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Sedative effects of roasted green tea aroma on autonomic nervous activity, central nervous activity, and subjective mood state in healthy adults

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ABSTRACT

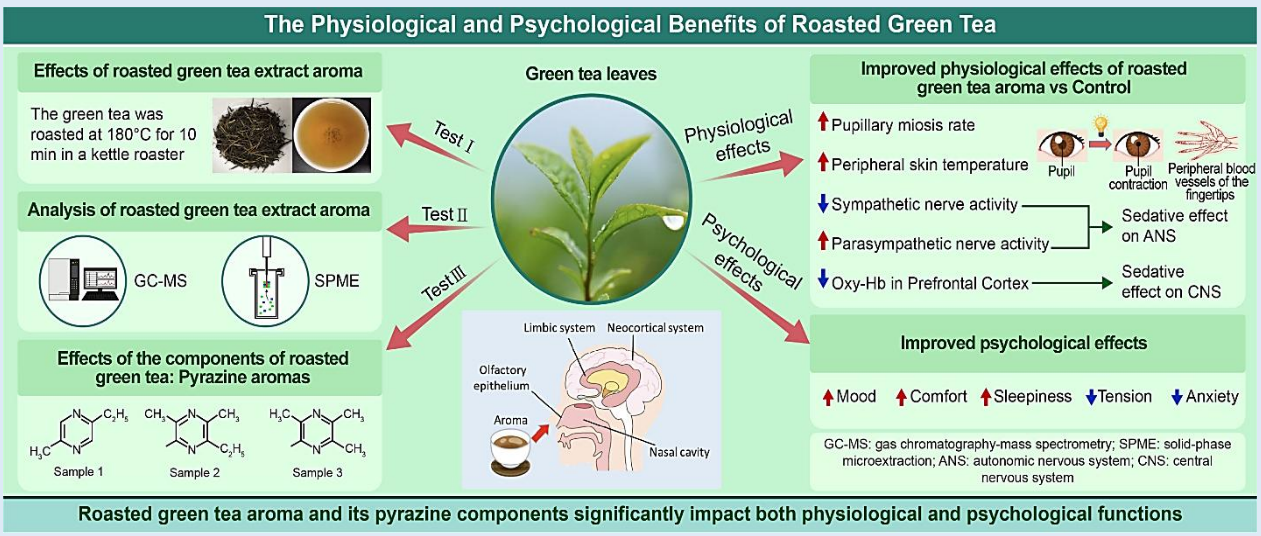
Introduction: To clarify the physiological and psychological effects of roasted green tea aroma, this study examined the effects of roasted green tea extract and its major aromatic components on autonomic nervous activity, central nervous activity, and subjective mood state.

Methods: Twenty healthy adults who were not averse to the aroma of green tea, did not smoke, did not display any flu symptoms, and were not receiving drug treatment participated in this study. The study samples comprised a roasted green tea extract from Shizuoka first-growth green tea (Test I) and three pyrazines, which are the main aromatic components of roasted green tea (Test III). The pupil miosis rate, peripheral skin temperature, and cerebral blood flow were measured after 2 min of inhalation of the study samples as physiological evaluations. Subjective mood state was investigated using a questionnaire for psychological evaluation.

Results: The results showed that inhalation of roasted green tea aroma significantly increased the pupillary miosis rate and peripheral skin temperature, similar to the effects observed with hot water. Furthermore, inhalation of roasted green tea aroma reduced the amount of oxygenated hemoglobin in the prefrontal cortex, suggesting that it also has a sedative effect on the central nervous system. In the psychological evaluation using the Visual Analog Scale, roasted green tea aroma inhalation reduced the scores for “tension”, and “anxiety” while increasing those for “mood” and “comfort” relative to those observed with hot water. In addition, the evaluation of the main aromatic components of roasted green tea, specifically three different pyrazines, demonstrated physiological effects similar to those of roasted green tea even at room temperature when tested at concentrations derived from the results of the analysis.

Conclusion: The aroma of roasted green tea has both physiological and psychological sedative effects that differ from those of hot water and suggest that these pyrazines play a role in producing these effects, with 2,3,5-trimethylpyrazine being the most important aromatic component. Furthermore, the findings suggest that roasted green tea aroma exerts a sedative effect on the autonomic nervous system by suppressing sympathetic nerve activity while increasing parasympathetic nerve activity.

Graphical Abstract:



INTRODUCTION

Functional foods play a significant role in reducing the risk of disease by providing additional health benefits beyond their basic nutritional value. These foods are enriched with bioactive compounds that can modulate physiological functions and improve overall health. Recently, functional foods have emerged as an important area of research, recognizing the potential impact on health outcomes [1]. Roasted green tea is generally produced by roasting green or coarse teas. The representative components of roasted green tea infusion include catechins, known for their antioxidant and antibacterial properties, caffeine, which has a stimulating effect, and theanine, which provides a relaxing effect similar to that of green tea. In recent years, studies have reported the anti-cancer effects of epigallocatechin gallate (EGCG), the primary catechin, along with its roles in reducing high blood pressure and its influence on heart rate variability, along with the physiological and metabolic responses associated with drinking matcha [2-5]. Roasted green tea also contains various volatile compounds that are produced during the roasting process through the Maillard reaction between sugars and amino acids [6]. Pyrazines, characterized by their savory aroma, and furanones, which have a caramel-like sweet aroma, are known to be aromatic components of roasted green tea and are said to contribute significantly to the composition of roasted green tea aroma [7-10]. In recent years, the savory aroma of roasted green tea has been increasingly used as a flavor in cafés and sweets and is believed to influence consumer preference significantly.

Conversely, studies on the functionality of pyrazines have long reported the vasorelaxant response of smooth muscle to alkyl pyrazines and the inhibition of platelet aggregation [11]. There have also been reports on the blood fluidity-enhancing effects of alkylpyrazines in barley tea and the relaxing effects of its aroma [12-14]. Recent studies have reported the sedative effects of 2,3-dimethylpyrazine and furaneol produced by the Maillard reaction [15,16]. Furthermore, studies have reported on the neuroscientific evaluation of the physiologically active components of roasted green tea and the impact of consuming roasted green tea on mental task performance [17,18].

However, there are still few reports on the functionality of roasted green tea aroma. Roasted green tea, such as barley tea, contains pyrazines and furanones [19], and physiological and psychological functionality can also be expected from roasted green tea aroma. Therefore, the purpose of this study is to investigate the effects of roasted green tea aroma on the body and clarify its physiological and psychological functions. In the future, the sedative effect of roasted green tea aroma could be leveraged to develop beverages and foods with functional properties aimed at stress reduction and tension relief. Based on previous studies evaluating the functionality of aroma, this study measured the pupil miosis rate (parasympathetic index) and peripheral skin temperature (sympathetic index) to evaluate autonomic nervous system activity [20,21]. In addition, to evaluate the central nervous system, near-infrared spectroscopy (NIRS) was used to measure the relative changes in the amount of oxygenated hemoglobin (Oxy-Hb), an indicator of brain activity. For psychological evaluation, questionnaires using the Visual Analog Scale (VAS) and the Multiple Mood Scale (MMS) (Test III only) were used to investigate subjective and multiple mood states [22]. These evaluations provide new information on the sedative effects of roasted green tea aroma and its components, which were clarified by a comprehensive evaluation of the autonomic nervous system, central nervous system, and subjective mood state. Furthermore, the clarification of the sedative effects of roasted green tea aroma and the involved components could contribute to the development of functional foods with claims such as “alleviation of stress and tension” and “reduction of fatigue.”

Outline of the study: This study examined the physiological and psychological effects of roasted green tea aroma in Test I, quantified the main aromatic components of roasted green tea in Test II, and conducted physiological and psychological evaluations of these components in Test III.

MATERIALS AND METHODS

Samples for human experiments

Test I, Roasted green tea extract: The raw material tea from Shizuoka first-growth green tea (JA Enshu Central Agricultural Cooperative, Shizuoka, Japan) was roasted at 180°C for 10 min in a kettle roaster. For the study sample, the roasted green tea was extracted with 50 times the volume of distilled water at 95°C for 2 min. Subsequently, the tea leaves were filtered through a 100-mesh sieve and the extract kept refrigerated. The study sample was heated to 75°C by an induction heater just before the start of the measurements and then served in a vacuum-insulated tumbler (JDP-300WH, 8 cm width × 8 cm depth × 10.5 cm height) (Thermos, Inc., Tokyo, Japan) at a volume of 200 mL. For comparison, commercially available hot drinking water, also heated to 75°C, was used. As the control, participants breathed normal air without exposure to green tea aroma or hot water vapor.

