



## Aroma Profile and Phenolic Content of Honey Wine Produced from Wild Rose Fruit

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In this study, two chromatographic techniques were applied to determine the aroma profile and phenolic compounds of “Honey wine” produced by addition of Wild Rose Fruits (*Rosa odorata*-Andrews-Sweet). In addition, another control wine was produced with sucrose and rose fruits in order to differentiate compounds produced by yeast fermentation from those originated from honey and rose fruits. After maturation aroma components, phenolic compounds and sugar composition of wines were identified. Aroma components were determined by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME/GC-MS) techniques in wine samples. The dominant aroma components in both wines were ethanol, ethyl acetate, isobutyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl caprylate, ethyl decanoate, capronate ethyl. Some minor differences in the percentage of major aroma components were determined between two wine samples. Phenolic and sugar compositions were identified by high performance liquid chromatographic (HPLC) method in fruit extract and wine samples. While the total amount of phenolic compounds were 3604.9 mg/l in fruit juice, 74.3 mg/l in control wine and 111.8 mg/l in honey wine, the most abundant phenolics in both wines were catechin, epicatechin and routine.

**Keywords:** Fermentation, HS-SPME/GC-MS, HPLC, *Rosa odorata* (Andrews) sweet, Yeast

### Introduction

The Rosaceae is one of the largest family of plants with more than 100 genera and 2830–3100 species.<sup>1</sup> Rose plant belongs to *Rosa* L. genus and member of Rosaceae family.<sup>2</sup> Fruits of some wild rose genus have been used as a food supplement and for pharmaceutical drugs, as an additive in probiotic beverages, soups, yogurt and as a health supplement for many years.<sup>3</sup> In addition, the fruits contain high amounts of bioactive components such as phenols, carotenoids (lycopene), vitamins and carbohydrates.<sup>4,5</sup> *Rosa odorata* (Andrews) Sweet also called wild rose hip has been used in the production of rose water and rose oil in some countries.<sup>6</sup>

Wine is an ancient drink made by the fermentation of grape must or some other fruit juices. Grape is the most widely used fruit in wine making since the ancient times. There have been other fruits also suitable for wine production other than grapes. In recent years, fruit wines have also been produced from various other fruits such as caja, mango, banana, apple, cherry, papaya, blackberry, orange and pineapple.<sup>7</sup> Regarding the fruity impression, it is closely related to the sense of

freshness in fruit wines. Consequently, the oxidation process in other words and the loss of fruity aroma compounds, may importantly affect the perfect balance of a high quality wine flavour.<sup>8</sup> Honey can be used in wine making, replacing sugar because of its antioxidant activity.<sup>9</sup>

Phenolic acids, catechins and other flavonoids, which are phenolic compounds have an essential role in the formation of the quality of wine. These compounds improve sensory properties and significantly contribute to colour formation, flavour, astringency and hardness.<sup>10</sup> In addition, phenolic compounds have some contributions to overall health, associated with the consumption of fruits and vegetables.<sup>11</sup>

Aroma, other than being one of the most noteworthy characteristics of a wine, is also a major indicator of its quality.<sup>12</sup> There are various methods used to determine the aroma compounds of wines.<sup>13</sup> In the present study SPME technique was used in order to determine the aroma components of wine samples. SPME is a solvent-free technique, in which the sample in liquid or gaseous phase is absorbed onto an absorbent like fused-silica fibre, and then either thermally sent directly into a gas chromatography

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equipment or the solvent is carried into a high-performance liquid chromatography equipment.<sup>14</sup> The technique is based on an extension of laser desorption from fused silica fibers, which provides extremely fast chromatographic separation.<sup>15</sup>

The main purpose of this study is to determine the differences in the characteristic features of two different wines made with sugar and honey prepared by using *Rosa odorata* (Andrews) Sweet fruit, which has not been used in winemaking before. Chemical and analytical properties of wild rose fruits were also determined before it was used in wine making. After fermentation, characteristic properties like total phenolic compounds, aroma components and sugar compositions of wines were determined.

