

Study on sensory evaluation instrument for describing
comprehensive palatability and preference

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2014

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GENERAL INTRODUCTION

Palatability refers to having an agreeable feeling on the palate, being pleasant to taste, and having a good flavor (The Oxford English Dictionary Second Edition, 1989). Palatability is not a simple reflection of a need state, but acts to promote intake through a distinct hedonic system, which has inputs from a variety of other systems including those regulating need (1). As past literature often uses compatible terms, including “pleasantness”, “liking” and “preference”, to refer to palatability, we sometimes incorporate such inferences to discuss palatability (Fig.1).

This study explored the possibility of generating a novel sensory evaluation instrument for describing palatability and preference.

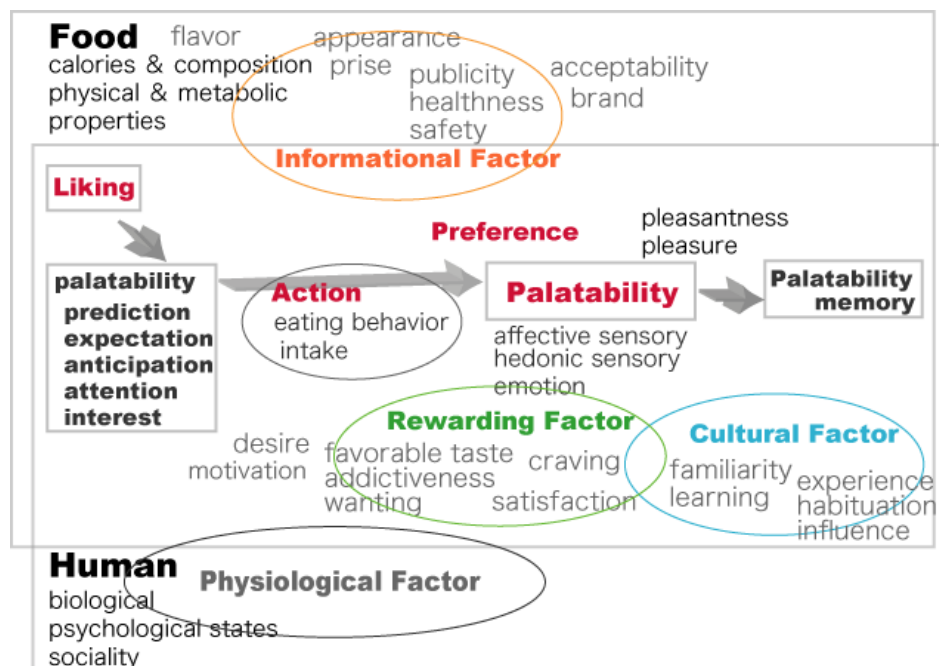


Fig. 1. Hypothesized structure of palatability.
Palatability is related to liking and preference.

In research on human food intake and acceptance, the term “palatability” has been used in its colloquial sense that reflects a positive hedonic evaluation under a given set of conditions e.g.,(1-3). Palatability has been viewed as a reflection of homeostatically driven motivation induced by some nutritional deficit. However, short-term overconsumption often resulting from palatability contradicts such a simplistic view (1). This leads to the more expansive view that palatability may be a reflection of a certain nutritional composition, fat and sugary, which may drive excessive intake (4). Although this view may well describe an important aspect of food palatability, it has also been suggested that palatability is based on past associations between flavors/textures and hedonic consequences (1, 5). Thus, palatability should not be regarded as a fixed food property intrinsic to a given food, but rather as the momentary evaluation of a food (2), which is largely influenced by experience (6).

Palatability has a composite nature involving various physiological and empirical factors. This led us to postulate the possibility that palatability may be dissected into its componential subdomains, which in turn may allow palatability to be reconstructed with explicit descriptions of the contribution of each subdomain. Specifically, we developed a questionnaire that would reflect the composite nature of palatability, and explore major factors that represent distinct aspects of palatability. In our subsequent analysis of the questionnaire responses, we ascribed comprehensive food palatability to its subdomains using multivariate

The author aimed to assess the feasibility of dissecting comprehensive palatability into the three componential subdomains. The author expected that the subsequent multivariate regression modeling could help interpret comprehensive palatability with respect to the differential contribution of its subdomains.

In research using animals, intake is usually used as an indicator of preference. Dietary fats enhance the palatability and preference of foods. The flavor of fats is considered to play an important role in increasing palatability or preference (7-10). Using a mouse behavioral model, the author explored the possibility that aroma components generated by the oxidation of olive oil may enhance the preference of olive oil.

Combining these, the author tried to explore analysis instrument for sensory evaluation in research on human as various application of sensory evaluation in research using animals.

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CHAPTER 1

Analyzing comprehensive palatability of cheese products by multivariate regression to its subdomains

In research on human food intake and acceptance, the term “palatability” has been used in its colloquial sense that reflects a positive hedonic evaluation under a given set of conditions, but its usage has not always been clear and consistent(1). For example, in meat studies, consumers are often asked to evaluate meat-related products (e.g., steaks) using hedonic scales for tenderness, juiciness, flavor, and palatability, which is often substituted with overall liking and pleasantness (2). Considering this ambiguity in terminology, in this article we use the term palatability to represent the positive hedonic reward provided by foods. However, as past literature often uses compatible terms, including “liking” and “pleasantness”, to refer to palatability, we sometimes incorporate such inferences to discuss palatability.

We should also note that palatability could either be a measure of food or of a person unless the source is specified. Ramirez (1) pointed out three different views on palatability. The first classical view proposes that palatability is an objective property of foods (3). This is consistent with our colloquial usage of the term that a certain food is more palatable than another: a palatable chocolate would be palatable to everybody. Conversely, in the second view, as far as food intake evokes the hedonic response of a human to sensory stimuli, the palatability should be regarded as a measure of the human (4). Accordingly, instead of saying that a food is palatable, we should specify that a food is palatable to any individual under certain defined conditions. In a sense, this is just a different side of the same coin: the former view focuses on the stimulus while the latter on the response. The third more holistic view

involves the effects of learning and experiences (1). Namely, the same chocolate would taste more palatable to one person than to another because they had undergone different chocolate experiences. From this perspective, palatability should not be regarded as a fixed food property intrinsic to a given food or an automatic physiological response, but rather as the context-dependent evaluation of a food by an individual (5), which is largely influenced by experience (6).

Although no individual has the same experiences with specific foods, some common factors affecting palatability seem present. If so, palatability may be dissected into componential subdomains, which in turn may allow its reconstruction with explicit descriptions of the contribution of each subdomain. To explore this possibility, we developed a questionnaire that would reflect the composite nature of palatability and explore major factors that represent distinct aspects of palatability. In our subsequent analysis of the questionnaire responses, we ascribed comprehensive food palatability to its subdomains using multivariate regression analyses. We will hereafter describe the theoretical background for this strategy.

Referring to a wealth of research on palatability and related food properties, we infer four factors: physiological, rewarding, cultural, and informational (Fig.1).

