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Sensory thresholds for natural flavoring extracts in different matrices

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Abstract

The information on sensory odor thresholds is of importance for food industry as necessary guidelines for the proper use of flavoring agents in food products that do not exceed an adequate perception. The objective of this study was to determine the best estimate threshold (BET) for detection of four commercial natural flavoring extracts (vanilla, almond, mint and lemon) in various matrices, i.e. water, sucrose solution, and pasteurized milk. Using the forced-choice ascending concentration method of limits (ASTM: E-679) with 8-trained sensory panelists and the general population (n=375), the mean BET for each extract evaluated by general population in the same matrix was higher than that done by the trained panel. Odor thresholds of each extract varied from a low concentration of 0.01 µg/l for mint in water, to a high concentration of 118.89 µg/l for almond in pasteurized milk. In addition, matrix effects of natural extracts were observed among medium of evaluation, which can considerably influence the odor perception. Analysis of variance by Friedman ranking test revealed that there were significant differences in odor thresholds among flavoring extracts and media of evaluation ($p<0.05$).

Keywords: *detection thresholds, flavoring extract, sensory, matrix, three-alternative forced choice*

บทคัดย่อ

ขีดเริ่มรู้สึกรับรู้กลิ่นโดยประสาทสัมผัสมีความสำคัญในอุตสาหกรรมอาหารโดยเป็นแนวทางการใช้สารให้กลิ่นรสให้เหมาะสมในผลิตภัณฑ์อาหารที่เพียงพอต่อการรับรู้ วัตถุประสงค์ของงานวิจัยนี้คือเพื่อประเมินขีดเริ่มรู้สึกรับรู้โดยประมาณของสารสกัดให้กลิ่นรสจากธรรมชาติที่มีจำหน่ายทางการค้า 4 ชนิดคือ วานิลลา อัลมอนด์ มิ้นต์และเลมอน ในตัวกลางที่แตกต่างกันได้แก่ น้ำ สารละลายซูโครสและนมสดพาสเจอร์ไรซ์ ด้วยวิธีบังคับเลือกตามความเข้มข้นแบบจำกัด (ASTM: E-679) โดยใช้ผู้ทดสอบที่ผ่านการฝึกฝนจำนวน 8 คนและผู้ทดสอบทั่วไปจำนวนรวม 375 คน พบว่า ค่าเฉลี่ยของขีดเริ่มรู้สึกรับรู้ที่ได้จากผู้ทดสอบทั่วไป มีค่าสูงกว่าค่าเฉลี่ยที่ได้จากผู้ทดสอบที่ผ่านการฝึกฝนในตัวกลางการประเมินเดียวกัน ขีดเริ่มรู้สึกรับรู้กลิ่นของสารสกัดให้กลิ่นรสแต่ละชนิด มีความแตกต่างจากค่าต่ำที่ 0.01 ไมโครกรัม/ลิตรของมิ้นต์ในน้ำไปจนถึงค่าสูงที่ 118.89 ไมโครกรัม/ลิตรของอัลมอนด์ในน้ำนม นอกจากนี้ยังพบผลของเมตริกซ์ตัวกลางในการประเมินที่มีต่อสารให้กลิ่นรสซึ่งมีอิทธิพลอย่างมากต่อการรับรู้กลิ่น การวิเคราะห์ความแปรปรวนโดยวิธี Friedman แบบเรียงลำดับพบว่า ขีดเริ่มรู้สึกรับรู้ที่ได้จากสารให้กลิ่นรสแต่ละชนิดในตัวกลางต่างกันมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

คำสำคัญ: *ขีดเริ่มรู้สึกรับรู้, สารสกัดให้กลิ่นรส, ประสาทสัมผัส, เมตริกซ์, บังคับเลือกแบบสามตัวอย่าง*