## Materials and Methods

### Winemaking, Fermentation Conditions and Monitoring

Fresh fruits of *Rosa odorata* (Andrews) Sweet used for wine production were harvested from Konya province in Turkey. Fully ripe fruits were chosen and they were separated from leaves, stems and stalks. After cleaning, the fruits were crushed without breaking the seeds and finally the seeds were removed. Two different wines were produced by addition using honey or sucrose and fruits of *Rosa odorata* (Andrews) Sweet. The first wine was produced with natural prairie honey and rose fruits. In order to make better comparison of compounds arising from honey, the second wine was produced for control purposes with sucrose and rose fruits only. A 750 g of cleaned and crushed fruits were placed in 2.5 liter anaerobic fermentation vessel. After the addition of the fruits, 2 liters of natural spring water was added and the mixture was macerated for 6 hours by gentle stirring. Thus, all soluble sugars and other soluble nitrogenous compounds in the fruits were transferred to the must. After maceration, the sugar amount in the must was measured 9°Brix (Bx) and honey was added until the sugar content was to reach 29 °Bx. Titratable acidity of the must was adjusted to 4.5 g/l with potassium tartrate and the pH was adjusted to 3.9 by addition of citric acid. The final °Bx of both musts were measured as 29 °Bx. The control wine was prepared by following the same protocols except that the sucrose replaced with honey. Both musts were pasteurized at 65°C for 15 min in water bath and then cooled down to room temperature. Freeze-dried yeast culture was activated for 2 hours in 10% sucrose at 20°C then 0.2 g/l (20 g/hl) of yeast culture (*Saccharomyces cerevisiae* var. *cerevisiae*, Fermivin

N°7013, Oenobrand, France) was added to both musts. After mixing the vessels were left for fermentation at room temperature. The fermentation process continued for around 15-20 days until most of the sugars were consumed. Fermentation vessels were kept in dark and the temperature was measured between 15 to 20°C during fermentation. During the fermentation process the amount of sugar was measured by refractometer (Atago, Japan) daily. After the fermentation process, the lees were removed from premature wines by racking. After first racking maturation of wines was done at 15°C for 3 months.<sup>16</sup> After the maturation process the second racking was done. Finally, wine analyses were applied just before bottling. In order to prevent further microbial activity bottles were pasteurized in 62°C water bath for 15 min.

### Chemical Analyses of Fruits and Wines

The total dry matter and ash analysis of fruits were determined by using the methods of AOAC.<sup>17</sup> The pH value of fruit juice and wine samples was measured at 20°C by pH meter (WTW, Germany). The total soluble solids (°Bx) of the samples were determined by refractometer (Atago ATC-1, Japan) according to the methods of AOAC.<sup>17</sup> The ebulliometer was used to determine the alcohol content of the wines (Alla, France). The total titratable acidity of fruit and wine samples were determined by 0.1 N NaOH titration and results were expressed as percent malic acid.<sup>18</sup> The ascorbic acid content of fruits was determined by titration with 2,6-dichloroindophenol (DCIF) according to the method of AOAC.<sup>19</sup> The HPLC system (Shimadzu SCL-10A, Japan) was used for the determination of sugar composition of the samples with the method described by Coelho *et al.* (2018)<sup>(20)</sup>. It is formed RID (Refractive Index Detector) detector, the system control unit (LC 20advp), the pump (10advp LC), gas separator (DG 20) and the column oven (CTO 10AVP). Sucrose (Sigma-Aldrich), glucose (D (+) Glucose, Sigma-Aldrich) and fructose (D (-) Fructose, Sigma-Aldrich) standards were separated with the Transgenomic COREGEL 87P column. Deionized pure water was used as mobile phase. The column temperature is set at 80°C and the flow rate is set at 0.6 ml/min. Standard chromatograms of monosaccharides have been established. The fruit juice was homogenized by dilution with pure water, and the wines are given directly to the system. 0.45 µm filtered samples were injected into the system in a volume of 20 µl. The amounts of sugar in the samples were calculated from

the equation of the standard curve generated using the external standard method.

