【Short Communication】

# The Influence of Olfactory Stimulation by Essential Oils on Salivary Alpha-Amylase Activity and State Anxiety Level

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**[ABSTRACT]**
It is considered that olfactory stimulation by fragrance inhalation is one of the methods of relaxation. We examined the possibility by using fragrance inhalation essential oils, such as sweet orange oil and peppermint oil. We measured salivary alpha-amylase activity and state anxiety levels in undergraduate students before and after inhalation. Salivary alpha-amylase activity levels have been utilized to assess the sympathetic nervous activity.
Our results indicate that both salivary alpha-amylase activity and state anxiety levels are significantly reduced after fragrance inhalation of the sweet orange oil. Consequently, our research suggests that sweet orange essential oil has a relaxation effect.

**[Key words]**
essential oil, salivary alpha-amylase activity, State-Trait Anxiety Inventory-Form JYZ

## INTRODUCTION

It has been shown that olfactory stimulation by fragrance inhalation using essential oil exerts physiological effects. In young healthy individuals, essential oil inhalation was found to be an effective method of relaxation. As an indicator of relaxation, the low frequency (LF) and high frequency (HF) components of heart rate variability were used to quantify modulation of the sympathetic and parasympathetic systems[1)]. Also, as another indicator, salivary alpha-amylase activity levels have been utilized to evaluate sympathetic nervous activity. Previous studies have indicated that salivary alpha-amylase activity levels rose in response to psychological and physical stress[2,3)]. Salivary alpha-amylase activity levels might reflect the acute psychological response by fragrance inhalation[4)].

Few experimental studies have examined the physiological and psychological effects of essential oil fragrance inhalation. It is expected that fragrance inhalation by using essential oil is an easy and noninvasive method to promote relaxation in humans. Therefore, we investigated the relaxation effect of fragrance inhalation on the sympathetic nervous activity by the measuring of salivary-alpha amylase activity and state anxiety level change by using the psychological index, the State Trait Anxiety Inventory (STAI)[5)].

## MATERIALS AND METHODS

### 1 Participants

Seventy-nine healthy undergraduate students (6 males and 73 females) aged 19 to 21 years old, participated in our study. All participants were informed about this study and consent

Accepted Date: October 12, 2012

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was obtained from each participant. This study was approved by the Ethics Committee of Hokkaido Bunkyo University.

**2 Methods**

Participants were randomized to inhale sweet orange oil or peppermint oil. Each participant was tested by control water and sweet orange oil or peppermint oil at the same time on separate days. Participants were unaware of what kind of essential oil was applied.

A tissue paper containing 100 µl of water was used as the control. Sweet orange (*Citrus sinensis* SIRIUS) oil and peppermint (*Mentha piperita L* SIRIUS) oil were used as essential oils for fragrance inhalation. The main constituents of sweet orange oil and peppermint oil were limonene (96.3%) and menthol (45.1%), respectively. For stimulation, 100 µl of the solution was applied to a piece of absorbent tissue paper (5 × 5 cm), and then placed under the participant's nose to inhale the fragrance of the essential oil and control water. Participants inhaled the control and the fragrance of the essential oils for 1 min during regular breathing.

Each participant took a seated position while inhaling the two samples, control water and essential oil. After inhalation of the control water, the salivary alpha-amylase activity and state anxiety levels were measured. Next, after the inhalation of the essential oil, the salivary alpha-amylase activity and state anxiety level were measured again.

As for the physiological parameters, the level of salivary alpha-amylase activity was measured by a Salivary Amylase Monitor (Nipro Co., Japan), which consisted of a testing-strip, a salivary transcription device and an optical analyzer. By adding maltose, a testing-strip is able to detect the salivary activity from 0 to 200 KU/L during a time of less than 150 seconds, and a salivary transcription device controlled the reaction time of the enzyme[7]. In order to evaluate the Salivary Amylase Monitor, salivary alpha-amylase activity between 0 and 200 KU/L, the calibration curve for the Salivary Amylase Monitor obtained a coefficient of $R^2$ value of 0.988 and a coefficient of variation of 10.2%[8].

The "state" component of the State-Trait Anxiety Inventory-Form JYZ[6] (STAI-JYZ) was used to evaluate each participant's anxiety levels before and after fragrance inhalation. STAI-JYZ included of two distinctive self-assessment subscales: the state-anxiety, which refers to a transitory emotional state or a state of stress and apprehension, and the trait-anxiety, which is able to assess relatively stable individual differences regarding anxiety tendencies. Both the state-anxiety and the trait-anxiety consist of 20 statements. Each answer has an intensity range from 1 to 4, and a sum total classified individuals according to the anxiety scores: low (20–44 points), moderate (45–54 points), high (55–80 points), concerning both the state and trait scores.