1. Introduction

Flavor plays an important role as the quality attribute that affects consumers' acceptability and repeat purchase. Food flavors are mixtures of complex volatile compounds which only a small number of these identified volatiles are of significance in determining the flavor (Grosch, 2001). To obtain information about their perception threshold is considered as the first approach in the selection of volatile compounds that contribute to a characteristic aroma of the food from those that do not (Teranishi, Buttery, Stern, & Takeoka, 1991). Generally, it is accepted that aroma quality changes with concentration and the sensory threshold of the odorants. The detection threshold is the lowest concentration of a substance in a medium relating to the lowest physical intensity at which a stimulus is detected (ASTM, 1997). Detection thresholds are not only useful measuring tools for specifying the potency of a flavor compound in food but also for measuring an individual's sensitivity to some flavor compounds of interest (Meilgaard, Civille, & Carr, 2007). Odor threshold values depend on the medium in which the component is dissolved as published threshold values mostly were reported in water, air or other matrices (van Gemert, 2003). One of the most common methods of threshold determination is the forced-choice ascending method of limits (ASTM: E 679) since it offers practical values for a group threshold with a minimum of tests (ASTM, 1997). Sensory detection threshold determined by this method was reported for off-flavors in milk (Santos, Ma, Caplan, & Barbano, 2003), acetic acid and ethyl acetate in ice wines (Cliff & Pickering, 2006), iron salts (Lim & Lawless, 2006), aromatic compounds in wine (Santos et al., 2010), and strawberry radiation dose (Filho et al., 2014). As part of quality evaluation for product improvement project, a preliminary survey on flavoring agents used in food products available in the markets was undertaken. The information on detection thresholds of selected flavoring agents in three different media of evaluation; water, sucrose solution and pasteurized milk would serve as necessary guidelines for proper use in food products without excessive perception.

2. Objectives

This study was undertaken to determine the detection thresholds of four selected flavoring agents in three different media of evaluation; water, sucrose solution and pasteurized milk to provide a basis for comparison for future studies.

3. Materials and methods

3.1 Materials

Four 100% pure natural flavoring extracts, vanilla, almond, mint, and lemon (McCormick, USA), were purchased from local supermarkets. Volatile compounds known as major contributors to the aroma of each natural flavoring extract were listed with their published threshold range in water (Table 1). The flavoring agents were used for sample dilution preparation in three different media of evaluation; bottled drinking water (Singha, Thailand), 10% (w/w) sucrose (Mitrphol, Thailand) in drinking water and 4% fat pasteurized milk (CP-Meiji, Thailand).

Table 1 Flavoring extracts tested, their major ingredient as certified by manufacturer, and published odor threshold range of predominant compound in that particular ingredient

Natural flavoring extract	Major ingredient	Predominant compound	Published threshold range in water (µg/l)*
Vanilla	Vanilla bean extractives	Vanillin	29-66,700
Almond	Oil of bitter almond	Benzaldehyde	350-4,600
Mint	Oil of peppermint	L-menthol	100-2,500
	Oil of spearmint	L-Carvone	6.7-820
Lemon	Oil of lemon	Limonene	4-1,000

*van Gemert (2003)

3.2 Sample preparation

The three-alternative forced choice (3-AFC) ascending concentration method of limits described by the American Society for Testing and Materials, designation E-679-91 was used to determine detection thresholds. Fresh stock solutions of each substance providing the highest concentrations (9x), where x represents an approximate odor detection threshold concentration predetermined by three panelists including the experimenter, were prepared and refrigerated overnight at 5 °C to stabilize the flavor. Samples were diluted in a four successive series by a factor of 3 immediately before tasting. The solutions with mean volume of 30 ml were presented at constant room temperature in a 2 oz. sip glass covered with glass slits and labeled with three-digit random numbers. One presentation consisted of five triangles, each containing two controls and one spiked sample.