#### Analysis of Phenolic Compounds

High Performance Liquid Chromatography (HPLC) method was used for identification of phenolic compounds. HPLC system (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan) consisted of diode-array detector (SPD, M20A), auto sampler (SIL-10ADvp), system controller (SCL-10Avp), pump (LC-10ADvp), degasser (DGU-14A) and column oven (CTO-10Avp). Separations were performed on a reverse phase column (Agilent® Eclipse XDB C-18, 250 mm × 4.6 mm, 5 µm) at 30°C and 0.8 ml/min flow rate. Two different mobile phases, 3% acetic acid and methyl alcohol, were used. Samples were dissolved in methanol and injected after filtration through a membrane filter (0.45 µm). Phenolic compounds were detected at a wavelength of 280 nm. The same steps were applied to all samples. In identification of phenolic compounds, authentic standards were used. Their retention times and ultraviolet-visible spectra were compared. On the contrary, external calibration standards were applied for determination of their amount.<sup>21</sup>

#### Analysis of Aroma Components

In the present study, headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was detected the aroma components of fruits and wines. SPME-GC analysis was performed on Shimadzu GC-2010 Plus (Japan) equipped with a flame ionization detector Shimadzu GCMS-QP2010 SE. Compounds were separated using a column (Restek Rx-5Sil MS) with 30 m length, 0.25 mm internal diameter, 0.25 µm coating thickness. After injection, the column

temperature was held at 40°C for 2 minutes. It was then programmed at 4°C/min to 250°C and held for 5 min. Helium used as carrier gas was adjusted to flow rate of 1.61 ml/min, injection temperature and detector temperature of 250°C. A 2 ml aliquot was placed in 4 ml vial (Supelco 27159 15 ml clear PTFE/Silicone septa Cap). Samples were kept for 15 min at 60°C with fiber and 15 min at 250°C without fiber. The fibre was then removed from the headspace and desorbed at the gas chromatograph. The peaks seen on the chromatogram were identified by scanning libraries named Wiley, Nist, Tutor and FFNSC. The ionization voltage was applied as 70 eV and mass spectra were obtained in electron impact (EI) mode.

#### Statistical Analysis

All statistical analyses were conducted in triplicates using Statistical Package for Social Science software (SPSS Inc. A.B.D.) 16.0 statistical package program. Means and standard deviations (SD) of the data were calculated and expressed. One-way ANOVA (analysis of variance) and Tukey multiple comparison test were used to determine statistical differences. The results were evaluated at 95% confidence level.

## Results and Discussion

#### Chemical Analysis

Some chemical properties of rose fruit juice were determined before wine production. The results are presented in Table 1. One of the important quality criteria of fruits to be used in winemaking is the amount of sugars present in the juice. Besides, the water and alcohol soluble materials other than sugars are directly related to the taste and odour of the final product. Total soluble solids of fruits used in this study were determined as 32.30 °Bx. In a study done

Table 1 — Chemical analysis of wines and fruit

Analyse	Fruit Juice	Control Wine	Honey Wine
pH	3.87 ± 0.03 <sup>a</sup>	3.91 ± 0.01 <sup>a</sup>	3.87 ± 0.02 <sup>a</sup>
Ascorbic acid (mg/100g, drymatter)	711.53 ± 1.00	ND	ND
°Brix	32.30 ± 0.30 <sup>a</sup>	8.7 ± 0.17 <sup>b</sup>	8.0 ± 0.31 <sup>c</sup>
Total Solids (%)	36.1 ± 0.06	ND	ND
Acidity (%) (as malic acid)	1.44 ± 0.01	ND	ND
Ash (% drymatter)	6.62 ± 0.01	ND	ND
Alcohol (Ebulliometer) (% v/v)	0.00 ± 0.00 <sup>b</sup>	11.05 ± 0.05 <sup>a</sup>	10.90 ± 0.10 <sup>a</sup>
Alcohol (HPLC) (%)	0.00 ± 0.00 <sup>c</sup>	11.86 ± 0.49 <sup>a</sup>	10.44 ± 0.03 <sup>b</sup>
Sucrose (g/10 mL)	0.69 ± 0.97 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>
Glucose (g/100 mL)	2.61 ± 1.85 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	3.78 ± 0.07 <sup>a</sup>
Fructose (g/100 mL)	2.88 ± 1.09 <sup>b</sup>	8.28 ± 0.31 <sup>a</sup>	7.92 ± 0.08 <sup>a</sup>

ND: not determined

by Ercisli (2007)<sup>(22)</sup> total soluble solids in fruits of *Rosa* spp. were determined between 29–37 °Bx. The ash content of the *Rosa odorata* (Andrews) Sweet fruits was found as 6.62% in dry matter.