Test III, Main aromatic components of roasted green tea: The three pyrazines used as the study samples were 2-ethyl-5(6)-methylpyrazine (purity 99.0%) (Combi Blocks, Inc., San Diego, California, U.S.A, Catalog No. QH-4887) (Sample 1), 2-ethyl-3,5(6)-dimethylpyrazine (purity ≥98.0%) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan, Catalog No. E1424) (Sample 2), and 2,3,5-trimethylpyrazine (purity ≥98.0%) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan, Catalog No. T0942) (Sample 3). All study samples were adjusted to 100 ppb based on the determination of the major aromatic components of roasted green tea in Test II. The samples were diluted with distilled water immediately before the start of the measurements and served in a vacuum-insulated tumbler (JDP-300WH) at room temperature (approximately 20°C). In addition, distilled water was used as a control in Test III.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

Test II, Determination of major aromatic components of roasted green tea extract: The roasted green tea extract prepared in Test I was used to determine its major aromatic components. We selected three pyrazines with relatively high FD factors—an index of aroma intensity identified using the Aroma Extract Dilution Analysis (AEDA) method —as key components based on the findings of previous studies [5,10]. Additionally, no pungent or earthy aroma components were included to maintain fragrance quality. As described above, three different pyrazines, 2-ethyl-5(6)-methylpyrazine, 2-ethyl-3,5(6)-dimethylpyrazine, and 2,3,5-trimethylpyrazine, were identified as the main aromatic components of roasted green tea. The pyrazines in the roasted green tea extract were analyzed using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Analysis samples were prepared by placing 10 mL of sample solution and 3 g of sodium chloride in a glass vial for GC-MS and adding 5 µL of internal standard (0.1% cyclohexanol) to the vial. The samples were extracted at 60°C for 30 min using Supelco SPME fiber (50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane) (Sigma Aldrich, St. Louis, Missouri, USA) after equilibrating the headspace gas in the glass vials at 60°C for 10 min. Gas chromatography was performed using an Agilent 7890A equipped with an Agilent DB-WAX column (0.25 mm I.D. × 60 m × 0.25 µm). Other parameters included carrier gas: helium (inlet pressure: 82.7 kPa), inlet temperature: 240°C, and injection method: splitless injection. The oven temperature was kept at 35°C for 3 min and then increased to 240°C at 5°C/min, and it was held at 240°C for 5 min. The mass detector was an Agilent 5975A MSD with the parameters of ionization voltage: 70 eV (EI), ion source temperature: 230°C, and measurement mode: SIM/scan (SIM mode: m/z: 81.1, 94.1, 108.1, 121.1, 122.1, 135.1; scan mode: m/z: 29–250). The concentrations of the aromatic components were calculated using calibration curves obtained from the respective standards.

Participants: The participants in this study were healthy Japanese adults residing in the vicinity of Tokyo who belonged to the subject bank of the Chiyoda Paramedical Care Clinic. Furthermore, the participants were selected based on their lack of aversion to the aroma of green tea, did not smoke, did not display any flu symptoms, and were not receiving drug treatment.

For Test I, 10 males and 10 females (43.3 ± 4.4 years old) were recruited (mean ± standard deviation), while Test III included 9 males and 11 females (42.0 ± 5.2 years old). The participants were instructed to get the same time and quality of sleep as usual the night before the test, refrain from stimulants at meals before the test, limit their intake of caffeine-containing beverages, and not wear any cosmetic products on the day of the test.

Experimental procedure

Test I: The participants entered the measurement room 30 min before the start of the test to acclimatize to the experimental environment. In addition, the participants were divided into two groups to eliminate order effects. Physiological evaluations were conducted in the following order: pupil miosis rate, peripheral skin temperature, and cerebral blood flow [20-21]. For the pupil miosis rate, the first measurement was taken as a control (air) after 2 min of dark adaptation. After another 2 min of dark adaptation, a second measurement was performed after 2 min of inhalation of hot water vapor or roasted green tea aroma. Finally, after 2 min of dark adaptation, a third measurement was performed after 2 min of inhalation of hot water vapor or roasted green tea aroma. Peripheral skin temperature and cerebral blood flow were measured in the same manner, except that the participants were not dark-adapted and were seated at rest. The tumbler containing the study sample was placed approximately 3 cm under the participant's nose, and the lid (1.5 cm long × 3.5 cm wide) was removed immediately before the participant inhaled the sample. The participants were instructed to breathe normally during inhalation.

Test III: As in Test I, the participants were acclimatized to the experimental environment by entering the measurement room 30 min before the start of the test. For the pupil miosis rate, after 2 min of dark adaptation, the first measurement was taken after 2 min of inhalation of the control sample (distilled water vapor). After a further 2 min of dark adaptation, a second measurement was performed after 2 min of inhalation of each pyrazine aroma. Peripheral skin temperature and cerebral blood flow were measured in the same manner, except that the participants were not dark-adapted and were seated at rest. Using this series of procedures, only one sample was evaluated per day, and three samples were tested by the same participant over a period of three days. Sample presentation was performed in the same manner as in Test I. The entire lid of the tumbler was removed immediately before the participants inhaled the aroma.

Experimental environment: The measurement room was maintained at 25°C and 50% humidity. Cerebral blood flow measurements using NIRS were performed in a measurement room isolated from the pupil miosis rate and peripheral skin temperature measurements with windows and doors closed to maintain quietness.

Measurements

Subjective evaluation of the aroma of the study samples: After inhaling the aroma of the study samples, the participants provided subjective evaluations for "preference," "strength of scent," and "comfort," all measured using a 100 mm VAS. The scale ranged from 0 (minimum intensity) to 100 (maximum intensity), with endpoints labeled as follows: "very dislike" to "very like" for preference, "very weak" to "very strong" for strength of scent, and "very uncomfortable" to "very comfortable" for comfort.

Measurement of pupil miosis rate: The pupil diameter was measured using an Iriscorder Dual C10641 (Hamamatsu Photonics, Inc., Shizuoka, Japan), a goggle-type electronic pupillometer. The pupil miosis rate was calculated from the change in pupil diameter after the pupils were dark-adapted for 2 min with light-shielding goggles on both eyes and then irradiated with light for 0.1 s. By comparing the changes in pupillary miosis due to light stimulation, the effect on autonomic nervous system activity, particularly parasympathetic activity, was evaluated. In this study, the pupil miosis rate was measured after 2 min of inhalation of the control or study samples. The pupil diameter in the initial state before light stimulation was D1, the pupil diameter after light stimulation was D2, and the change, D1−D2, was used to calculate the pupil miosis rate (CR = (D1−D2)/D1). Furthermore, the pupil miosis rate after inhalation of the control was CR1, while the rate after inhalation of the study sample was CR2, and the miosis rate change (ΔCR = CR2−CR1) between the control and study samples was also compared [20-21].

Measurement of peripheral skin temperature: The peripheral skin temperature was evaluated by measuring the skin temperature (FT) of the index finger using an LT-8A temperature logger (Gram Corporation, Saitama, Japan). The effect on autonomic nervous activity, particularly sympathetic activity, was evaluated by comparing changes in skin temperature at peripheral sites. In this study, the skin temperature at the center of the ventral side of the fingertip after 2 min of inhalation of the control was FT1, while the skin temperature in the same area after 2 min of inhalation of the study sample was FT2, and the change in skin temperature (ΔFT = FT2−FT1) between the control and test samples was compared [20-21].

Evaluation of subjective mood state: As a psychological evaluation, the participants rated their subjective mood state based on eight items: “mood,” “comfort,” “tension,” “anxiety,” “anger,” “fatigue,” “activity,” and “sleepiness” using a VAS after 2 min of inhalation of the study samples [23]. Each item was rated on a linear scale (0–100 mm) from “very bad” to “very good” or from “very weak” to “very strong.” In Test III, “concentration” was added to the above items, and nine items were evaluated. In Test III, participants’ mood states were evaluated using the MMS, which consists of 40 questions classified into eight subscales: “depression/anxiety,” “hostility,” “fatigue,” “activity,” “inactivity,” “affinity,” “focus,” and “surprise.” Each item was rated on a four-point scale, ranging from “not feeling at all” to “feeling clearly.” In this study, after evaluating the MMS, the scores from the 40 questions were grouped by subscale and summed to compare multiple mood states [22].