3.3 Sensory panels

The panel consisted of 375 tasters recruited from Siam University's staff and students based on their health and availability. Of these 375 panelists, eight were students from Department of Food Technology, Siam University who had been trained and had previously participated in sensory analysis. The proportion of gender was in the range of 40-50% for male and 50-60% for female. The majority age of panelists was in the range of 18-23 years old. Panelists were instructed to take three short sniffs of the sample headspace presented from left side to right side of the tray. They were asked to indicate which one was different from the other two. Panelists then sipped and chose the odd sample. The set of triangle test started with the lowest concentration and spaced with the 3 scale step higher concentration. Panelists waited for 30 seconds between each set of one triangle, sniffed their sleeves to clear the nasal passageways and rinsed their palates with water. All evaluations were conducted in individual booths within a controlled sensory panel room (25±2ºC). Panelists evaluated all flavoring agents in three matrices over several afternoon sessions until completed.

3.4 Sensory threshold determination

The individual best estimated threshold (IBET) was calculated as the geometric mean of the last concentration, with an incorrect response, and the first concentration with a correct response. The group best estimated threshold (GBET) was calculated as the geometric mean of the individual best estimated thresholds. The detection threshold data obtained from general population and from experienced sensory panel were analyzed by the Friedman two-factor ranked analysis of variance followed by the Fisher's LSD test (O'Mahony, 1986).

4. Results

The individual BET ranges of each natural flavoring extract for each matrix were listed in Table 2. A wide range of thresholds among panelists was observed. The lower detection values in this study can probably be partly due to the fact that the panel included the eight experienced panelists who were able to recognize these flavoring agents commonly used in the food products especially in ice-creams and jellies and were also acquainted with the test protocol. A panel trained extensively with a particular substance may have a profound influence on the threshold obtained with that substance (ASTM, 1997). The IBET ranges tended to increase with matrix possessing viscosity. Depending on the flavoring agents, the most sensitive panelists were 250, 280, and 240 times as sensitive as the least acute panelists evaluated in water, sucrose solution, and milk respectively. The proportion of correct responses of the IBET from panels demonstrated the left skewed distribution (data not shown). There were variations of sensitivity of individuals as seen from the log standard deviation which varied from the least uniform at 1.01 for mint to the most uniform at 0.35 for vanilla. Since the normality and the independence consumption did not hold for the obtained data, therefore, a nonparametric test was applied for further data analysis (Kuehl, 2000).

Table 2 Panelist individual best estimated threshold (IBET) range (μg/l) determined for each flavoring extract in various matrices; (A) aqueous solution; water (B) 10% sucrose solution and (C) pasteurized milk

Natural flavoring extract	Panelist IBET range (μg/l)		
	A	B	C
Vanilla	0.16-39.28	1.04-84.18	2.60-210.44
Almond	0.59-47.30	9.47-766.95	3.94-958.69
Mint	0.01-1.40	0.001-0.281	0.20-47.70
Lemon	0.18-45.27	0.37-90.41	4.49-515.35

Results of the group best estimated detection thresholds (GBET) for the four flavoring extracts in three matrices for all panelists (general population) and trained panel are shown in Table 3 and 4, respectively. Generally, population thresholds were higher than trained panel thresholds, and odor thresholds perceived through the nose (orthonasally) were higher than those done through the mouth (retronasally) (Plotto, Margaria, Goodner, Goodrich, & Baldwin, 2004). Threshold values determined in the complex system could differ significantly from those reported in the simpler system (van Gemert, 2003) as medium of evaluation has been reported to affect aroma perception (Tandon, Baldwin, & Shewfelt, 2000). Thresholds determined in this study were in a similar fashion as indicated but were several orders of magnitude lower than published threshold values especially those evaluated in water matrix. Detection thresholds for vanilla extracts in water were approximately between 180 and 1700 times lower than the reported vanillin values in water (van Gemert, 2003). Likewise, thresholds for almond extract in water were approximately between 97 and 590 times lower than the published values for benzaldehyde whereas 22 times lower than the reported limonene values belonged to the lemon extract thresholds, respectively (van Gemert, 2003). Since major ingredients for mint extract, as declared by the company, were the blending of oils from spearmint and peppermint. Therefore, results obtained in this study were 585-670 times and 1,700-10,000 times lower than published threshold values of l-carvone and l-menthol, respectively. (van Gemert, 2003). When matrix was changed, odor threshold values for each flavoring extract were higher except those for mint extract. This could suggest there is a mechanism between flavor compounds and food matrix components including binding, partitioning and releasing (Adhikari, Hein, Elmore, Heymann, & Willott, 2006). In addition, decreasing perceived intensities of volatile compounds in media containing varying concentration of compounds has been resulted from increasing viscosity (Hollowood, Linforth, & Taylor, 2002); a similar mechanism may be accounted for this as well. The lower values of thresholds perceived retronasally than orthonasally (Table 3 and Table 4) were probably due to the temperature in the mouth which would activate more volatiles into the headspace and thus decrease retronasal odor thresholds (Plotto et al., 2004).