The amount of ascorbic acid in fruits is considered as an important criterion for the formation of the colour due to its antioxidant effects. The amount of ascorbic acid in fruits used in this study was determined as 711.53 mg/100 g. The ascorbic acid results were similar to that of studies about fruits of various Rosaceae families.<sup>23</sup> Ascorbic acid content of *Rosa* species may show significant changes depending on various factors such as region, climatic conditions, altitude.<sup>24</sup>

The results of analytical tests of wines and fruit juice were presented in Table 1. As it can be seen, pH, alcohol and °Bx values of both control and honey wines were similar. In a review done by Gupta and Sharma (2009)<sup>(9)</sup>, the pH in wines made with sugar and honey was measured 3.86 and 3.59. The Brix measurements were also close to each other which were 8.7 and 8.00, respectively. Ethyl alcohol content of wines was 11.05% (v/v) in control wine made with sucrose and 10.90% (v/v) in honey wine. Gupta and Sharma (2009)<sup>(9)</sup> stated that the alcohol content of similar wines were between 10.5 and 9.8% (v/v).

The sugar composition in wines and fruit juice was examined by HPLC. Three major sugars were determined namely sucrose, glucose and fructose in the samples (Table 1). Most of the sugars in Rose fruits were glucose and fructose as expected. During fermentation, yeasts metabolize most of the glucose and fructose to ethanol and CO<sub>2</sub>.<sup>(25)</sup> Although glucose and fructose are the two major fermentable sugars used simultaneously during alcohol fermentations, fructose is the dominating sugar present in the later stages of alcoholic fermentation.<sup>26</sup>

#### Total Phenolic Compounds

A total of 11 phenolic compounds were identified and quantified in two different wines made with addition of *Rosa odorata* (Andrews) Sweet fruits (Table 2). The total amount of phenolic compounds was 3604.9 mg/l GAE (Gallic Acid Equivalent) in fruit juice. Czyzowska *et al.* (2015)<sup>(27)</sup>, determined concentrations of polyphenols in musts from *Rosa canina* L. and *Rosa rugosa* fruits were between 7400–9007 mg/l GAE. In another study conducted by Ercisli (2007)<sup>(22)</sup>, the total phenolics contents of two rose species were found 73 mg GAE/g-DW in *Rosa villosa* and 96 mg/g-DW GAE in *Rosa canina* L. The

large differences in total phenolic content are probably as a result of growing conditions, geographical location, maturity, species, and climate conditions.

The results indicated that the total amount of phenolic compounds of two different wines made by addition of sugar and honey were found as 74.3 mg/l and 111.8 mg/l, respectively. In a study, the phenolic contents of wines made from *Rosa canina* L. and *Rosa rugosa* Thunb fruits were determined between 2786–3456 and 3389–3990 mg/l GAE in aged and young wines respectively.<sup>27</sup> In a similar study the total phenolic compounds of wine made from *Cherokee* rose was found 2529 mg/l GAE.<sup>28</sup>

In our study, the most abundant phenolics determined in fruit juice were catechin (1991.8 mg/l) which gives the bitter taste in wines<sup>29</sup>, epicatechin (470.3 mg/l), rutin (347.5 mg/l) and benzoic acid (203.7 mg/l). Although these substances were also been determined in both wines, there was a significant decrease in their amounts. Polyphenols such as catechins, proanthocyanidins, cinnamic acids and their derivatives are generally subject to oxidation in white

Table 2 —Phenolic compounds of wines and fruit (Results are given in mg/L)