**3 Statistical analysis**

Paired t-test was used to examine differences between before and after fragrance inhalation in the state-anxiety. The Wilcoxon signed-rank tested differences between before and after fragrance inhalation in salivary alpha-amylase activity levels. Logarithmic-transformation was performed for levels of salivary alpha-amylase activity. The statistical significance level was set at 0.05 of p-value. The analyses were performed with a commercially available statistical package (SPSS Ver. 16.0).

## RESULTS

Change in salivary alpha-amylase activity levels induced by fragrance inhalation of sweet orange oil and peppermint oil was measured. As shown in Fig. 1, comparison of measured levels before and after inhalation of sweet orange oil decreased from 3.90 to 3.55 KU/L (median value: by logarithmic-transformation) in salivary alpha-amylase activity ($p < 0.05$). In contrast, fragrance inhalation of peppermint oil did not show significant change.

The "state" component of STAI-JYZ was used to evaluate before and after inhalation of sweet orange oil and peppermint oil. The state anxiety score decreased from 48.2 point to 41.2 point after fragrance inhalation of sweet orange oil. This change was statistically significant ($p < 0.001$). On the other hand, the state anxiety score by inhalation of peppermint oil did not change after fragrance inhalation (Fig. 2).

## DISCUSSION

As shown in Fig. 1 salivary alpha-amylase activity levels were significantly lower than before fragrance inhalation of sweet orange oil. Previous studies have indicated that fragrance inhalation has an influence on salivary alpha-amylase activity or sympathetic nervous activity. It has been shown that salivary alpha-amylase activity decreased significantly by lavender oil inhalation[4]. In another study, fragrance inhalation of rose oil resulted in a 30% decrease of adrenaline plasma concentration[9]. On the other hand, fragrance inhalation of pepper oil

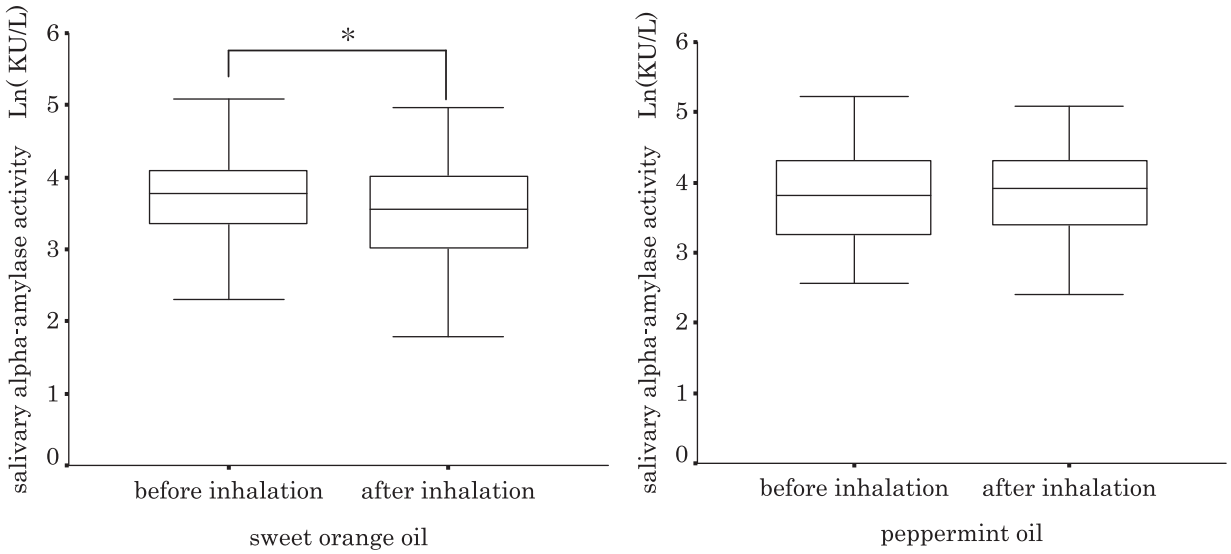


**Fig. 1** Change of salivary alpha-amylase activity levels (logarithmic-transformation) before and after fragrance inhalation of essential oils.
*p<0.05 (Wilcoxon signed-rank test).
sweet orange oil (n=44), peppermint oil (n=31)