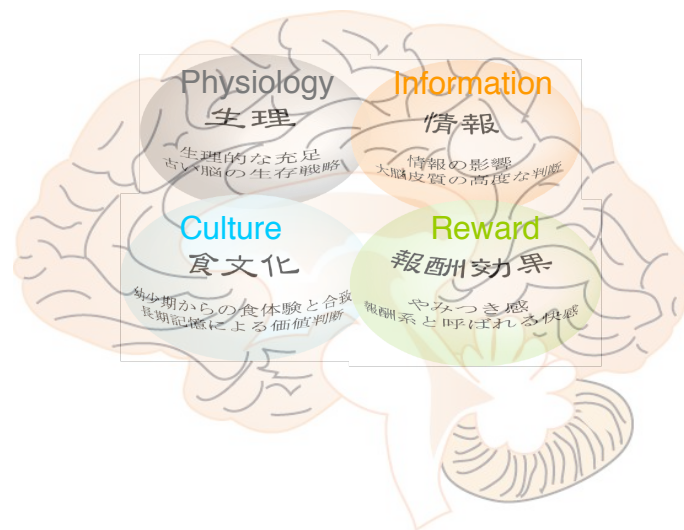


Fig. 1. Hypothesized four factors of palatability.

Among the putative subdomains of palatability, the most influential physiological factor is kept constant to focus on the effects of other factors. Informational, rewarding and cultural factors are assessed in the current study.

First, the physiological factor plays a pivotal role in determining palatability. Five basic tastes, sweetness, sourness, saltiness, bitterness, and umami (savoriness), elicit relatively fixed hedonic responses (7, 8). For example, humans prefer sweetness and are averse to bitterness. These responses appear as early as from birth, and thereafter last throughout the lifetime (9). Additionally, nutritional deficit affects palatability through a homeostatically driven motivational system. It has been shown that physical exercise that necessitates calorie consumption increases preference for sucrose (10). When animals, including humans, detect deficient or imbalanced protein intake, a sparing of protein and a search for the deficient materials are initiated to maintain the necessary level of dietary protein intake (11). Thus, the alteration of the physiological state due to nutritional deficit, fatigue, and/or hunger can affect food palatability.

The second factor is the reward elicited by the intake of high-calorie foods. This has been well evidenced by studies on food craving, a strong desire to eat a particular food that may lead people to go out of their way to satisfy it (12). Although the intake of high fat content foods, sweets, carbohydrates/starches, and fat-containing fast-foods(13) often induces excessive caloric consumption and leads to obesity, they are frequently preferred. Underlying the over consumption of high calorie foods, animal studies have revealed the role of the reward system involving the dopaminergic and opiate systems in the brain (14, 15). Hence, the activation of the reward system by high calorie foods may be the dominant factor that underlies food palatability.

Third, food is influenced by cultural factors established as part of the acquisition of culture, including beliefs, culinary traditions, and special occasions (16). For example, a recent implicit association experiment revealed that positive attitudes toward traditional diets relate to the type of breakfast eaten in childhood in young Japanese (17). Also, elderly Italians' favorite foods are not only based on the sensory aspects of dishes, but also on tradition and familiarity from youth (18). A subject's current vegetable consumption is known to be predicted by their previous vegetable intake at home (19), and another study demonstrated that the intake frequency of fruits and vegetables at home were positively associated with the intake

of fruits and vegetables 5 years later (20). This evidence collectively suggests that food palatability is influenced by cultural factors, and past eating habits seem to be the most effective predictors.

Fourth, taste expectations formed based upon information can dramatically bias the sensory perception of food. Information such as the name of the item, its shape, and how it is packaged would have a great impact on forming expectations, which can either raise or lower liking ratings (21, 22). Indeed, a series of experimental studies about the effects of information on food intake performed by Wansink and colleagues clearly demonstrated the importance of informational factors. For example, environmental cues including ambience, lighting, and sounds can create expectations and generate an intake bias. It is believed that expectations may lead a person to focus on particular aspects of taste that strengthen their initial expectations (23, 24). Environmental cues of food quality can take many forms, including price, labels, appearance, or names (25). Moreover, it has been revealed that specific colors influence the perception of specific tastes, liking, and intensity ratings (26-29). Even names can influence the perception of unimodal basic tastes (30).

Among the four factors presented above, physiological is considered the most influential. However, this poses a serious experimental problem: individuals tend to attribute their own food intake to a highly influential physiological state such as hunger ignoring other important but less influential factors (31). Alteration of the physiological state due to nutritional deficit, fatigue or hunger may lead to individual differences regardless of food. Thus, we decided not to pursue the obvious effects of physiological factors. Rather, we controlled to minimize the effect of physiological variance by making the time of day for the experiment and the temperature invariant. The remaining three factors were psychometrically assessed in reference to the overall palatability of a food sample.

We aimed to assess the feasibility of dissecting comprehensive palatability into the three componential subdomains. We developed a questionnaire that reflected the composite nature of palatability, and explored major factors representing its distinct aspects. In our subsequent analysis of the questionnaire responses, we

ascribed comprehensive food palatability to its subdomains using multivariate regression analyses.

Materials and Methods

Participants and procedure

Seventy-five Japanese participants (43 females, aged 19–79 years, median 20–39), with written informed consent, voluntarily participated.

To minimize physiological and physical interference, the experiment was conducted during off-meal hours (around 11:00 or 15:00) in a room set at 23 degrees Celsius. The absence of health issues, hunger and satiety among participants was verified. Participants were asked to sit in front of a table, and to take three bites from one of three different types of cheese. Immediately after tasting a sample, participants were asked to respond to a questionnaire. Only one sample was tasted per day in a randomized order.

The study was approved by the institutional ethics committee of the Graduate School of Agriculture, Kyoto University.

Food samples

Three commercially available cheeses (Cheeses A, B, and C) were sampled. Cheese A was a soft and natural Camembert cheese (Hokkaido Tokachi Camambert Kireteru, Meiji Co., Ltd., Tokyo, Japan). Cheeses B and C were processed cheeses accentuating natural flavor (B: Hokkaido Tokachi Smart Cheese; C: Hokkaido Tokachi 6P Cheese, Meiji Co., Ltd.); they were made of identical ingredients in the same ratios, but differed in the size of each piece, labeling, and wrapping design: this information was confirmed by the cheese manufacturer. In particular, Cheese B was thinner, had a more sophisticated wrapping design, and was more widely advertised on TV.

Questionnaire

In the first part of the questionnaire, demographics including age, gender, hometown, existence of company to eat with, and physical conditions were measured.

Questionnaire items were sampled to suitably reflect three hypothetical subdomains of palatability (rewarding, cultural, and informational). First, using 5-point Likert type scales (1 = not at all / 5 = extremely), experts in nutrient chemistry and food research sampled items to explore their representation of the subdomains of palatability and compare their various perspectives. After the examination of content validity, 15 items were retained.