Compound Name	Fruit Juice	Control Wine	Honey Wine
Gallic Acid	—	—	—
Protocatechic Acid	64.4	3.3	—
Catechin	1991.8	6.5	32.3
p-Hydroxy Benzoic Acid	341.1	2.4	5.7
Chlorogenic Acid	—	—	—
Caffeic Acid	—	—	—
Epicatechin	470.3	9.2	25.6
Syringic Acid	32.6	1.4	—
Vaniline	54.7	—	—
P-Coumaric Acid	25.6	0.4	0.3
Ferulic Acid	—	0.4	0.3
Sinapic Acid	—	—	—
Benzoic Acid	203.7	—	—
O-Coumaric Acid	6.7	—	—
Rutin	347.5	47.3	44.4
Hesperidine	—	—	—
Rosmarinic Acid	66.5	—	—
Eriodictiol	—	—	—
Cinnamic Acid	—	—	—
Quercetin	—	3.4	3.2
Luteolin	—	—	—
Campherol	—	—	—
Apigenin	—	—	—
Total Phenolic Compounds	3604.9	74.3	111.8

grape wines. Thus, they are responsible for the conversion of the initial light yellow colour to deep golden yellow first and finally the typical brown color.<sup>30</sup> High amounts of phenolic compounds like *p*-coumaric acid, quercetin, kaempferol etc. are originated from honey can be transferred to honey wine.<sup>31</sup>

#### Aroma Components

The aroma substances and their amounts determined by HS-SPME/GC-MS in wines are given in Table 3. The employment of HS-SPME/GC-MS ensured for the identification of 41 aroma compounds in control wine and 36 aroma compounds in honey

Table 3 —Volatile compounds of wines

Name	Control Wine		Honey Wine	
	R.Time (min.)	Concentration (mg/L)	R.Time (min.)	Concentration (mg/L)
Ethanol	1.185	98399.64	1.162	88267.55
1-Propanol	1.387	0.29	1.391	0.24
2-Methylbutanal	1.559	0.68	—	—
Ethylacetate	1.632	17.26	1.634	18.66
Isobutylalcohol	1.715	3.37	1.72	4.29
Heptane	2.358	0.82	—	—
3-Methylbutanol	—	—	1.934	0.13
3-Methyl-1-butanol	2.879	21.50	2.888	33.08
2-Methyl-1-butanol	2.935	6.16	2.941	8.21
Ethylisobutyrate	3.263	0.11	3.267	0.24
Isobutylacetate	—	—	3.56	0.13
Ethylbutyrate	4.144	0.95	4.15	0.92
Furfural	4.901	0.58	4.912	0.16
Ethylisovalerate	5.559	0.08	5.569	0.11
Hex-3(Z)-enol	5.612	0.21	5.61	0.34
o-Xylene	—	—	5.969	0.18
Hexanol	6.042	1.50	6.044	2.03
Isoamylacetate	6.253	0.79	6.258	1.82
2-Methyl-1-butyl acetate	6.318	0.24	6.321	0.34
Styrene	6.631	0.18	—	—
alpha-Pinene	8.075	0.63	—	—
Benzaldehyde	9.068	0.21	9.07	0.53
Vinylamylcarbinol	9.895	0.03	9.89	0.16
6-Methyl-5-hepten-2-one	10.029	0.21	10.032	0.32
Ethylcapronate	10.589	25.08	10.595	24.90
Ethyl 3-hexenoate	10.774	0.21	10.776	0.13
dl-Limonene	11.653	0.26	—	—
Benzeneacetaldehyde	—	—	12.162	0.18
Ethyl 2-hexenoate	12.285	0.08	12.296	0.13
Caprylalcohol	13.362	0.24	13.362	0.29
Phenethylalcohol	14.824	0.87	14.822	2.40
Methyloctanoate	15.355	0.32	15.365	0.21
Ethylbenzoate	17.038	0.13	17.046	0.26
Nonanol	17.222	0.11	17.22	0.18
Caprylicacid	17.374	0.24	17.374	0.50
Ethylsuccinate	17.477	0.97	17.485	0.45
Ethylcaprylate	18.166	60.58	18.167	55.35
Vitispirane	21.075	0.58	21.081	0.29
Ethyldecanoate	25.206	14.97	25.214	16.16
Butanoicacid<3-methyl-, octyl-> ester	26.466	0.32	—	—
Octanoicacid<3-methylbutyl-> ester	26.895	0.21	26.903	0.37
Farnesene<(E)-, beta->	27.106	0.37	—	—
Ethyl laurate	31.56	1.34	31.561	1.26
Curcumene	40.27	0.50	—	—
Tributylacetyl citrate	48.532	1.53	—	—