Measurement of cerebral blood flow: The relative amount of Oxy-Hb was measured as cerebral blood flow using a Spectratech OEG-16 (Spectratech Inc., Tokyo, Japan) brain function measurement device on NIRS. In this study, a 16-channel light-receiving sensor was attached to the prefrontal area of the participant, and the amount of Oxy-Hb was continuously measured during 2 min of inhalation of the control and study samples. The measured data were baseline corrected for each channel for each participant and averaged to compare the change in Oxy-Hb between the control and study samples [20-21].

Statistical analysis: All data in the text are expressed as mean ± standard deviation. Continuous data were assessed for normality using the Shapiro-Wilk test and for the homogeneity of variance using Levene's test. In Test I, multiple comparisons were performed using the Bonferroni method after a one-way analysis of variance (ANOVA) for differences among the three groups: control, hot water, and roasted green tea. In Test III, the data for each control and pyrazine were statistically analyzed using the paired t-test (two-tailed test). Statistical analyses were performed using SPSS Statistics 25 (IBM Corporation, Armonk, New York, USA). A probability (p) value of < 0.05 was considered a statistically significant difference, and values between 0.05 and 0.10 were considered to indicate a significant trend.

Ethical considerations: This study was conducted in accordance with the ethical regulations of the Declaration of Helsinki. The participants were informed of the purpose, content, and possible risks of the study, and informed consent was obtained using a consent form. This study was conducted under the review and approval of the ethical review committee of Chiyoda Paramedical Care Clinic, a contract clinical trial organization. The ethics review approval numbers were ITE23C1 (Test I) and ITE24C1 (Test III). The study contents were registered in the University Hospital Medical Information Network, and the UMIN registration numbers were UMIN000051292 (Test I) and UMIN000053673 (Test III).

RESULTS

Subjective evaluation of roasted green tea aroma: Figure 1 shows the results of the subjective evaluation of hot water and roasted green tea aroma. Compared to hot water, the evaluation of roasted green tea aroma showed significantly higher scores for all the items of "preference" ($p < 0.01$), "strength of scent" ($p < 0.01$), and "comfort" ($p < 0.01$).

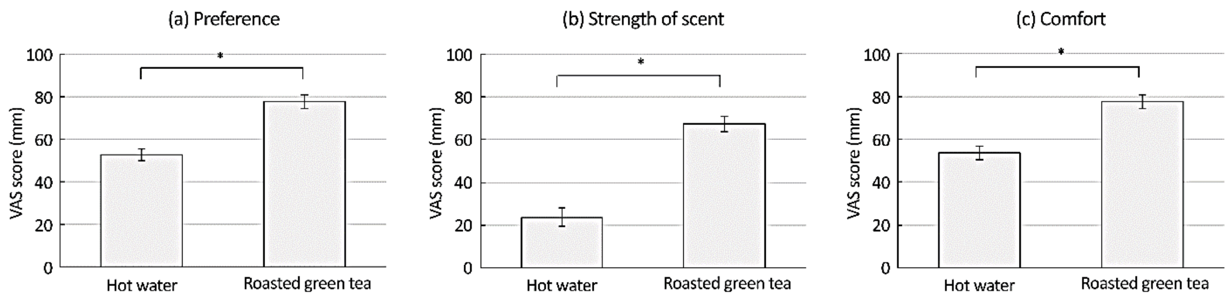


Figure 1. Subjective evaluation of hot water and roasted green tea aroma.

VAS scores for (a) preference, (b) strength of scent, and (c) comfort after 2 min of inhalation of hot water vapor and roasted green tea aroma were measured using a 100 mm VAS from 0 (minimum intensity) to 100 (maximum intensity). Data are shown as mean ± SEM (n = 20). * $p < 0.05$, as determined by paired t-test.

Measurement of pupil miosis rate and peripheral skin temperature: Figure 2 shows the results of pupil miosis rate after inhalation of hot water vapor and roasted green tea aroma (Control 0.386 ± 0.077, Hot water 0.414 ± 0.064, Roasted green tea 0.403 ± 0.067). Compared to the control group, the pupil miosis rate after inhalation of hot water vapor and roasted green tea aroma increased significantly (Hot water, $p < 0.01$; Roasted green tea, $p < 0.01$). The results of peripheral skin temperature measurements are shown in Figure 3 (Control 32.4 ± 1.6℃, Hot water 33.3 ± 1.8℃, Roasted green tea 33.3 ± 2.0℃). Similar to the pupil miosis rate, there was a significant increase in fingertip skin temperature after inhalation of hot water vapor and roasted green tea aroma compared to the control (Hot water, $p < 0.01$; Roasted green tea, $p < 0.01$).

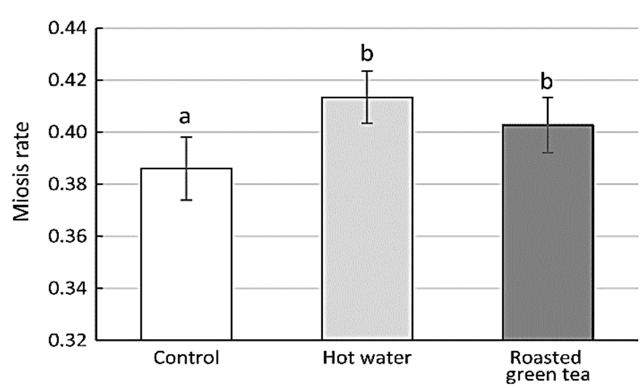


Figure 2. Effects of hot water and roasted green tea aroma on pupil miosis rate.

The pupil miosis rate after 2 min of inhalation of the control (air), hot water vapor, and roasted green tea aroma. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, by multiple comparisons using the Bonferroni method. There are significant differences between the different letters.

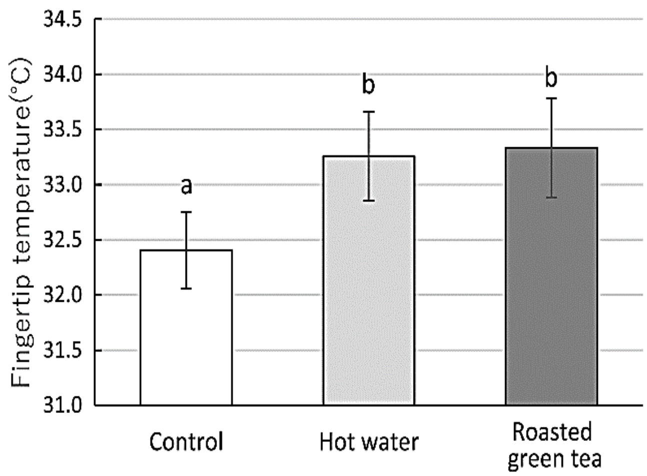


Figure 3. Effects of hot water and roasted green tea aroma on peripheral skin temperature.

The fingertip temperature after 2 min of inhalation of the control (air), hot water vapor, and roasted green tea aroma. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, by multiple comparisons using the Bonferroni method. There are significant differences between the different letters.

Evaluation of subjective mood state: The results of the subjective mood state after the inhalation of hot water vapor and roasted green tea aroma are shown in Figure 4. Compared to the control, the scores of "tension" and "anxiety" were significantly reduced after inhalation of hot water vapor and roasted green tea aroma ("tension," Hot water, $p < 0.05$; Roasted green tea, $p < 0.01$; "anxiety," Hot water, $p < 0.05$; Roasted green tea, $p < 0.05$). In addition, after inhalation of roasted green tea aroma, there was a significant increase in scores for "mood", "comfort", and "sleepiness" ("mood," Control, $p < 0.05$; Hot water, $p < 0.01$; "comfort," Control, $p < 0.01$; Hot water, $p < 0.01$; "sleepiness," Control, $p < 0.05$).