Of these 15 items, five were developed to measure the rewarding factor, which was measured by the degree of 1) desire caused by the addictiveness of a food, 2) level of difficulty in inhibiting urges to eat, 3) level of difficulty in inhibiting eating a food, 4) sense of satiety recognized by eating a food, and 5) sense of rewarding ingredients perceived by eating a food. Another five items were developed to measure the cultural factor, which was measured by the degree of 1) repeated exposure to a food, 2) dietary accustomedness to a food, 3) similarity with an accustomed food, 4) embeddedness of a food as a home-cooked taste, and 5) entrenched preference for a certain food. Finally, the remaining five items were developed to measure the informational factor, which comes from the 1) visual information from a food, 2) publicity of a food, 3) health information of a food, 4) perceived safeness of a food, and 5) perceived value for the price of a food (Table 1).

Visual analogue scales to measure comprehensive palatability

Comprehensive palatability for a cheese sample was measured in mm using 100-mm line visual analogue scales (VAS) with descriptive anchors at each end (*dislike extremely* for the left extremity, *like extremely* for the right extremity). VAS were used because their utility in measuring comprehensive palatability judgments was indicated in (8).

Table 1. Fifteen questionnaire items for the three componential factors of food palatability

a: Items putatively related to reward

- a1 Is the taste likely to be addictive to you?
 - a2 Does the taste make you feel compelled to pick up the food?
 - a3 Does the taste make you take another bite if you take a bite?
 - a4 Are you satisfied with the taste?
 - a5 Do you think the food tastes good because of rich fat sweetness or umami?
-

b: Items putatively related to culture

- b1 Are you used to the taste?
 - b2 Have you had a food that has the same or a similar taste to the food?
 - b3 Have you eaten food like this many times?
 - b4 Do you think your family (your parents, siblings, spouse, etc.) would like the taste of the food?
 - b5 Have you liked the taste of the food since your childhood?
-

c: Items putatively related to information

- c1 Does the food appear tasty?
 - c2 Have you ever seen this food in advertisements or heard of it by word-of-mouth?
 - c3 Have you ever heard anything good about the healthfulness of the food?
 - c4 Do you feel secure about the ingredients of the food?
 - c5 Do you think that the food seems expensive?
-

Psychometrical establishment of hypothetical subdomains of palatability (Cheese A data)

PASW statistics 19.0 was used throughout the analyses described hereafter. Data obtained for Cheese A was explored first. The psychometric adequacy of the items in the three hypothetical subdomains that reflect palatability was examined based on the classical testing theory (CTT; (32). Specifically, an exploratory factor

analysis with a promax rotation was performed to categorize the 15 items. The criteria for extracting factors were based on (a) Kaiser's rule (33), (b) the scree test (34), and (c) interpretability of the extracted factors (35, 36). Stringent criteria for factor loadings at .45 were used based on criteria by (37). After the extraction, the correlations among the factors were explored.

Examination of unidimensionality of subdomains (Cheese B & C data)

Data obtained for Cheeses B & C was used in the subsequent analyses. The unidimensionality of the questionnaire for each sample was examined using parallel analysis where random data sets that contained the same number of items and participants as in the actual data sets were simulated to conduct an exploratory factor analysis. In order to confirm a unidimensionality, the first factor estimated from the observed data should be larger than that of simulated data, and the subsequent factors estimated from observed data should not be larger than those of the simulated data (38). The questionnaire was re-examined for Cheese B and C data. Criteria were set to be equal to those of Cheese A.

Regression analyses and comparison of comprehensive palatability and its subdomains (Cheese B & C data)

A paired *t*-test (two-tails) was performed to compare VAS scores of comprehensive palatability between Cheeses B and C. In addition, paired *t*-tests (two-tails, Bonferroni-corrected) were performed to compare rewarding and cultural subdomain scores for Cheeses B and C.

Upon establishing the unidimensionality of the subdomains, multiple regression analyses with backward elimination were respectively conducted for Cheeses B and C to explore whether the subdomains of palatability accounted for the comprehensive palatability.

Results

Psychometrical establishment of hypothetical subdomains of palatability (Cheese A data)

For Cheese A, an exploratory factor analysis with a promax rotation was performed on the 15 questionnaire items reflecting the three hypothetical subdomains of palatability. As shown in the scree plot, the scree region seemed to begin at the fourth or fifth factor. In addition, the first three factors exceeded an Eigen value of 1. The three-factor structure could be extracted on Kaiser's rule and scree test. Ten items exhibited factor loading above .45 (Table 2).

Table 2.
Exploratory factor analysis with promax rotation for 15 items of Cheese A

	Communality (h^2)	Factor loadings for food palatability		
		Rewarding	Cultural	Informational
a				
a1	.838	.920		
a2	.887	.913		
a3	.885	.929		
a4	.792	.909		
a5	.468	.608		
b				
b1	.999		1.032	
b2	.521		.625	
b3	.670		.625	
b4	.362			
b5	.248			
c				
c1	.238			
c2	.358			.608
c3	.315			
c4	.289			
c5	.263			.466

A criterion of 0.45 for factor loading was used as the cutoff for inclusion of items in a factor. Only factor loadings for items over the criterion are shown.

The factor loadings of the items finally included in the three factors are in bold.

In the first factor, two items exhibiting excessively high inter-item correlation were excluded. As a result, the remaining 8 items retained the three-factor structure, and were respectively interpreted as “rewarding” (*Cronbach’s* $\alpha = .88$, $n_{items} = 3$), “cultural” (*Cronbach’s* $\alpha = .82$, $n_{items} = 3$), and “informational” (*Cronbach’s* $\alpha = .20$, $n_{items} = 2$) in accordance with the hypothesized subdomains of palatability. The factors respectively accounted for 23.5%, 22.3%, and 8.4% of variance in the items. Correlation between the first and the second factors, between first and the third, and between the second and the third were, $r(75) = .55$, $p < .01$, $r(75) = .28$, $p < .05$, and $r(75) = .24$, $p < .05$ respectively. Although internal consistencies of the first two factors were sufficiently high, the third factor indicated a low internal consistency.

Examination of unidimensionality of subdomains (Cheese B & C data)

The parallel analysis indicated the unidimensionality of the rewarding and cultural factors, whereas it failed to detect unidimensionality of the informational factor. Multicollinearity among items or factors was absent. Internal consistency for the rewarding factor was .88 and .86 for Cheeses B and C, respectively, and for the cultural factor was .70 and .81, respectively. The internal consistencies for the first two factors were sufficiently high for Cheeses B and C, but were only .19 for Cheese B and .06 for Cheese C. These analyses led us to conclude that the rewarding and cultural factors are reliable subdomains, accounting for comprehensive palatability.

Regression analyses and comparison of comprehensive palatability and its subdomains (Cheese B & C data)

Comprehensive palatability as measured by VAS was 65.6 ± 24.2 (mean $\pm$ standard deviation) for Cheese B and 56.2 ± 26.4 for Cheese C. A paired *t*-test revealed that comprehensive palatability was significantly higher for Cheese B ($t(74) = 2.42$, $p < 0.05$, $ds = 0.37$), despite the fact that the ingredients in both types of cheese were identical.

