = 11.85 s (SD = 6.26 s). [e] SD.
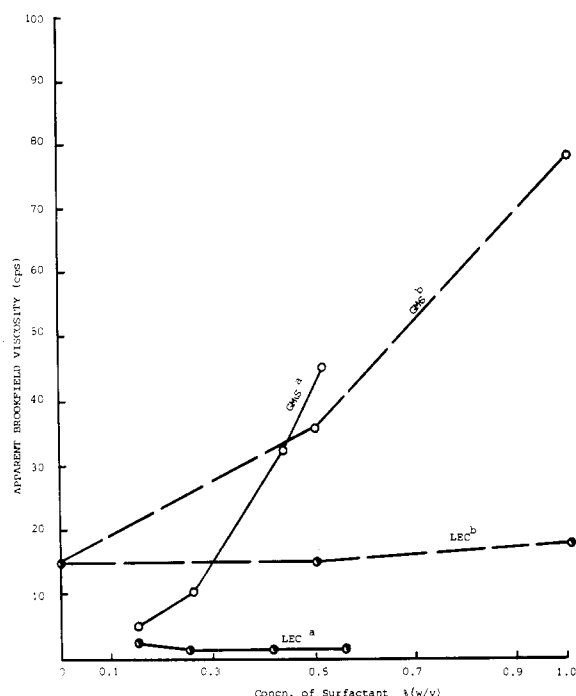

Figure 1. Plots of apparent viscosities vs. concentration of surfactants in (a) aqueous solutions of 5% (w/v) sucrose, (b) modified cocoa drinks containing 5% (w/v) cocoa powder and 10% (w/v) sucrose for surfactants GMS and LEC, respectively. (O) Glycerol monostearate (GMS); (◑) lecithin (LEC).
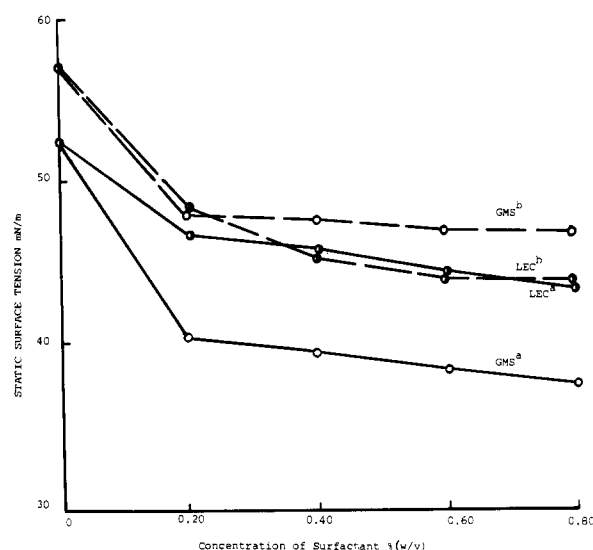

Figure 2. Plots of static surface tension values vs. concentration of surfactants in (a) aqueous solutions of 5% (w/v) sucrose, (b) modified cocoa drinks containing 5% (w/v) cocoa powder and 5% (w/v) sucrose for surfactants GMS and LEC, respectively. (O) Glycerol monostearate (GMS); (◑) lecithin (LEC).

effects of log sweetness intensity, surface tension, and viscosity, using the results presented in Tables I, II, and III. Individual panellist responses rather than the means presented in Table III were used in the analysis.

Surface Tension and the Sweetness Response. Figure 2 shows the depression of surface tension caused by increasing concentrations of surfactants GMS and LEC in aqueous sucrose solutions as well as modified cocoa drinks. Over the range of 0–0.8% (w/v), the surfactants cause a decrease of surface tension of 5% (w/v) sucrose solutions amounting to 23–28% for GMS and 11–17% for LEC, but the depression achieved in the same range in solutions of cocoa drinks containing 5% (w/v) cocoa powder, 5% (w/v) sucrose, and surfactant is found to be 15.7–18.5% for GMS and 15.4–23.2% for LEC. At high concentrations of added sucrose (10–30%) as shown in

Table II, a lowering of surface tension ranging from 13.9 to 14.8% for GMS solutions and 23.7 to 25.5% for LEC solutions is observed. In spite of this apparently large difference between the two surfactants, it may be seen from Table III that there are no significant sensory differences between cocoa drinks containing either GMS or LEC at the same concentration (panellist responses for sweetness in terms of either subjective intensity or persistence time), which indicates that the *accession efficiency* of the sweet stimulus to the receptor is independent of the nature of the surfactant. Apparently, however, it is related to the concentration of each surfactant because there is a significant difference in sweetness between all concentration steps.