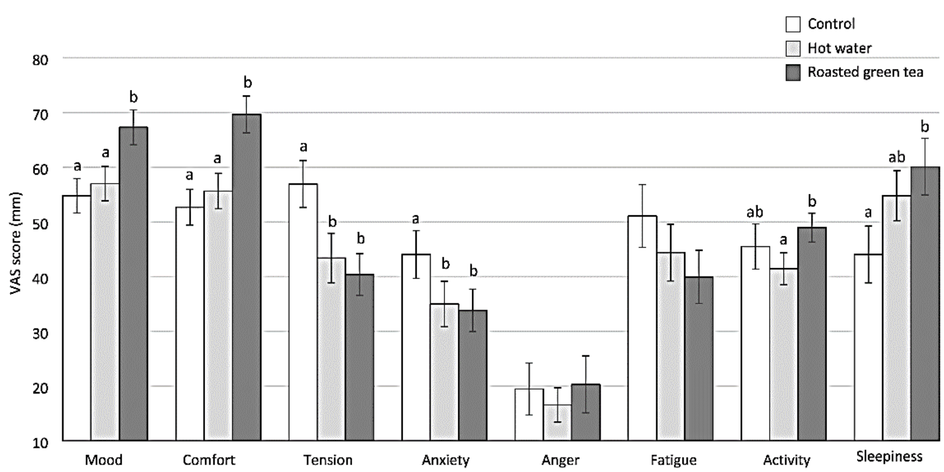


Figure 4. Effects of hot water and roasted green tea aroma on subjective mood state.

The VAS scores for mood, comfort, tension, anxiety, anger, fatigue, activity, and sleepiness after 2 min of inhalation of control (air), hot water vapor, and roasted green tea aroma were measured using a 100 mm VAS from 0 (minimum intensity) to 100 (maximum intensity). Data are shown as mean ± SEM (n = 20). * $p < 0.05$, by multiple comparisons using the Bonferroni method. There are significant differences between the different letters.

Measurement of cerebral blood flow: Figure 5 shows the Oxy-Hb concentrations after inhalation of hot water vapor and roasted green tea aroma. Compared to the control group, the amount of Oxy-Hb in the prefrontal cortex of the brain was significantly decreased in all channels after inhalation of both hot water vapor and roasted green tea aroma. In addition, when comparing the effects of hot water vapor and roasted green tea aroma, the amount of Oxy-Hb was lower in many channels for the roasted green tea aroma (except in ch1, 3, 4, 6, and 13).

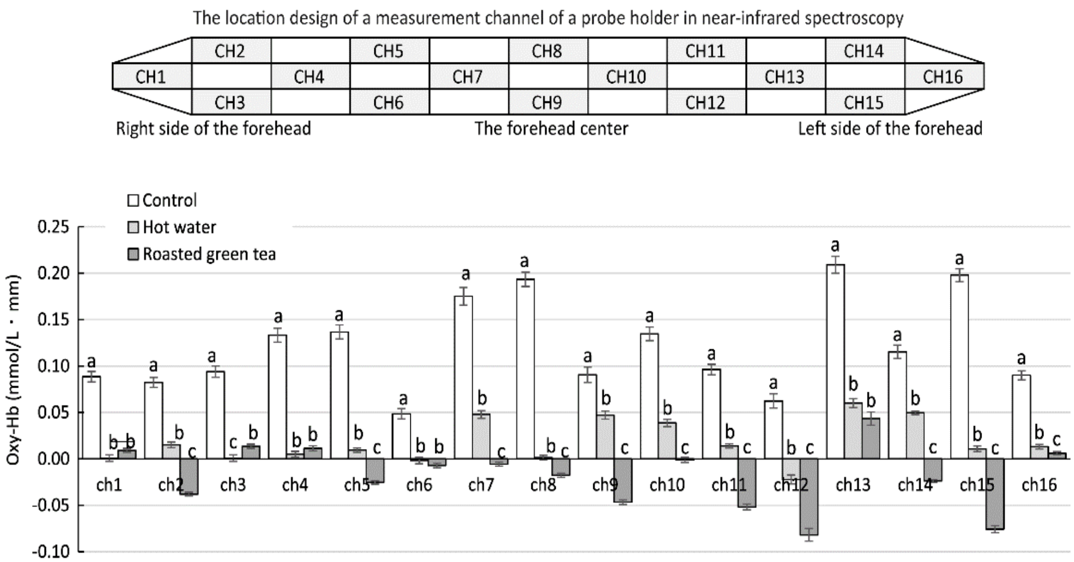


Figure 5. Effects of hot water and roasted green tea aroma on cerebral blood flow.

The relative amount of Oxy-Hb during 2 min of inhalation of the control (air), hot water vapor, and roasted green tea aroma. Data are shown as mean ± SEM (n = 20). *$p < 0.05$, by multiple comparisons using the Bonferroni method. There are significant differences between the different letters.

Test II

Results of the determination of major aromatic components of roasted green tea extract*:* Table 1 shows the results of GC-MS analysis of the three different pyrazines, the primary aromatic components of roasted green tea, including the equations for the calibration curves of the respective pyrazines and their correlation coefficients (R^2). The results showed that 2-ethyl-5(6)-methylpyrazine was 140 ppb, 2-ethyl-3,5(6)-dimethylpyrazine was 133 ppb, and 2,3,5-trimethylpyrazine was 105 ppb. In addition, the table shows the retention index (RI) and their corresponding aroma qualities.

Table 1. Quantitative values and aroma quality of three different pyrazines of roasted green tea extract

Pyrazine	Retention index	Calibration curve	R^2	[ppb]	Aroma quality
2-Ethyl-5(6)-methylpyazine	1396	y = 0.0341x + 0.1028	0.9945	140	Sweet and nutty
2-Ethyl-3,5(6)-dimethylpyrazine	1466	y = 0.0417x + 0.4791	0.9749	133	Sweet, caramel, and savory
2,3,5-Trimethylpyrazine	1410	y = 0.0168x - 0.0464	0.9991	105	Sweet and savory

TEST III

Subjective evaluation of three different pyrazine aromas*:* The results of the subjective evaluation of the three pyrazine aromas are shown in Figure 6. No significant differences were found in the items of "preference" and "comfort". For "strength of scent", 2-ethyl-3,5(6)-dimethylpyrazine (Sample2) scored significantly higher than the other pyrazines (Sample1, $p < 0.01$; Sample3, $p < 0.01$).

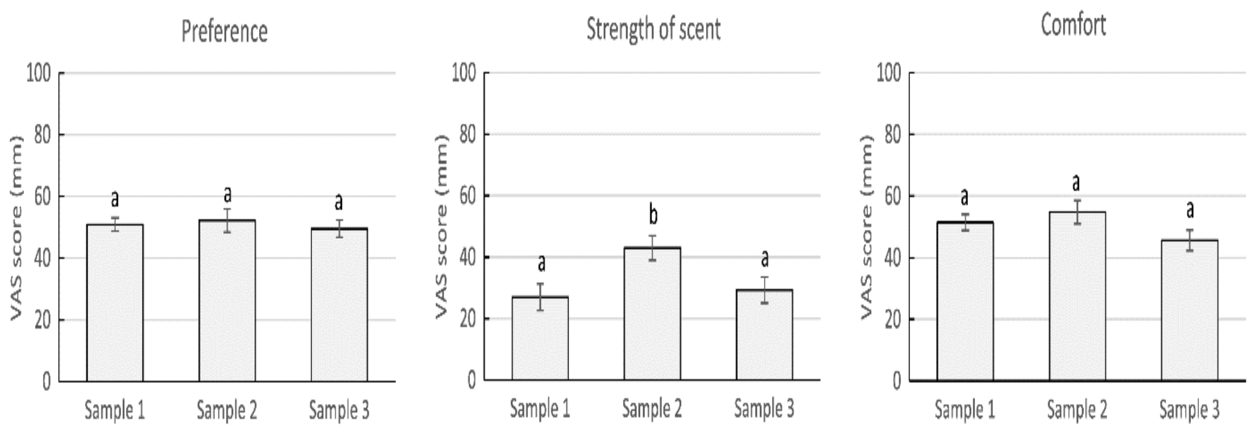


Figure 6. Subjective evaluation of three pyrazine aromas.

VAS scores for (a) preference, (b) strength of scent, and (c) comfort after 2 min of inhalation of three pyrazine aromas were measured using a 100 mm VAS from 0 (minimum intensity) to 100 (maximum intensity). Sample 1: 2-ethyl-5(6)-methylpyrazine; Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, by multiple comparisons using the Bonferroni method. There are significant differences between the different letters.