Scores for the rewarding and cultural subdomains averaged across items were 3.24 ± 1.07 and 3.25 ± 0.92 , respectively, for Cheese B and 2.83 ± 1.01 and 3.00 ± 1.04 , respectively, for Cheese C. Paired *t*-tests revealed that the rewarding factor score was significantly higher for Cheese B ($t(74) = 2.37, p < 0.05, ds = 0.39$). No significance was found for the cultural factor.

Accountability of the rewarding and cultural factors on comprehensive palatability measured using VAS for Cheeses B and C, which were then respectively examined using multiple regression with the backward elimination method. The resulting equation for each cheese is shown in Table 2. Although accountability of the models was high for both types of cheese, there were striking differences: only the rewarding factor accounted for the palatability of Cheese B, while the cultural as well as rewarding factors accounted for the palatability of Cheese C (Table 3).

Table 3. Multiple regression analyses with backward elimination method to account for palatability of Cheeses B and C by subdomains

Predictor variable	Cheese B			Cheese C		
	R^2	β	$F_{model} (df_1, df_2)$	R^2	β	$F_{model} (df_1, df_2)$
Sequence 1	.715		90.446* (2, 72)	.688		79.296* (2, 72)
Rewarding		.801*			.740*	
Cultural		.079			.163*	
Sequence 2	.711		179.428* (1, 73)			
Rewarding		.843*				

F model df_1 , stands for degree of freedom for effect; df_2 , degree of freedom for error; * $p < .05$; Backward elimination was terminated at Sequence 1 for Cheese C.

Taken together, these differences in comprehensive palatability could be a reflection of a larger contribution of the cultural factor in Cheese C than in Cheese B in the resulting equations.

Discussion

The current study explored the possibility of generating a novel sensory evaluation instrument for describing palatability. Although palatability has only been vaguely described as a single food attribute, the current study successfully dissected palatability into subdomains and quantitatively associated their relation, presenting a novel, quantitative approach for assessing food palatability.

Subdomains of palatability

As exemplified in the proverb, “hunger is the best spice”, the most influential subdomain of palatability is obviously the physiological factor. However, the predominant influence of the physiological factor has prevented the decomposition of palatability, as the food intake of individuals tends to be more affected by a highly influential physiological state such as hunger than by other important but less influential factors (31). To overcome this issue, the current study employed a unique attempt to eliminate the possible effects of physiological factors by controlling the physiological states of the participants and focusing on the analyses of contributions of other less influential but important factors.

Consequently, a factor analysis, applied on the sensory evaluation of a cheese sample employing the 15 palatability-related items, extracted three factors as predicted. The subsequent adjustment processes of eliminating seemingly duplicated items and those with insufficient factor loading still yielded three factors, which were reasonably interpreted as rewarding, cultural, and informational, consisting of 3, 3, and 2 items, respectively.

We suggest that the rewarding and cultural factors are stable and reliable subdomains of palatability, and that although the third factor related to information may be present, further exploration is required to establish it as robust.

Multivariate regression model for palatability

The results of the factor analyses and subsequent construct validation suggested the appropriateness of using the two-variable regression model to account for total palatability with its subdomains. The samples were both available commercial cheese products sold in different packages, with different names, serving sizes and shapes, but they actually consisted of exactly the same ingredients. Participants were not informed of this fact. Use of these samples was expected to contrast out the relative importance of each palatability subdomain and their net contribution to the formulation of the total palatability.

Interestingly, although the samples were made of the same ingredients, the comprehensive palatability was significantly different. Comparison of rewarding and cultural factor scores between Cheeses B and C revealed that the rewarding factor score was significantly higher for Cheese B, while the cultural factor score was similar. Moreover, multiple regression analyses exhibited a predominant contribution of the rewarding factor in explaining the comprehensive palatability of Cheese B, while both factors were shown to be appropriate for Cheese C. We thus concluded that the greater comprehensive palatability of Cheese B was attributed to the greater contribution of the rewarding factor of Cheese B.

This observation clearly demonstrates that the subdomains of food palatability can have substantially large effects: so much so as to alter the total palatability of a food. To our knowledge, this is the first experimental demonstration quantifying the effects of food palatability subdomains and their contribution to the formation of comprehensive palatability.

The observed difference in the overall palatability and the cultural factor could be interpreted from the perspective of flavor preference conditioning, in which omnivorous animals including humans learn to prefer flavors that are associated with positive consequences (39, 40). Namely, Cheese C was perceived as more palatable because it was more associated with past eating experiences that had positive consequences. Actually, Cheese C is sold in a package that looks similar to the conventional processed cheese products in the Japanese market. Its product name is coherent enough to allow its inclusion in the conventional processed cheese category.

Thus, Cheese C is likely to be perceived as an extension of conventional processed cheese products. On the other hand, Cheese B is intended to offer a more compact and slim package, and appears with a different product name, together emphasizing its convenient usage. Thus, this type of processed cheese product is new to the Japanese market, and Cheese B might be perceived less in association with past eating experiences of conventional processed cheese products compared to Cheese C.

Conclusion

The current study presents the first experimental demonstration that food palatability can be dissected into its subdomains, which in turn can reconstitute comprehensive palatability with an explicit description of the contribution of each componential subdomain. Such a quantitative approach using a multivariate regression model would be effective in analyzing detailed aspects of palatability when designing and evaluating food products, and would provide a novel sensory evaluation instrument for describing palatability.

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CHAPTER 2

Quantitative approach for analyzing palatability using multivariate regression model

The previous study presented the first experimental demonstration that food palatability can be dissected into its subdomains, which in turn can reconstitute comprehensive palatability with an explicit description of the contribution of each componential subdomain (1). As an intriguing application, the multivariate model may be sensitive enough to quantitatively illustrate differences in palatability perception across age, gender, or generations, and thus would analyze the structure of palatability and preference.

In this study we tried to investigate differences in palatability perception across generations and gender by the application of multivariate regression model.

We used three commercially available cheese products for several reasons. Major domestic food companies apply stringent quality control to food products, enabling stable food-sample presentation throughout the experiment. Moreover, cheese products are expected to offer the three putative palatability factors in a balanced way: First, cheese products are rich in reward-related ingredients including fats, proteins, and umami-generating small molecules such as dipeptide, amino acids and nucleotides (2, 3). Second, cheese is a relatively new but popular food in Japan, with an average annual consumption rate of approximately 2 kg per person. This is a four-fold increase over the last three decades, but still remains approximately one tenth of that of typical Western countries (4, 5); Thus, the incorporation of cheese products into dietary habits is expected to vary across individuals, probably providing insight into the importance of sociocultural factors. Third, cheese products greatly

vary in informational factors including nutritional and health values, package, and shape, and these factors are repeatedly conveyed to consumers through advertisement.