In solutions of sapid molecules, any depression of surface tension could significantly enhance taste response by increasing diffusion of stimulus molecules to the taste receptor environment. However, for a solution of surface-inactive stimulus molecules like the sugars, an increase in concentrations leads to *increased* surface tension, and since an increase in concentrations gives greater taste intensity,
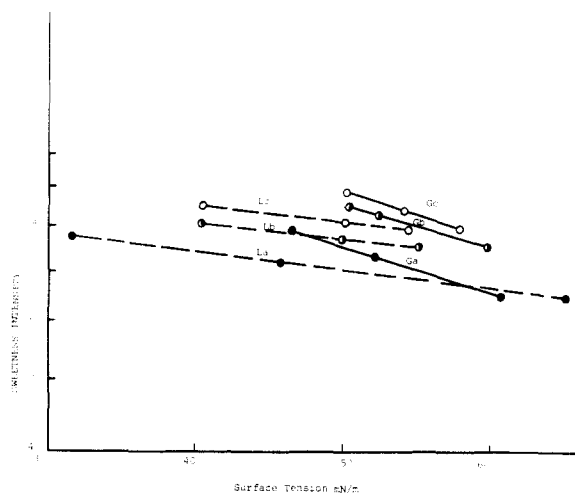
Figure 3. Log-linear plots of fitted regression models for subjective intensity of sweetness vs. surface tension showing depression of surface tension with increasing sweetness for modified cocoa drinks. Points on each line correspond to 1.0, 0.5, and 0% (w/v) surfactant addition (from left to right), and mean values of 10 panellists are plotted. Ga, Gb, and Gc and La, Lb, and Lc lines correspond to 10, 20, and 30% (w/v) sucrose with GMS and LEC, respectively.

Figure 4. Log-linear plots of fitted regression models for persistence time vs. surface tension showing depression of surface tension with increasing persistence time (seconds) for modified cocoa drinks. Points on each line correspond to 0, 0.5, and 1.0% (w/v) surfactant addition (from left to right), and mean values of 10 panellists are plotted. Ga, Gb, and Gc and La, Lb, and Lc lines correspond to 10, 20, and 30% (w/v) sucrose with GMS and LEC, respectively.

conclusions opposite to our own might be drawn for solutions without added surfactant.

By use of the usual techniques of regression analysis for comparisons of treatment effects (Bennett and Franklin, 1954), the regression of log subjective sweetness intensity or persistence time against surface tension was examined and the lines were parallel (i.e., constant slopes for increasing concentrations of sucrose), implying that the surface tension effect is constant for increasing concentrations of added sucrose. Fitted models were obtained from which log–linear plots of sweetness intensity vs. surface tension and persistence time vs. surface tension are derived (Figures 3 and 4, respectively). The fitted models are as follows (SI = sweetness intensity; PT = persistence time; γ = surface tension): for GMS, $\log_e$ SI = 2.7597–0.0148γ (for 10% w/v sucrose), 2.8855–0.0148γ (for 20% w/v sucrose), and 2.9298–0.0148γ (for 30% w/v sucrose), and $\log_e$ PT = 5.3388–0.0409γ (for 10% w/v sucrose), 5.5923–0.0409γ (for 20% w/v sucrose), and 5.6852–0.0409γ (for 30% w/v sucrose); for LEC, $\log_e$ SI = 2.2324–0.00567γ (for 10% w/v sucrose), 2.3269–0.00567γ (for 20% w/v sucrose), and 2.3809–0.00567γ (for 30% w/v sucrose), and $\log_e$ PT = 4.031–0.0185γ (for 10% w/v sucrose), 4.204–0.0185γ (for 20% w/v sucrose), 4.352–0.0185γ (for 30% w/v sucrose).

Differences between the surfactants in terms of the rate at which log SI or log PT changes as surface tension is decreased were found to be significant ($p < 0.05$) for log SI but not significant for log PT. This seems to indicate that each surfactant is specific in its efficiency of taste modification. Since different surfactants would modify the lipophilic characteristics of sapid stimulus molecules to different extents and at different rates depending on the characteristics of the complexes formed, these differences in rates of change of log SI with decreasing surface tension are to be expected for the different surfactants.

Thus, in highly dispersed colloidal systems of surface-active molecules, such as exist in cocoa drinks, an inverse relationship appears to exist between log subjective intensity or persistence time and surface tension, while the effects of low viscosities (up to 112.5 cP) are less significant. McLaughlin and Dilger's (1980) suggestions from studies on the adsorption of weak acids to membrane–solution
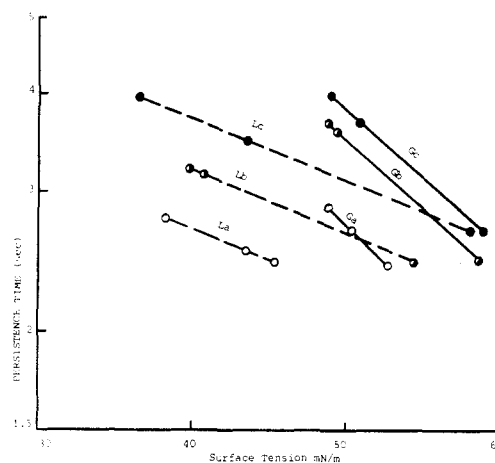
interfaces, that molecules may adsorb to the interface by means of hydrophobic forces and that energy barriers may be encountered at membrane–solution interfaces which may affect the flux of stimuli across membranes, seem to support the importance of surface effects since reduced surface tension means a reduction in surface energy. If, in addition, resulting elevated lipophilicity is also a requirement for taste enhancement (Kier, 1972), then the importance of surface tension effects in solutions of sapid molecules can be readily appreciated.