Measurement of pupil miosis rate and peripheral skin temperature: Figure 7 shows the pupil miosis rate after inhalation of three different pyrazine aromas (Control1: 0.345 ± 0.079, Sample1: 0.372 ± 0.069; Control2: 0.351 ± 0.069, Sample2: 0.386 ± 0.076; Control3: 0.332 ± 0.085, Sample3: 0.391 ± 0.058). Compared to that in the control, the pupil miosis rate significantly increased after the inhalation of each pyrazine aroma (Sample1, $p < 0.01$; Sample2, $p < 0.01$; Sample3, $p < 0.01$). The results of peripheral skin temperature are shown in Figure 8 (Control1: 31.6 ± 2.5, Sample1: 32.2 ± 2.9; Control2: 32.1 ± 2.2, Sample2: 32.7 ± 2.7; Control3: 31.9 ± 2.6, Sample3: 32.5 ± 2.8). Similar to the pupil miosis rate, there was a significant increase in fingertip skin temperature after inhalation of each pyrazine aroma compared to the control (Sample1, $p < 0.01$; Sample2, $p < 0.01$; Sample3, $p < 0.01$).

Furthermore, after performing a one-way ANOVA on the change in pupil miosis rate (ΔCR) between each control and pyrazine (Sample1: 0.027 ± 0.049; Sample2: 0.035 ± 0.050; Sample3: 0.059 ± 0.065), multiple comparisons using the Bonferroni method revealed a significant trend in the difference in ΔCR between 2-ethyl-5(6)-methylpyrazine and 2,3,5-trimethylpyrazine ($p < 0.1$) (Figure 9). Similarly, one-way ANOVA was performed on the change in fingertip skin temperature (ΔFT) between each control and pyrazine (Sample1: 0.56 ± 0.67; Sample2: 0.53 ± 0.62; Sample3: 0.53 ± 0.55), but no significant difference was found (Figure 10).

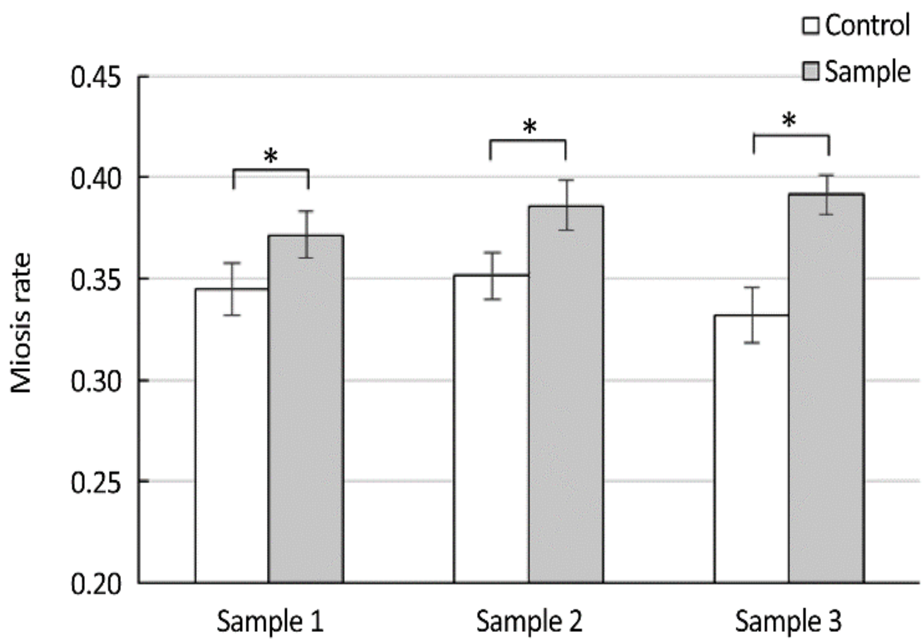


Figure 7. Effects of pyrazine aromas on pupil miosis rate.

The miosis rate after 2 min of inhalation of the control (water) and three pyrazine aromas. Sample 1: 2-ethyl-5(6)-methylpyrazine; Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 19). * $p < 0.05$, as determined by paired t-test.

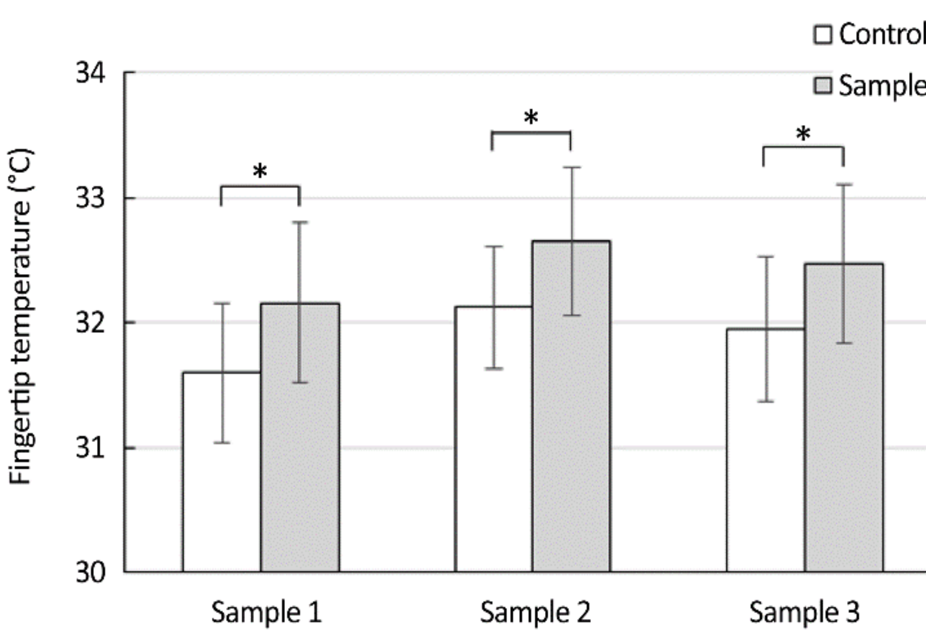


Figure 8. Effects of pyrazine aromas on peripheral skin temperature.

The fingertip temperature after 2 min of inhalation of the control (water) and three pyrazine aromas. Sample 1: 2-ethyl-5(6)-methylpyrazine; Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, as determined by paired t-test.

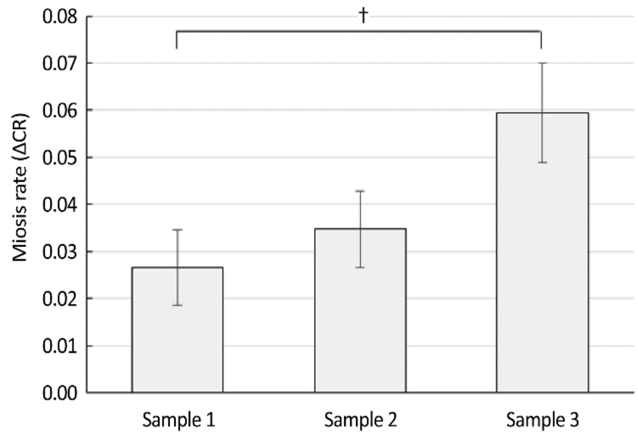


Figure 9. Comparison of change in pupil miosis rate among three pyrazine aromas.

Sample 1: 2-ethyl-5(6)-methylpyrazine; Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 19). † $p < 0.1$, by multiple comparisons using the Bonferroni method.

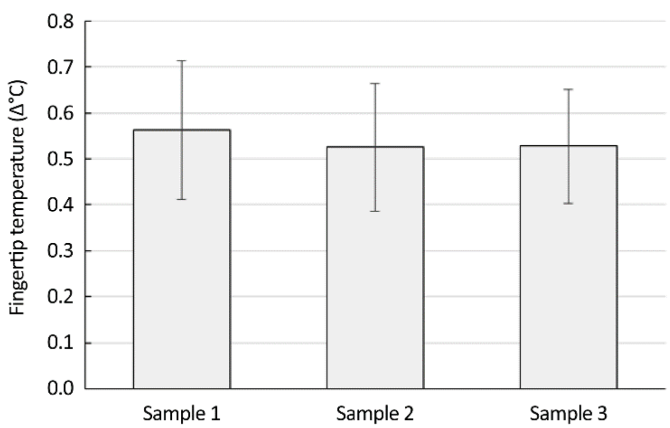


Figure 10. Comparison of change in peripheral skin temperature among three pyrazine aromas.

Sample 1: 2-ethyl-5(6)-methylpyrazine; Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 20).