Table 3 Group Best Estimated Thresholds (GBET) by different perception, log standard deviation (in parenthesis) determined by general population (n=375) for each flavoring extract in (A) aqueous solution; water (B) 10% sucrose solution and (C) pasteurized milk

Perception	Matrix	GBET (µg/l)			
		Vanilla	Mint	Almond	Lemon
Orthonasal	A	8.69 (0.72)	0.18 (0.83)	9.45 (0.67)	4.67 (0.56)
	B	28.06 (0.50)	0.03 (0.88)	91.28 (0.59)	12.99 (0.73)
	C	55.16 (0.58)	6.09 (0.77)	118.89 (0.64)	43.30 (0.62)
Retronasal	A	6.98 (0.70)	0.08 (0.80)	7.46 (0.71)	1.49 (0.77)
	B	18.08 (0.59)	0.02(0.70)	36.13 (0.64)	3.56 (0.70)
	C	35.30 (0.55)	4.12 (0.77)	51.20 (0.67)	31.93(0.55)

Table 4 Group Best Estimated Thresholds (GBET) by different perception, log standard Deviation (in parenthesis) determined by trained sensory panel (n=8) for each flavoring extract in (A) aqueous solution; water (B) 10% sucrose solution and (C) pasteurized milk

Perception	Matrix	GBET (µg/l)			
		Vanilla	Mint	Almond	Lemon
Orthonasal	A	9.94 (0.42)	0.10 (1.01)	4.59 (0.78)	3.82 (0.50)
	B	18.58 (0.35)	0.01 (0.93)	64.79 (0.56)	7.62 (0.71)
	C	40.49 (0.57)	5.30 (0.81)	80.94 (0.42)	36.31 (0.65)
Retronasal	A	6.58 (0.36)	0.05 (0.72)	3.99 (0.56)	0.83 (0.72)
	B	16.20 (0.44)	0.02 (0.70)	28.41 (0.44)	2.21 (0.76)
	C	30.77 (0.49)	4.04 (0.71)	46.72 (0.71)	30.30 (0.63)