More recently van der Heijden et al. (1979) have demonstrated that the sweetness of aspartyl dipeptide methyl esters is dependent on the parachor ([P]) which is in turn related to surface tension (γ) by the formula

$$[P] = V\gamma^{1/4}$$

where V = molecular volume.

The same authors have also shown that hydrophobic and steric factors are of great importance in the sweetness response, and these conclusions may apply to a common receptor site for all classes of sweet stimuli. While our own results with cocoa drinks certainly indicate that surface tension is more important that viscosity as far as taste modification is concerned, it is clear that an interplay of many factors related to surface tension may be involved in the taste chemoreception process. Thus, it is not yet possible to decide whether surface tension changes alone may be expected to modify taste or whether complexation of the sweet stimulus with a surfactant is a prerequisite. The answer to this question must await the outcome of further sweetness studies in which the possibility of stimulus complexation is eliminated.

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Isolation and Identification of Banana-like Aroma from Banana Shrub (*Michellia figo* Spreng)

Hisayuki Toda, Kenji Yamaguchi, and Takayuki Shibamoto*

Banana shrub (*Michelia figo* Spreng) flowers were collected in late April 1980. The aroma constituents of the petals of these flowers were extracted with organic solvents and analyzed by using GC/MS techniques. The banana-like aroma of this flower was due to the high content of ester derivatives. The composition of the first fraction obtained from ethanol distillation was 65% esters, 1.8% monoterpenes, and 19.1% sesquiterpenes. The main component, which gives a fruity banana-like aroma, was isobutyl acetate (47.1%). One novel ketone, (8Z,11Z,14Z)-8,11,14-heptadecatrien-2-one, was isolated and identified by high- and low-resolution MS, ^{13}C NMR, ^{1}H NMR, and IR. This ketone could be derived from linolenic acid, which came from the wax of the flowers.

Banana shrub (*Michelia figo* Spreng) is an evergreen low-growing shrub (approximately 5 m) native to Southern China. The aroma constituents of its flowers have not been analyzed prior to this study. The flower is approximately 3 cm in diameter and consists of six light yellow petals. It blooms in March and April and gives a pleasant banana-like fragrance on warm, clear days. In its native country, China, it is called the "flower containing a laugh" and has been used as an accessory by young girls for their hair. The aroma from banana shrub flowers has long attracted people, and its fruity, banana-like fragrance is quite unusual among flower aromas. In this study, the volatile chemicals related to the banana-like fragrances were isolated and identified by GC/MS techniques. A novel ketone isolated from this organic solvent extract was identified by using high- and low-resolution MS, ^{1}H NMR, ^{13}C NMR, and IR. The volatile aroma constituents of natural banana and those of banana shrub were compared.

EXPERIMENTAL SECTION

Sample Preparation. Fresh flowers of the banana shrub were collected in Nishinoomote City, Kagoshima, Japan, at the end of April. The flower petals (1.5 kg) were placed in a 5-L round-bottomed flask with 1 L of ethanol. The flask was connected to a condenser, and the volatile constituents were distilled from the ethanol solution.

The distillates were collected into five fractions (100 mL each). Each fraction was marked as 1–5 according to its elution order. Distilled water (100 mL) was added to each fraction. Each fraction was extracted with 100 mL of

Department of Environmental Toxicology, University of California, Davis, Davis, California 95616 (T.S.), and Ogawa & Co., Ltd., 6-32-9 Akabanenishi, Kita-Ku, Tokyo, Japan (H.T. and K.Y.).

Table I. Yields and Odor Description of Fractions 1–5

fraction	yield, %[a]	odor description[b]
1	0.007	banana-like, floral
2	0.017	spicy
3	0.011	sweet
4	0.005	woody
5	0.004	sweet–woody

[a] (Quantity of oil recovered)/(quantity of petals used) × 100. [b] Examined by five trained perfumers.

isopentane and subsequently reextracted with 100 mL of an isopentane–ether (65:35) solution by using a liquid-liquid continuous extractor. Extraction were continued for 48 h each. The isopentane and isopentane–ether extracts were combined, and the solvents were removed by distillation. The yields of oils (obtained from each fraction) relative to the weight of petals used and the odor descriptions of each fraction are shown in Table I.

Identification of the Aroma Constituents. The oil obtained from fraction 1, which gave the most banana-like odor, was analyzed by GC/MS. Identification of gas chromatographic peaks of the oil was made by comparison of their mass spectra and gas chromatographic retention indexes to those of authentic compounds. For some compounds, standard samples were not available to confirm positive identification. If the mass spectrum matched precisely that of published data and the retention could be estimated from the published data, the compound was listed as tentatively identified.

A Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector was used for routine work. The gas chromatograph was fitted with an all-glass injector splitter of our own design to avoid any contact with metal surfaces in order to avoid artifacts and was operated with an injector split ratio of 100:1. Peak