Evaluation of subjective mood state: The results of the subjective mood state after inhalation of the three different pyrazine aromas are shown in Figure 11a–c. Compared to the control, the scores for “tension” and “anxiety” were significantly decreased after inhalation of 2-ethyl-5(6)-methylpyrazine (“tension,” $p < 0.05$; “ anxiety,” $p < 0.01$) (Figure 11a). In addition, a significant decrease in “concentration” scores was observed with 2-ethyl-3,5(6)-dimethylpyrazine (“concentration,” $p < 0.05$) (Figure 11b), while a decrease in “fatigue” scores and an increase in “sleepiness” scores were observed with 2,3,5-trimethylpyrazine (“fatigue,” $p < 0.05$; “ sleepiness,” $p < 0.05$) (Figure 11c).

The MMS results are shown in Figure 12a–c. Compared to the control, the scores for “affinity” decreased significantly after inhalation of 2-ethyl-5(6)-methylpyrazine (“affinity,” $p < 0.05$) (Figure 12a). In addition, after inhalation of 2-ethyl-3,5(6)-dimethylpyrazine (Figure 12b), there was a significant decrease in “fatigue” scores (“fatigue,” $p < 0.05$), while after inhalation of 2,3,5-trimethylpyrazine (Figure 12c), there was a decrease in “focus” scores (“focus,” $p < 0.05$).

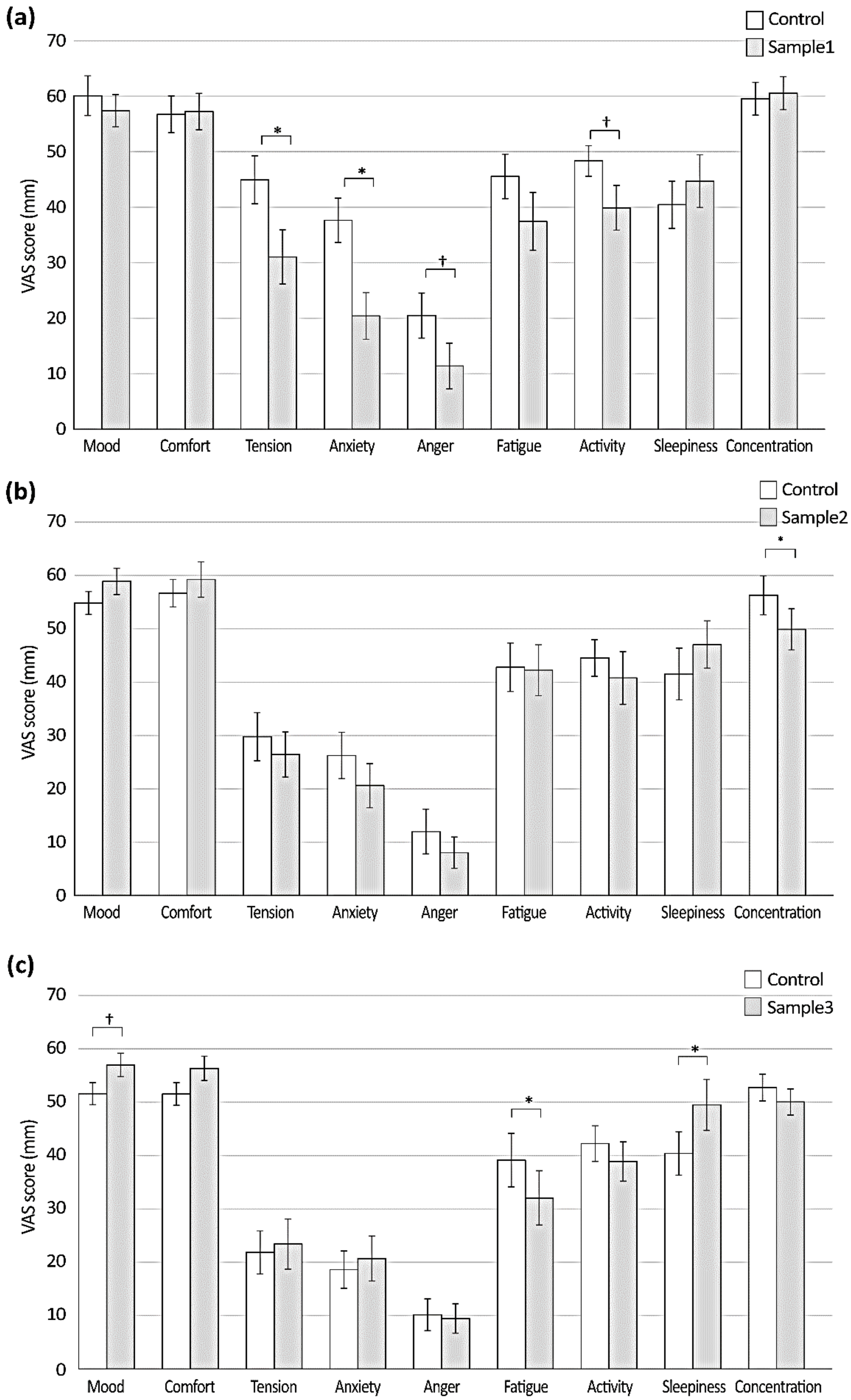


Figure 11. Effects of the three pyrazine aromas on subjective mood state.

VAS scores for mood, comfort, tension, anxiety, anger, fatigue, activity, sleepiness, and concentration after 2 min of inhalation of the control and the three different pyrazine aromas. (a) Sample 1: 2-ethyl-5(6)-methylpyrazine; (b) Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; (c) Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, † $p < 0.1$, by paired t-test.

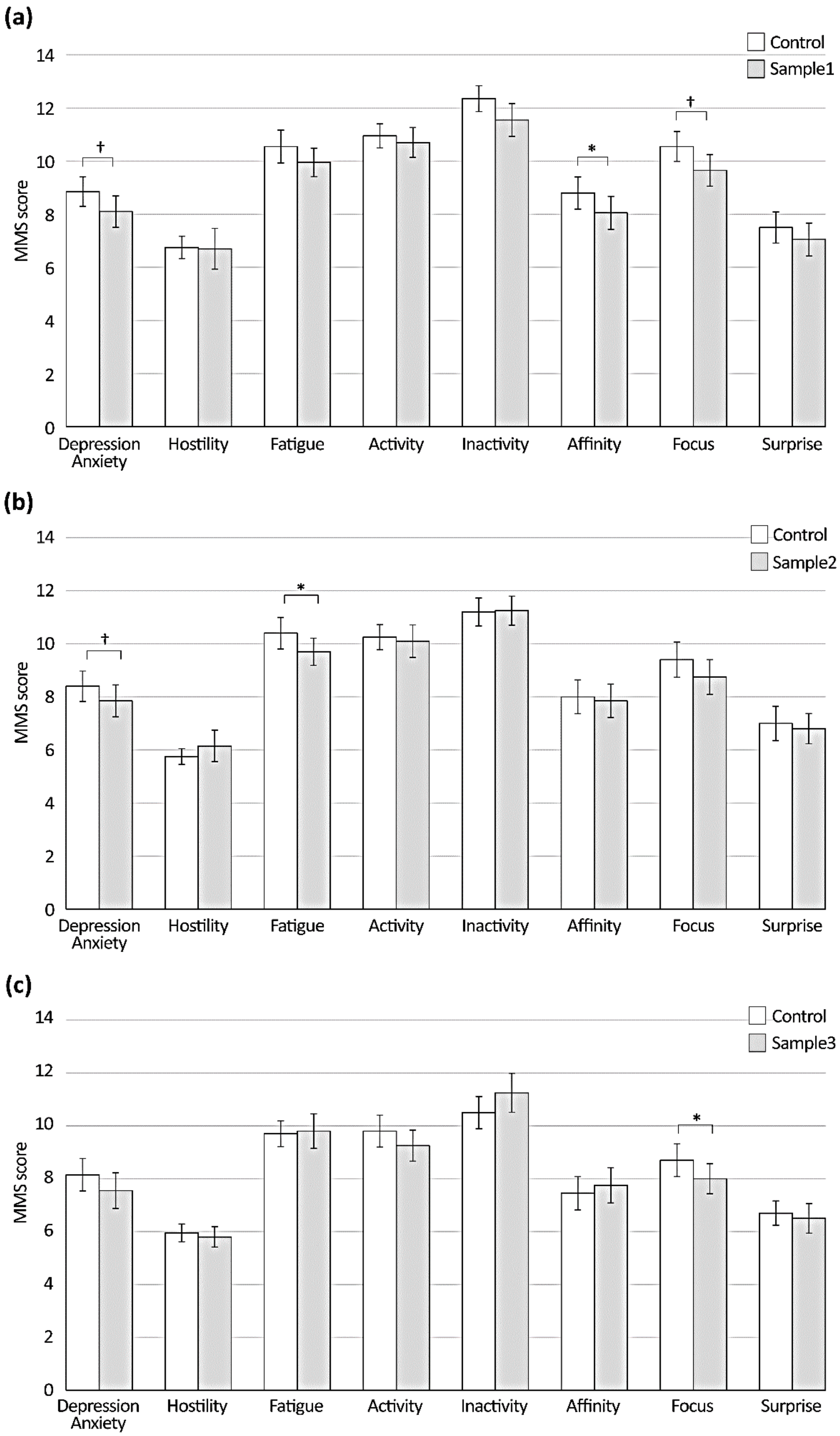


Figure 12. Effects of the three pyrazine aromas on multiple mood states.