Methods

Participants and procedure

Seventy-five Japanese participants from the Agricultural Department of Kyoto University (43 females and 32 males, aged 19–79 years, median 20-39) voluntarily participated in the current study. The number of participants was determined based on the recommended sample size for an exploratory phase of correlational analyses.

The experiment was conducted in temperature-controlled (23 degrees Celsius) rooms during off-meal hours (around 11:00 or 15:00) to ascertain a uniform physiological state for participants. First, the experiment was explained to participants, and written informed consent was obtained. Then, participants were asked to take three bites from any one of three different types of cheeses on a table. One sample was tasted per day, and each tasting session took about 10 minutes. The order of tasting samples was randomized. Immediately after tasting each sample, participants were asked to respond to a questionnaire. This procedure was devised as a result of a preliminary study where the optimum amount of a sample and timing of tasting necessary to avoid a reduction in the interest in the food and to avoid satiety during the experiment were examined.

After the experiment, experimenters verified that participants had followed the instructions precisely by asking free answer questions. The entire procedure took approximately 30 minutes altogether. All of the participants completed the study.

The study was approved by the institutional ethics committee of the Graduate School of Agriculture, Kyoto University.

Food samples

Three kinds of commercially available cheeses were used in this study. Cheese A was a soft and natural Camembert cheese product (Meiji Hokkaido Tokachi

Camambert Kireteru, Meiji Co., Ltd., Tokyo, Japan). Cheeses B and C were processed cheeses accentuating natural flavor (B: Meiji Hokkaido Tokachi Smart Cheese, Meiji Co., Ltd., Tokyo, Japan; C: Meiji Hokkaido Tokachi 6P Cheese, Meiji Co., Ltd., Tokyo, Japan). Ingredients in both samples B and C were identical though the cheeses differed in size, labeling, and wrapping design. In particular, Cheese B was thinner, had a more sophisticated wrapping design, and was more highly advertised on TV compared with Cheese C.

Questionnaire form

Demographics

In the first part of the questionnaire, demographics including age, gender, hometown, existence of company to eat with, and physical conditions were measured.

Three hypothetical subdomains of palatability

Questionnaire items were chosen to suitably reflect three hypothetical subdomains of palatability (rewarding, cultural, and informational). Using 5-point Likert type scales (1 = not at all / 5 = extremely), each item was examined to explore its representation of the subdomains of palatability and compare their various perspectives.

Visual analogue scales to measure comprehensive palatability

Comprehensive palatability for a cheese sample was measured in mm using 100-mm line visual analogue scales (VAS) with descriptive anchors at each end (*dislike extremely* for the left extremity, *like extremely* for the right extremity).

Data analyses

Three sequences of analyses were performed as described below. PASW statistics 19.0 was used throughout the analyses.

Regression analyses of subdomains of palatability accounting for comprehensive palatability

Upon establishing the reliability and validity of the subdomains, we used multiple regression analyses to explore whether they could describe comprehensive palatability.

Results

Psychometrical establishment of hypothetical subdomains of palatability (Cheese A data)

For Cheese A, an exploratory factor analysis with a promax rotation was performed on the 15 questionnaire items reflecting the three hypothetical subdomains of palatability. As shown in the scree plot (Fig. 1), the scree region seemed to begin at the fourth or fifth factor. In addition, the first three factors exceeded an Eigen value of 1. The three-factor structure could be extracted on Kaiser's rule and scree test. Ten items exhibited factor loading above .45

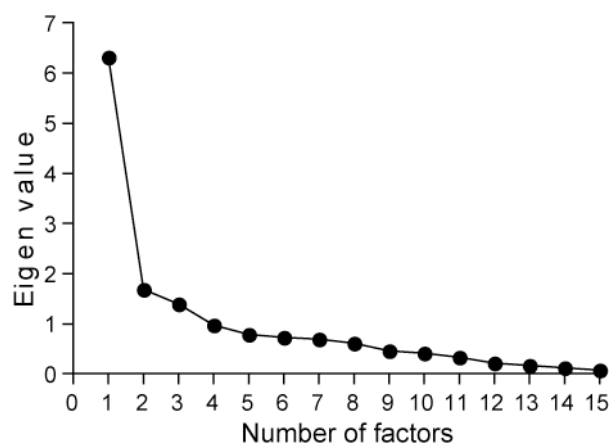


Fig. 1. Scree plot for 15 questionnaire items (Cheese A data).

The initial steep slope is obvious at the first factor, and seems to continue at least to the third factor. Beyond the fifth factor, the scree region seems obvious.

Note that the first three factors have Eigen values over 1.

Examination of unidimensionality of subdomains (Cheese B & C data)

The parallel analysis indicated the unidimensionality of the rewarding and cultural factors (Fig. 2).

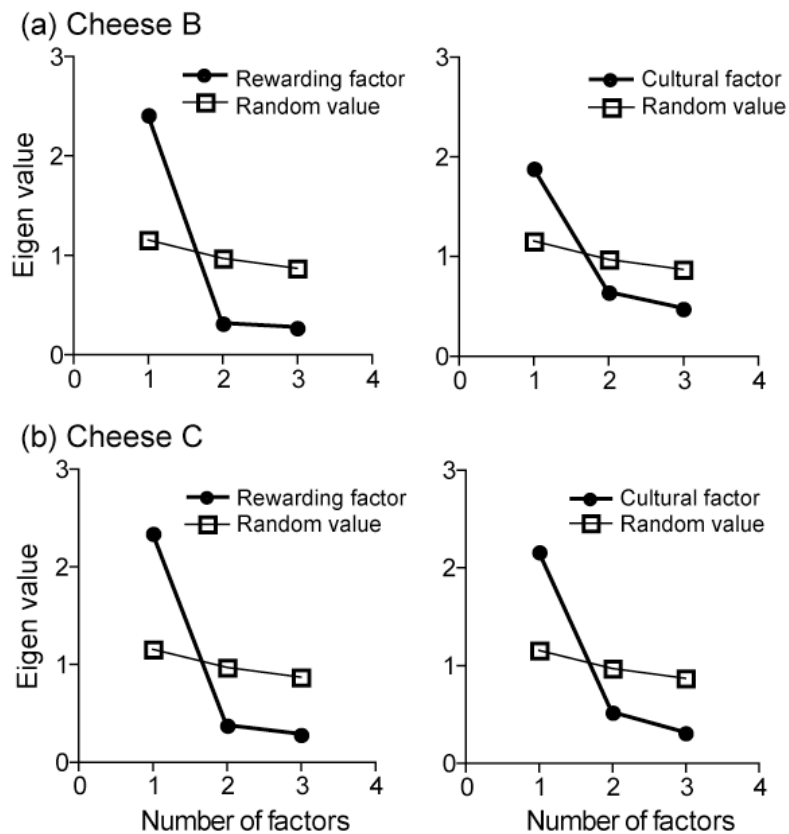


Fig. 2. Parallel analysis of the rewarding and cultural factors in Cheeses B (a) and C (b) to examine unidimensionality.