Individual volatile compounds are known to act differently in different matrices according to their nature, especially hydrophilicity/hydrophobicity, which possibly decrease compound mobility as well as the dynamics of flavor release for sensory perception (McGorrin, 1996). Aromatic aldehydes (benzaldehyde and vanillin), ketone (l-carvone) demonstrate the lesser polarity of molecules than that of alcohol (l-menthol) whereas terpene compounds (limonene) are very lipophillic (Plotto et al., 2004). In sucrose solution, sugar molecules probably accounted for the decrease of volatility of major compounds by hydrogen bonding (Hollowood et al., 2002) except for those in mint extract, which resulted in the increase of odor thresholds. The results were in agreement with those reported by Godshall (1997). However, in the case of mint extract of which the proportion between major ingredients is not declared, it is possible that there might be some synergistic effects of the two major compounds listed to increase the intensity of perception (Meilgaard et al., 2007). Since proteins and fats are food matrix components that are also reported to have interactions with flavor compounds (McGorrin, 1996), pasteurized full fat milk was used in this study as a medium of odor threshold evaluation due to its wide utilization in numerous food products, including dairy products, bakery and confectionary. Therefore, each natural extract noted an increase in odor thresholds. Most flavors exhibit hydrophobic, reversible interaction between milk proteins and flavor, including hydrogen bonds, hydrophobic interactions and ionic bonds (Kuhn, Considine, & Singh, 2006). As a result, the presence of proteins in a food matrix has been reported to decrease aroma perception (Hansen & Heinis, 1991). Similarly, fat is attributed to the low headspace concentration of lipophilic flavor compounds by influencing their vapor pressure (Schirle-Keller, Reineccius, & Hatchwell, 1994). Consequently, it can be assumed that major compounds in mint extract are more soluble in water than compounds in the three others; hence they were released faster in high fat matrix. It is worth noting that the pure flavor extracts used in this study were commercially made by dissolving the major ingredients with ethanol and water to increase compound solubility. This was probably another reason why the odor thresholds obtained in this study were substantially different from the published data at which individual aroma compounds of highest percentage of purity were used. Ethanol is commonly used as flavor carrier due to its high volatility, but the ethanol threshold in water is extremely low at 100,000 µg/l and thus not perceivable (van Gemert, 2003). Generally, analysis of variance requires the normality assumes options which are commonly violated by threshold test. Therefore, it is recommended to transform the scale of observations to conform more closely to the assumptions of the linear model and provide more valid inferences for the analysis of variance (Kuehl, 2000) or to use non-parametric statistics. Analysis of data using the Friedman ranked analysis of variance detected a significant difference in the group threshold among flavoring extracts as well as matrices. The subsequent LSD test revealed that the detection thresholds of mint flavor were significantly lower than those of the other three flavor extracts within the same medium of evaluation and among types of extracts (Table 5).

Table 5 Rank sum scores* of the odor detection thresholds by different perception for each flavoring agent determined in (A) aqueous solution; water (B) 10% sucrose solution and (C) pasteurized milk

Perception	Matrix	Vanilla	Mint	Almond	Lemon
	A	122.18 $^{a, C}$	3.82 $^{c, B}$	120.29 $^{a, C}$	66.99 $^{b, C}$
Orthonasal	B	205.76 $^{b, B}$	0.59 $^{d, C}$	946.87 $^{a, B}$	169.63 $^{c, B}$
	C	623.52 $^{c, A}$	131.92 $^{d, A}$	994.2 $^{a, A}$	774.33 $^{b, A}$
	A	66.89 $^{b, C}$	1.75 $^{d, C}$	77.06 $^{a, C}$	23.43 $^{c, C}$
Retronasal	B	224.46 $^{b, B}$	0.27 $^{d, B}$	340.89 $^{a, B}$	67.69 $^{c, B}$
	C	436.46 $^{c, A}$	62.39 $^{d, A}$	915.27 $^{a, A}$	505.24 $^{b, A}$

*Rank sum scores carrying the different letters in the same row are significantly different whereas those carrying the different capital letters in the same column are significantly different ($p \leq 0.05$)

5. Conclusion

In conclusion, based on the conditions of the study, the geometric mean best estimated detection thresholds for four selected flavoring agents; vanilla, mint, almond, and lemon determined by trained panelists appear to be lower than those did by the general population in the same media of evaluation. The study justifies that when flavoring agents are applied to food products, the amount added should not exceed the detection thresholds for adequate perception. This work provided a basis guideline of thresholds for understanding perception limits necessary for future studies. The knowledge of interactions between flavoring agent and ingredients is of importance to explore and will further help in the development of more tasteful products since a decrease in flavor perception may also reduce consumer acceptability in a variety of food products.

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