MMS scores for depression/anxiety, hostility, fatigue, activity, inactivity, affinity, focus, and surprise after 2 min of inhalation of the control and three pyrazine aromas. (a) Sample 1: 2-ethyl-5(6)-methylpyrazine; (b) Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; (c) Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 20). * $p < 0.05$, † $p < 0.1$, by paired t-test.

Measurement of cerebral blood flow: The Oxy-Hb concentrations after inhalation of the three different pyrazine aromas are shown in Figure 13a–c. Compared to the control, the amount of Oxy-Hb was significantly increased in all channels of the prefrontal cortex after inhalation of 2-ethyl-5(6)-methylpyrazine (Figure 13a). In addition, inhalation of 2-ethyl-3,5(6)-dimethylpyrazine tended to decrease the amount of Oxy-Hb in the upper frontal regions (ch2, 4, 5, 7, 11, and 14) and increase it in the lower regions (ch3, 6, 8, 9, 12, 15, and 16) (Figure 13b). In contrast, inhalation of 2,3,5-trimethylpyrazine caused a significant decrease in Oxy-Hb in all channels except ch11 compared to the control (Figure 13c) (data from three participants who showed a significant decrease in Oxy-Hb in the control were excluded here).

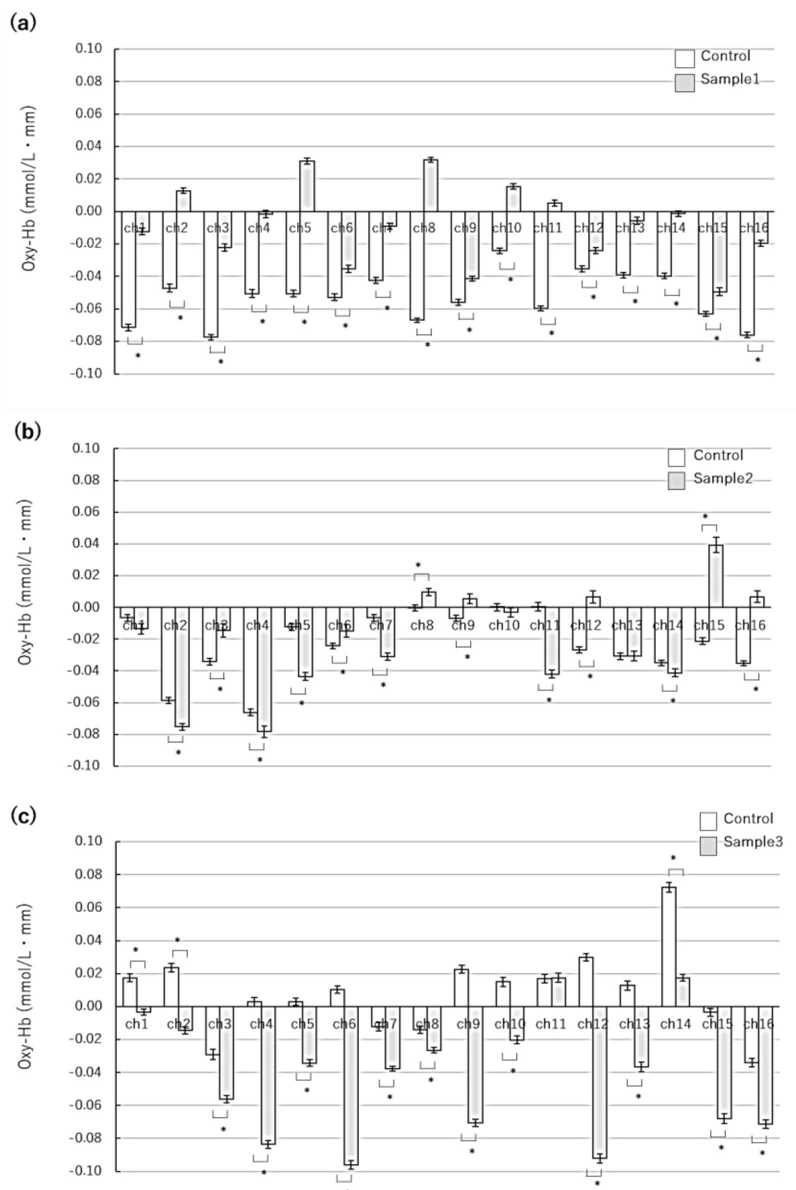


Figure 13. Effects of the three pyrazine aromas on cerebral blood flow.

The relative amount of Oxy-Hb during 2 min of inhalation of the control and three pyrazine aromas. (a) Sample 1: 2-ethyl-5(6)-methylpyrazine; (b) Sample 2: 2-ethyl-3,5(6)-dimethylpyrazine; (c) Sample 3: 2,3,5-trimethylpyrazine. Data are shown as mean ± SEM (n = 17). * $p < 0.05$, as determined by paired t-test.

DISCUSSION

To clarify the physiological and psychological effects of roasted green tea aroma, this study examined the effects of roasted green tea extract and its main aromatic components on autonomic nervous activity, central nervous activity, and subjective mood state.

First, a subjective evaluation of the aromas of the study samples was conducted using the VAS. In Test I, the participants found the roasted green tea aroma to be more preferable and comfortable than hot water vapor (Figure 1). Regarding the evaluation of hot water, participants detected no aroma other than the vapor itself, which resulted in a score around the median (50 mm) for both "preference" and "comfort." Similarly, in Test III, there was no difference in "preference" and "comfort" scores among the three pyrazines, with ratings also around 50 mm, similar to those for hot water. This suggests that the participants perceived little to no aroma from the pyrazines (Figure 6). The reason for this may be that, in Test III, the pyrazines were presented at room temperature; therefore, their volatilization was lower than that of the roasted green tea extract.

Next, the effects of the aroma of roasted green tea on autonomic nervous system activity were examined by measuring the pupil miosis rate, which reflects parasympathetic nervous system activity, and peripheral skin temperature, which reflects sympathetic nervous system activity. Unlike heart rate variability analysis, which requires controlled respiration for the evaluation of the autonomic nervous system, these evaluation methods can be performed with high accuracy in a relatively short measurement time under normal breathing conditions [20,21].

In Test I, inhalation of hot water vapor and roasted green tea aroma increased the pupil miosis rate and fingertip skin temperature, indicating that both samples had a sedative effect that increased parasympathetic activity by suppressing sympathetic activity (Figures 2 and 3). However, this result also raises the possibility that the effect of roasted green tea aroma may be influenced by temperature (hot water vapor). In contrast, in Test III, an increase was observed in the pupil miosis rate and fingertip skin temperature in response to the aromas of the three different pyrazines at room temperature, with concentrations based on the analysis results (Figures 7 and 8).

These findings indicate that these main aromatic components of roasted green tea also have a sedative effect that suppresses sympathetic nerve activity and increases parasympathetic nerve activity [16, 21, 23, 24]. Although Test I could not determine whether the sedative effect of roasted green tea aroma was due to the action of its aromatic components or the hot water vapor, Test III confirmed the sedative effect of three pyrazines at room temperature. This demonstrates that the sedative effect of roasted green tea aroma is distinct from that of hot water vapor and is specifically derived from its aromatic components, with these pyrazines playing a role in the sedative effect. Among the three pyrazines, 2,3,5-trimethylpyrazine increased the pupil miosis rate the most, suggesting that differences in the functional groups of pyrazines may influence the differences in physiological effects (Figure 9).