In a parallel analysis, random data sets that contain the same number of items and participants as observed data were simulated. In order to confirm unidimensionality, the first factor estimated from the observed data should be obviously larger than that from the simulated data, and the subsequent factors estimated from the observed data should not be obviously larger than those of the simulated data.

Regression analyses and comparison of comprehensive palatability and its subdomains (Cheese B & C data)

Comprehensive palatability as measured by VAS was 65.6 ± 24.2 (mean $\pm$ standard deviation) for Cheese B and 56.2 ± 26.4 for Cheese C. A paired t -test revealed that comprehensive palatability was significantly higher for Cheese B ($t(74) = 2.42, p < 0.05, ds = 0.37$), despite the fact that the ingredients in both types of cheese were identical (Fig. 3).

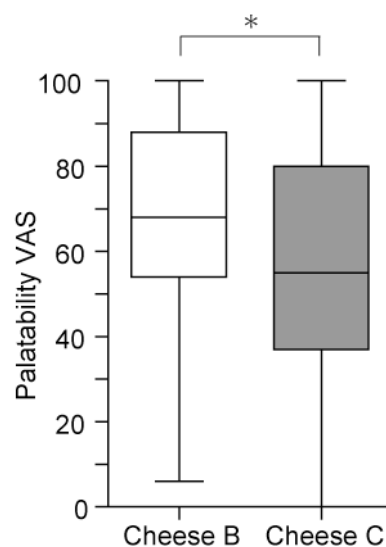


Fig. 3. Box-and-whisker plots of comprehensive palatability measured with VAS for Cheeses B and C (n = 75).

Middle line in the box, median; upper and lower edges of the box, upper and lower quartile; upper and lower ends of whiskers, maximum and minimum values within 1.5 interquartile range from the first and the third quartiles. An asterisk indicates significance ($p < 0.05$), a paired t -test.

Subdomains of palatability accounting for comprehensive palatability

Accountability of the factors on comprehensive palatability across generations and gender measured using VAS for Cheeses A, B and C (Fig.4.), which were then respectively examined using multiple regression. The resulting equations for each cheese across generations are shown in Table 1, and across gender in Table 2.

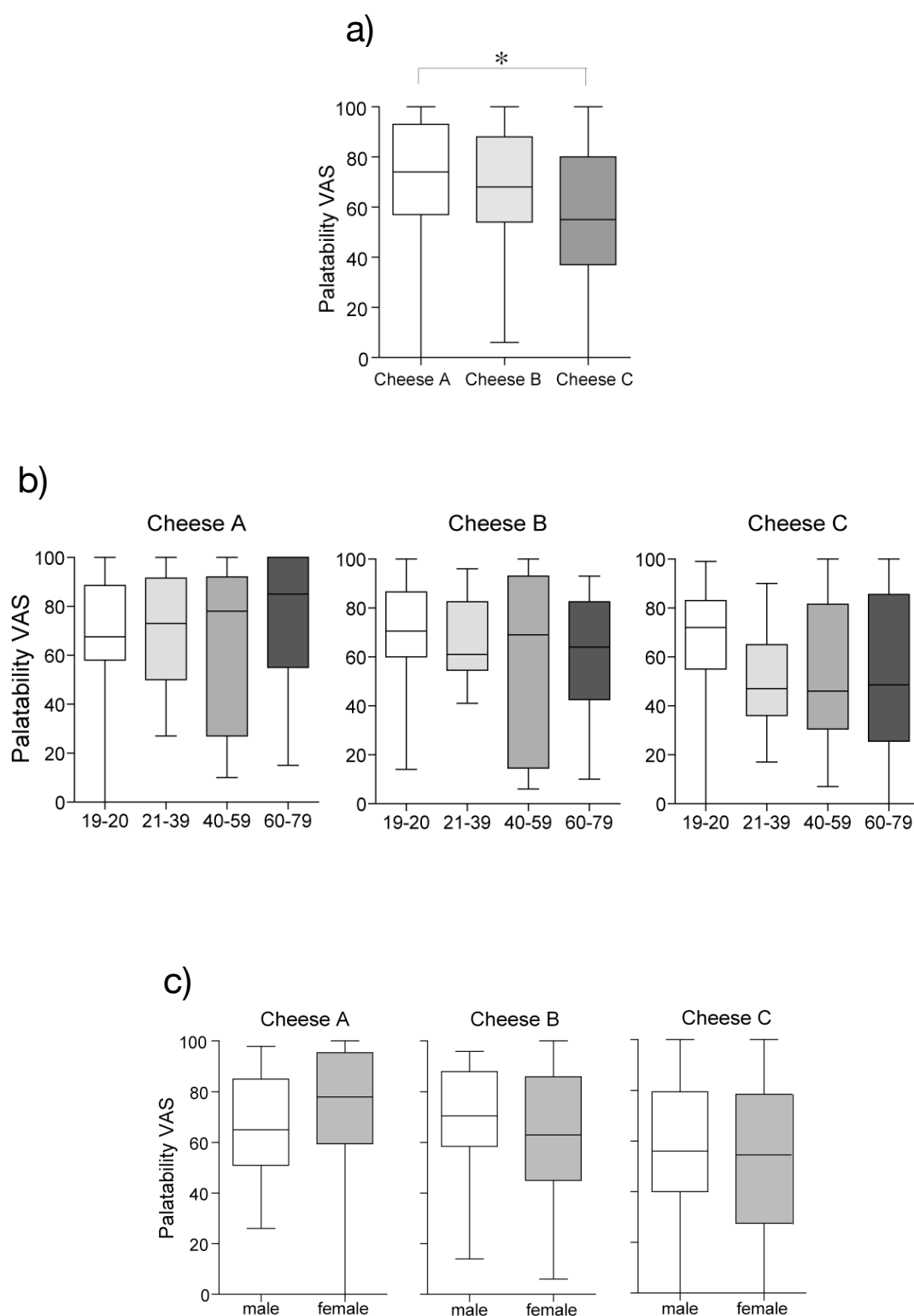


Fig. 4. Box-and-whisker plots of comprehensive palatability measured with VAS all age (a) across generations (b) gender (c) for Cheeses A,B and C.

Middle line in the box, median; upper and lower edges of the box, upper and lower quartile; upper and lower ends of whiskers, maximum and minimum values within 1.5 interquartile range from the first and the third quartiles.