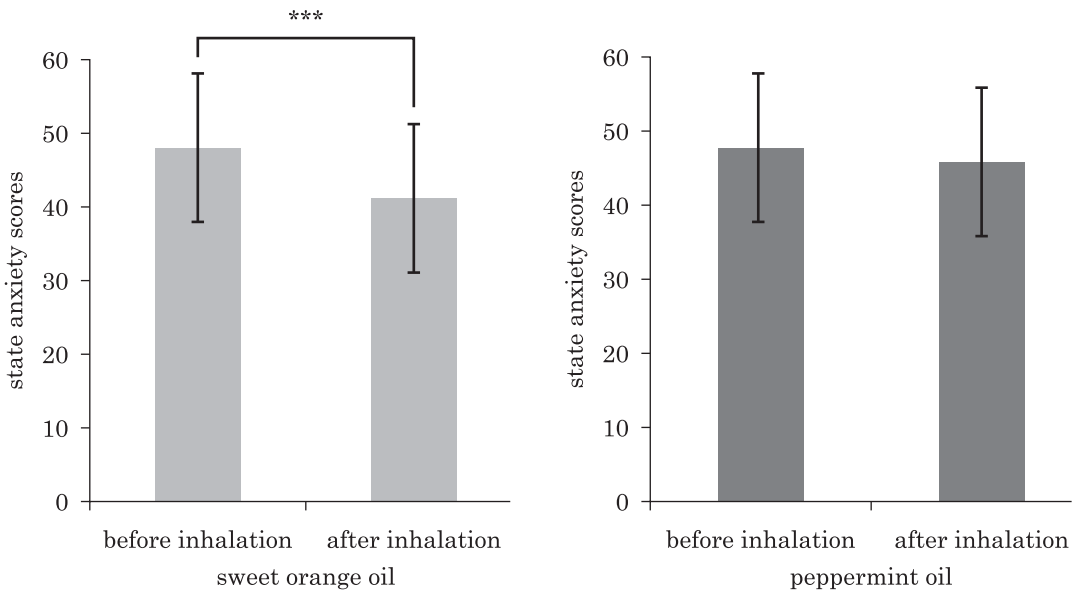


**Fig. 2** Change of state anxiety scores before and after fragrance inhalation of essential oils.
***p<0.001 (paired t-test).
sweet orange oil (n=39), peppermint oil (n=31)

induced a 1.7-fold increase in plasma adrenalin concentration[9]. Furthermore, after fragrance inhalation of bergamot oil, there were significant decreases in blood pressure and heart rate along with the ratio of the low frequency (LF) power to high frequency (HF) components of heart rate variability (LF/HF) showing an increase in heart rate variability and HF power percentage[10]. These findings indicate that fragrance inhalation of essential oil stimulates or depresses the sympathetic nervous activity. Accordingly, it may be considered that the results from this study showing salivary alpha-amylase activity levels decrease after fragrance inhalation were caused by depressing sympathetic nervous activity.

From our results, the score of the state anxiety also decreased significantly from before fragrance inhalation of the sweet orange oil (Fig. 2). In other words, state anxiety level changed from moderate score (48.2) to a low score (41.2) after

1 min of fragrance inhalation of the sweet orange oil. It is conceivable that sweet orange oil might induce relaxation through psychological effects and decrease sympathetic nervous system activity. In experimental study on Wister rats, anxiolytic effects were observed using sweet orange[11]. The effect of some essential oils, including sweet orange oil, and lavender oil were reported to reduce anxiety through use as aromatherapy[12,13]. In patients with breast cancer, the anxiety scores reduced after massage of the neck, thorax, back, shoulders, arms, hand and upper legs using by sweet orange oil in the STAI test[14].

In contrast, our results show that there are no significant differences before and after fragrance inhalation of peppermint oil. Peppermint oil has been used to calm pruritus and relieve irritation[15]. Research indicates that peppermint oil and its components, such as menthol, produce moderate inhibitory activity against human pathogens[16]. Effects of peppermint oil may be different from relaxation.

Limitations of this study included that participants were only undergraduate students and a large majority were female students. Various age groups and males will need to be studied in the future. Also, another indicator of sympathetic nervous system activity will be required in future studies, such as LF/HF.

In conclusion, our findings suggest that fragrance inhalation of sweet orange oil may be utilized as a method of relaxation in undergraduate students.

## ACKNOWLEDGEMENTS

The authors would like to thank the participants who cooperated in this study. This research was supported by a grant-in-aid from Hokkaido Bunkyo University. The authors declare that there are no conflicts of interest associated with this study.

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# 要　旨

## 精油嗅覚刺激による唾液 α-アミラーゼ活性と状態不安に及ぼす影響

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精油の香り吸入による嗅覚刺激は，リラクゼーションの方法の一つと考えられている．スウィートオレンジの嗅覚刺激後に，唾液 α-アミラーゼ活性と状態不安レベルは有意な低下を示した．ペパーミントでは，有意な変化はみられなかった．ある種の精油による嗅覚刺激によって，リラックス効果が得られる可能性が示唆された．

**キーワード**：精油，唾液 α-アミラーゼ活性，状態・特性不安検査（新版）