The effect of roasted green tea aroma on central nervous system activity was examined using NIRS to measure changes in the amount of Oxy-Hb, which reflects brain activity. In Test I, inhalation of hot water vapor and roasted green tea aroma resulted in a decrease in the amount of Oxy-Hb in the prefrontal cortex, suggesting that cerebral blood flow decreased (Figure 5). This effect was more pronounced for the roasted green tea aroma than for hot water vapor. These results clearly demonstrate the sedative effect of the roasted green tea aroma on the central nervous system. In addition, in Test III, 2-ethyl-3,5(6)-dimethylpyrazine and 2,3,5-trimethylpyrazine decreased the amount of Oxy-Hb

(Figure 13b, c). These findings indicate that the aroma of roasted green tea and its main aromatic components have a sedative effect that decreases the amount of Oxy-Hb in the prefrontal cortex of the brain and decreases cerebral blood flow. Among the three pyrazines, the effect of 2,3,5-trimethylpyrazine was the most pronounced. In contrast, 2-ethyl-5(6)-methylpyrazine tended to increase the amount of Oxy-Hb. This may have occurred because, during the first half of the control measurement, the Oxy-Hb levels significantly decreased across all channels, likely due to participant drowsiness. Then, the act of presenting the sample may have acted as a stimulus, producing an arousal effect. We observed that three participants experienced obvious drowsiness during the NIRS measurement, corresponding with a significant decrease in Oxy-Hb levels. Therefore, 2-ethyl-5(6)-methylpyrazine did not directly increase Oxy-Hb levels, but the presentation of the aroma may have stimulated a transient increase in the amount of Oxy-Hb. Similarly, 2-ethyl-3,5(6)-dimethylpyrazine caused a further decrease in Oxy-Hb, particularly in the upper frontal region, indicating a mild sedative effect by decreasing cerebral blood flow. Overall, these findings clarified the sedative effects of roasted green tea aroma on both the autonomic and central nervous systems, with 2,3,5-trimethylpyrazine showing the strongest sedative effect among the three pyrazines.

Finally, the effects of the roasted green tea aroma on subjective mood state were evaluated. In Test I, the scores for “tension” and “anxiety” were decreased by hot water vapor and roasted green tea aroma, while the scores for “mood,” “comfort,” and “sleepiness” were increased only by roasted green tea aroma (Figure 4). These results reveal the sedative effect of roasted green tea aroma on psychological aspects. In addition, in Test III, 2-ethyl-5(6)-methylpyrazine decreased the scores for “tension” and “anxiety,” and 2,3,5-trimethylpyrazine decreased the scores for “fatigue” but increased the scores for “sleepiness” (Figure 11a–c). These results indicate that the main aromatic components of roasted green tea also have a psychological sedative effect. However, the aromas of the individual pyrazines did not elicit effects as remarkable as those of roasted green tea aroma and were relatively weak. This may be because the participants did not perceive the aromas of these pyrazines during the subjective evaluations (Figure 6b) [16]. In addition, the MMS results showed that 2-ethyl-5(6)-methylpyrazine decreased the “affinity” score, 2-ethyl-3,5(6)-dimethylpyrazine decreased the “fatigue” score, and 2,3,5-trimethylpyrazine decreased the “concentration” score, but all aromas had weak overall effects (Figure 12a-c).

Ohata et al. revealed the sedative effects of the aromatic compounds 2,3-dimethylpyrazine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), which are produced by the Maillard reaction of glycine and glucose, by measuring the pupil miosis rate and fingertip skin temperature [16]. Regarding subjective mood changes, they found that DMHF reduced the scores for anger hostility and tension anxiety, while 2,3-dimethylpyrazine had no effect on mood state. On the other hand, Ohno et al. examined the sedative effects of the second flush of Darjeeling black tea (SFDJ) and reported that the physiological sedative effects of SFDJ aroma occur independently of preference or psychological factors [23]. They also reported that hotrienol, a characteristic aromatic component of SFDJ, plays a crucial role in its sedative effects. The physiological effects of hotrienol were observed even at low concentrations, although most participants were unable to perceive the aroma [24]. Furthermore, hotrienol showed little psychological action in reducing stress and depression/anxiety, similar to the aroma of SFDJ. The results of the current study showed that three different pyrazines, the major aromatic components of roasted green tea, had physiological sedative effects like those of the roasted green tea aroma, even though these aromas were barely perceived. However, the psychological effects were relatively weak compared to those of the roasted green tea aroma, which is similar to the results of the previous study. Therefore, the physiological effects on the autonomic and central nervous systems observed in this study are direct and not influenced by the perception or preference of the aroma. In contrast, the psychological effects are likely influenced by the participants' subjective evaluation of the aroma, including their preference and sensitivity to it.

In this study, the aroma of roasted green tea extract and its main aromatic components were found to have a sedative effect on the autonomic nervous system. Furthermore, the findings suggest that roasted green tea aroma and 2,3,5-trimethylpyrazine also have sedative effects on the central nervous system (Figures 5 and 13c). Pyrazines, such as 2,3,5-trimethylpyrazine, are known for their roasted nut-like aroma and contribute to the sweet-savory aroma characteristic of roasted green tea. Therefore, the use of these aromas in beverages and foods to increase preference can be expected to produce psychological and physiological effects [25-27]. Furthermore, the fact that these pyrazines exhibit physiological efficacy even at low concentrations, which cannot be perceived as aromas, suggests their potential as functional aromatic materials, regardless of preference. This study explored both the physiological and psychological effects of roasted green tea aroma and its main components, including three different pyrazines. Future research should investigate other aromatic components in roasted green tea and develop their applications in food and beverages. In the next phase, we plan to investigate the effects of consuming the product under actual stressful conditions. Future research could contribute to consumer health and quality of life by and has implications for reducing daily stress through the consumption of beverages and foods containing functional aromatic compounds. The findings of this study, which elucidated the physiological and psychological effects of roasted green tea aroma, hold significant potential for achieving these goals. A potential limitation of this study includes managing participant-related variables during NIRS measurements to ensure consistency and minimize bias. To accommodate this, participants need sufficient sleep the day before to avoid feeling drowsy on the day of the test. Additionally, addressing the effect of the order in which samples are presented and the challenge of evaluating multiple samples in a single day to eliminate the difference between days, also need consideration in future studies.

CONCLUSION

The aroma of roasted green tea increased the pupillary miosis rate and peripheral skin temperature, although this effect was also observed with hot water. However, the aroma of roasted green tea reduced the amount of Oxy-Hb in the prefrontal cortex of the brain more than hot water, indicative of its sedative effect on the central nervous system. Upon psychological evaluation, roasted green tea aroma inhalation reduced the scores for "tension" and "anxiety" while increasing those for "mood" and "comfort" when compared with the effects of hot water. Additionally, the three different pyrazines, the main aromatic components of roasted green tea, similarly increased the pupil miosis rate and peripheral skin temperature at room temperature. The amount of Oxy-Hb was reduced by 2,3,5-trimethylpyrazine. These results suggest that the aroma of roasted green tea exerts physiological and psychological effects associated with sedation superior to those of hot water. Furthermore, three pyrazines were involved in the sedative effect of roasted green tea, with 2,3,5-trimethylpyrazine being the most important aromatic component. The physiological effects of the aroma identified in this study were considered direct effects and remained unaffected by the participants' perception or preference, whereas the psychological effects were thought to be influenced by the subjective evaluation of the aroma.

Abbreviations: EGCG: epigallocatechin gallate; NIRS: near-infrared spectroscopy; Oxy-Hb: oxygenated hemoglobin; VAS: Visual Analog Scale; MMS: Multiple Mood Scale; AEDA: Aroma Extract Dilution Analysis; SPME: solid-phase microextraction; GC-MS: gas chromatography-mass spectrometry; ANOVA: Analysis of variance; DMHF: 2,5-dimethyl-4-hydroxy-3(2H)-furanone; SFDJ: second flush of Darjeeling black tea; UMIN: University Hospital Medical Information Network

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Authors' contributions: Akio Sugimoto: Conceptualization, Methodology, Investigation, Analysis, Writing; Yuka Tatsumi: Investigation, Analysis; Masaki Ichitani: Project administration

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