Table 1. Multiple regression analyses to account for palatability of Cheeses A, B and C across generations by subdomains

Ages	<u>19-20</u>		<u>21-39</u>		<u>40-59</u>		<u>60-79</u>	
Predictor variable	<i>R</i> ²	β	<i>R</i> ²	β	<i>R</i> ²	β	<i>R</i> ²	β
<u>Cheese A</u>								
	.607		.570		.815		.672	
Rewarding		.760*		.813		.663		.804*
Cultural		.106		.092		.259		.080
Informational		-.144		-.103		.082		-.045
<u>Cheese B</u>								
	.646		.238		.961		.838	
Rewarding		.796*		.458		.965*		.848*
Cultural		.059		.016		.025		.308
Informational		.007		.232		-.112		-.261
<u>Cheese C</u>								
	.620		.510		.687		.679	
Rewarding		.745*		.576		.672		.848*
Cultural		.155		.366		.219		.308
Informational		-.099		-.292		-.015		.085

p < .05

Table 2. Multiple regression analyses to account for palatability of Cheeses A, B and C across gender by subdomains

Multiple regression analyses to account for palatability of Cheeses by subdomains

Gender	<u>male</u>		<u>female</u>	
Predictor variable	<i>R</i> ²	β	<i>R</i> ²	β
<u>Cheese A</u>				
	.514		.715	
Rewarding		.693*		.816*
Cultural		.082		.090
Informational		.039		-.068
<u>Cheese B</u>				
	.496		.844	
Rewarding		.656*		.923*
Cultural		.204		-.014
Informational		-.161		.040
<u>Cheese C</u>				
	.637		.740	
Rewarding		.699*		.817*
Cultural		.317*		.099
Informational		-.283*		.063

p < .05

Discussion

This study explored the possibility to quantitatively illustrate differences in palatability perception when participants were dissected into generations or gender by various applications of multivariate regression model. These results suggest that palatability is not merely determined by the physical and chemical properties that are intrinsic to a food product itself, but also depends on psychological properties that can arise through interaction between humans and the food product.

Subdomains of palatability accounting for comprehensive palatability

Rewarding factor

Initially, we devised five items that were expected to relate to reward of food intake. As expected, the exploratory factor analysis extracted them to constitute a single factor, which was interpreted as the rewarding factor. Among the five items, two that seemed to semantically overlap both each other and other items were excluded. Accordingly, the remaining three items, which represented (1) tastes that elicit a strong desire to eat, (2) tastes that cause satisfaction, and (3) the presence of a taste attribute that may stimulate the reward system. While these are semantically related to each other, they are different enough to be distinctive. This three-item factor exhibited a high internal consistency with a Cronbach's α above 0.8 for all cheese samples tested. In addition, the unidimensionality of the factor was confirmed for all cheese samples. Taken together, it is reasonable to consider that these items constitute a single factor and reflect reward-related food attributes.

Cultural factor

Concerning cultural aspects of food consumption, we first devised five items. The exploratory factor analysis extracted a factor, which was highly loaded with three of the five items. We eliminated two items with a factor loading below 0.45. Consequently, the remaining three items seemed to represent (1) familiarity with the taste of the food, (2) experience of eating the food or similar foods, and (3) habit and frequency of eating the food or similar foods. The three items constituted a single

factor which was interpreted as a cultural factor. The two eliminated items related to family relationship, which is reasonable since the liking of particular foods has been known to vary with age, and similarity in food liking between parents and children has been known to be smaller than generation effects (6). The internal consistency of the three-item factor was sufficiently high with Cronbach's α of above 0.7 for all cheese samples. Moreover, the unidimensionality of the factor was confirmed for all cheese samples. Thus, the cultural factor was considered a reasonable subdomain of palatability.

Informational factor

Although the rewarding and cultural factors were found to be reliable and robust, the informational factor was less stable. The factor analysis extracted one factor that may represent informational aspects of food. Nevertheless, among the five items that presumably constitute the informational factor, only two items, exemplifying advertisement and price, exhibited factor loading above 0.45. The internal consistency of the factor was small with a Cronbach's α of below 0.2 for all three cheese samples. Even allowing for the decrease of Cronbach's α due to the use of only two items, the two items were not coherent enough to constitute a reliable single factor. However, the result of the factor analysis suggested the existence of a factor, and this was moderately associated with the items presumably related to informational aspects of food.

The failure of convergence of the information-related items may be unavoidable, as how information is perceived tends to vary among individuals. For example, it has been shown that information about healthy food aspects such as reduced-fat labels could either enhance positive or negative effects on liking, or exert no effect at all. Depending on the type of products tested, way the information is conveyed or the expectations of a consumer about the food products, consumers' attitudes toward a food sample may differ (7), and this would have a substantial effect on their ratings. Considering the variable nature of the effect food-product information has on individual consumers, the failure to extract a stable informational factor does not necessarily imply that information has little effect on food palatability,

but in order to stably handle the putative informational factor, a more elaborate approach is necessary.

Other potential palatability subdomains

One important factor that was untested in this study is the social factor. We may empirically feel that foods are more palatable when eating them with family or friends. For example, it has been shown that certain external social factors such as the behavior of others can influence people's food intake without them being aware of it (8). Even the presence of others can lead to increased food intake through social facilitation (9, 10, 11). In addition, social factors would further result in different mental stress levels, which can influence food consumption in certain individuals, and can shift their food choices, for example from lower fat to higher fat foods (12). However, the rewarding, cultural, and informational factors examined in the current study, as well as the physiological factor, are intra-individual determinants, and can be distinguished from interpersonal or social factors that reflect family and group influences (13). Therefore, as in the case of the physiological factor, it would be better to control to minimize or render constant the effects of social factors by setting appropriate experimental conditions.

As an intriguing future application, the multivariate regression model would serve as a valuable tool in food product development.

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CHAPTER 3

Effects of aroma components from oxidized olive oil on preference

Dietary fats enhance the palatability of foods. It has been reported that several factors, including texture, flavor, orosensory chemical detection of fats, and post-ingestive effects, might contribute to food palatability.(1-7) Among these, the flavor of fats is considered to play an important role in increasing palatability.(8-11)

Dietary fats contain many types of flavors. Certain flavors can be contained in unmodified fats in their original form, while others can be modified by environmental manipulation, as by cooking or oxidation. As an example of environmental modification of flavors, deep-fat frying of potato slices reportedly generates important flavor attributes of potato chips.(12)

People frequently sense attractive flavors in fatty foods such as hamburgers, doughnuts, and tempura or deep-fried foods, and responses to these attractive cues are especially enhanced during fasting.

These flavors are generated by increases in volatile compounds as fats are oxidized by heat during the cooking process.(11) Volatile compounds generated from fat during storage, processing, and cooking significantly contribute to the aroma of oxidized fat. Understanding the formation and degradation of these volatile compounds can thus clarify their significant contributions to aroma.(13)

Generally, since dietary fats constantly undergo oxidation, most dietary fats contain the aroma of oxidized fat to varying degrees. People unconsciously smell the aroma and eat oxidized fats. Thus it is possible that the oxidized fat aromas act as signals for sensing the presence of fats.

For example, olive oil is frequently used in cooking to exploit the typical flavor of olive fruits, and for the health benefits provided by the high content of unsaturated fatty acids and phenolic compounds.(14) This is highly appreciated by consumers not only in countries of the Mediterranean basin where olive oil production is concentrated but also in countries all over the world,(11) and consumption of olive oil has increased by 228% during the past 20 years.(15)

It has been reported that the positive attributes and sensory effects of olive oil are associated with volatile compounds that are produced mainly by the oxidation of fatty acids.(13) The aroma of oxidized fat present in the characteristic flavor of olive oil.