wine. Environmental factors like climate and soil, fermentation conditions (yeast flora, pH and temperature), wine production technologies and aging were the main factors affecting the formation of aroma compounds.<sup>32</sup> Volatile profiles of wines generally include esters, alcohols, hydrocarbons, aldehydes and ketones. Regarding the wines produced in this study, the dominant aroma components in the control wine were ethyl acetate, isobutyl alcohol, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl capronate, ethyl caprylate. The aroma profile of honey wine was similar with minor differences. However, some differences were significant.

Aroma compounds of wines were analyzed in various studies by using HS-SPME/GC-MS method. More than 400 volatile compounds have been identified in rose cultivars. Sixty two volatile compounds were determined in fruits of rosehip (*Rosa L.*) species which were 19 acids, 9 aldehydes, 6 ketones and 5 esters, 2 terpenes, 2 phenols and 18 alcohols.<sup>33</sup> The chief aromatic components of the pink, orange and yellow rose flowers were hexanol, benzyl alcohol and hexenyl acetate.<sup>34</sup> It was thought that some of the volatile compounds were originated from the fruits of *Rosa odorata* (Andrews) itself. Aromatic compounds add "fruity" and "floral" sensory properties to wines, thus contributing to the sensory quality of the wine.<sup>35</sup>

In this study, various types of alcohols were identified in both control and honey wine. Alcohols present in both wines were also found in former studies in terms of variety and concentration. Ethanol plays a role in the stability and aging of wines. In this way, it affects the sensory properties. In addition, the increased amount of ethanol during fermentation inhibits the growth of microorganisms that can produce off-odors.<sup>29</sup> Most of the aroma compounds are released into the environment as secondary products of yeast metabolism. Alcohols other than ethanol were also identified and quantified in the wines.<sup>36</sup> Those are the fusel alcohols namely o-butanol, is o-amyl alcohol, 2-phenylethanol and propanol. Fusel alcohols cause pungent odour and taste.<sup>37</sup> Yeast synthesized these compounds using two different mechanisms. The first occurs with the main anabolic pathway starting with glucose, and the second with the catabolic pathways of amino acids (valine, leucine, o-leucine and phenylalanine).<sup>38</sup>

Esters are formed during fermentation by yeasts. Ethyl esters, characterized by fruity and floral notes

are the main ones among them.<sup>39</sup> Ethyl acetate is the most ample ester in wines. The amount of ethyl acetate was determined in control wine 17.26 mg/l and 18.66 mg/l in honey wine produced in this work. The ethyl acetate concentration in wines usually does not reach 50–100 mg/l. At low levels, it may be suitable and add complexity to the fragrance.<sup>29</sup> A high amount of ethyl acetate can be considered a sign of wine spoilage. The study determined by Rodriguez-Bencomo *et al.* (2003)<sup>(40)</sup>, limits of detection 9.6 mg/l in ethyl acetate.

### Conclusions

Fruit and honey wines are special type of wines in which sensory properties are quite variable. The unique taste and aromatic features of such wines are influential in wine preference of some people. These properties depend on some factors such as the type of honey, fruit species used, climate, soil conditions, fermentation process and aging.

HS-SPME/GC-MS techniques are novel methods for detailed determination of aroma and fragrance substances especially in aromatic and fruit wines give better results. It has been found that some minor components which cannot be determined by other methods can be detected by such methods. In addition, some major components such as ethanol, other alcohols and sugars can also be identified with higher sensitivity, thus providing more reliable results when compared to conventional methods.

Fruits of the Rosaceae family were used in wine production in the past; however studies focused on their combined production with honey are quite limited. We determined that wide variety of phenolic and aroma compounds in wines were originated from honey, fruit and yeast activity all together. In addition, the results also showed the usage of honey in fruit wines to improve aromatic characteristics, thus increasing the sensory quality. Wines made with honey or sucrose contained such substances in different concentrations. The results also showed that, fruit, honey and yeast activity used in wine making have been found to be effective in the enrichment of phenolic compounds and aroma substances.

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