The aim of this study was to investigate the possibility that aroma components generated by the oxidation of olive oil enhance the palatability of olive oil in a mouse behavioral model.

Materials and Methods

Animals

Eight-week-old male BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan) for each experiment. The mice were housed individually in a vivarium maintained at $23 \pm 2^{\circ}\text{C}$ under a 12:12 h light/dark cycle (lights on 06:00–18:00 h). Commercial standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water were available *ad libitum*. All experiments were carried out during daytime (14:00–16:00 h). This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee, and was in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

POV test paper

POV test paper was purchased from Sibata Scientific Technology (Saitama, Japan). The peroxide value (POV) can be measured in a range of 0-50 meq/kg *via* a

color reaction after the test paper is soaked in oils for 3 min. Accordingly, the POV test paper serves as a simple test for measuring POV, as the value is reflected in the three colors. Although not quantitative, the test paper is useful for rough confirmation of the oxidation process.

Oxidized commercial olive oil

Commercial olive oil was purchased from Ajinomoto (Tokyo, Japan), and was oxidized at room temperature. Specifically, the oil was left at about 26°C for 1, 2, or 3 weeks after opening the package. POVs were about 5 meq/kg after 1 week, 10 meq/kg after 2 weeks, and 25 meq/kg after 3 weeks, as assessed by the test paper. In some experiments, the commercial olive oil was oxidized by heating at 180°C for 7 min, and the POV was about 50 meq/kg.

Oxidized refined olive oil (16)

Refined olive oil was purchased from Moreno, S.A. (Cordoba, Spain). Oxidized refined olive oil and oxidized refined olive oil flavoring preparation were obtained from T. Hasegawa Co., (Tokyo, Japan). Commercial olive oil contains many volatile compounds except the aroma of oxidized oil. Refined olive oil was used to exclude the effects of aromas of unmodified olive oil and volatile compounds. The preparation system consisted of a reactor, heater, reflux condenser, stirrer, thermometer, and an air-inlet. Two traps were sequentially connected from the top of the reflux condenser; the first trap was filled with medium-chain triglycerides (120 g), and the second trap was chilled with dry ice/acetone. The reactor was filled with refined olive oil (2400 g) and water (240 g), and the oxidation reaction was carried out at 100-105°C with air supplied at a flow rate of 480 mL/min. The reaction was stopped when the POV of the oil reached 250 meq/kg or higher.

Oxidized refined olive oil flavoring preparation (16)

Volatile compounds from the oxidized refined olive oil in the exhaust air from the reactor were collected in the two traps. The contents of the two traps were mixed to prepare the flavor. The oxidized refined olive oil flavoring preparation (hereafter

referred to as flavoring preparation) was dissolved in medium-chain triglycerides (Nissin Oillio, Tokyo, Japan).

Non-oxidized commercial olive oil and non-oxidized refined olive oil

New packages or vials of non-oxidized olive oil were used. The POVs of these new oils were lower than the limit of detection by test paper.

The test oils were suspended in 0.3% xanthan gum (Sigma Chemical Co., St. Louis, USA) in water. The vehicle was 0.3% xanthan gum in water. All the oils were stored in vials, degassed, sealed, and stored at -20°C until use. Oxidation was minimized under these storage conditions, and we confirmed that the POVs of the oils were lower than the limit of detection by test paper. Test fluids were made fresh.

Two-bottle choice test

In two-bottle choice training, mice were provided with a pair of bottles containing either 1% non-oxidized refined olive oil solution or vehicle control 10 min daily for 3 days in their home cages. After confirming that the mice had learned to select the non-oxidized refined olive oil solution for 3 consecutive days, we administered two-bottle choice tests. Food and water were removed 1 hour before the test.

In the first two-bottle choice test, 1% non-oxidized commercial olive oil was compared with 1% commercial olive oil oxidized at room temperature. In the second two-bottle choice test, non-oxidized commercial olive oil was compared with commercial olive oil oxidized by heat treatment, at concentrations of 0.1% and 1%. We imposed these concentrations because the POV of the olive oil oxidized by heat treatment was higher than that oxidized at room temperature, and we assumed that the concentration of aroma from olive oil oxidized by heat treatment would be higher than that oxidized at room temperature. In the third two-bottle choice test, we used a different set of mice, repeating the procedure described above with 0.1% non-oxidized refined olive oil and 0.1% oxidized refined olive oil. In the fourth two-bottle choice test, we used a different set of mice, repeating the procedure described above. Based on preliminary experiments, we determined that the appropriate concentrations

of the flavoring preparation were 0.00025, 0.0005, and 0.001%. Non-oxidized refined olive oil (1%) containing the flavoring preparation was compared with non-oxidized refined olive oil (1%) without the flavoring preparation. To exclude the effects of the medium-chain triglycerides used to dissolve the flavoring preparation, we added an equal concentration of medium-chain triglycerides to the 1% non-oxidized refined olive oil.

Once per day, mice were given a pair of non-oxidized refined olive oil and oxidized refined olive oil, as well as a pair of different concentrations of medium-chain triglycerides and the flavoring preparation. Each mouse was given every pair of the test fluids during the test period. The left-right positions of the test fluids were alternated daily at random to avoid any side preference.

The intake of each fluid in each cage was measured by weight and was expressed in g per mouse. After olfactory blockade treatment, the mice were reared one per cage, and preferences for fluids containing 0.1% oxidized refined olive oil or 1% non-oxidized refined olive oil with 0.0005% flavoring preparation were measured in a similar manner.

Olfactory blockade.

Olfactory blockade was carried out by bilateral olfactory nerve transection surgery. Anesthetized mice were placed in a supine position, and 0.25 mL of 0.34 M ZnSO₄ dissolved in physiological saline was infused into each nasal cavity with a blunted needle. The drain to the pharynx was aspirated with a capillary pipette. The mice were used in behavioral tests after recovery from anesthesia. Before and after behavioral testing, olfactory sensitivity was evaluated functionally by a potato chip localization test, slightly modified from previously described methods.⁽¹⁷⁾ In brief, a 22-h food-deprived mouse, which had been exposed to the potato chip test in advance to avoid neophobia, was placed in a test cage (182 × 260 × 128 mm) with flooring made of a thin layer of nesting paper chips. Under the paper chips one fragment of a potato chip was buried, and the latency in finding the potato chip was measured. Untreated mice showed a mean time of about 30 s to find the target, while the post-olfactory blockade mice could not find the potato chip. The cut-off time used as a

criterion for the olfactory blockade mice was 180 s, and data obtained for mice not meeting this criterion were excluded.

Gas chromatography analysis

Gas chromatography (GC)-mass spectrometry (MS) analysis was performed with the following equipment and settings: GC 6890 N (Agilent Technologies) coupled to MS 5975B (Agilent Technologies); column, TC-WAX (film thickness 60 m $\times$ 0.32 mm $\times$ 0.25 μ m); gas, helium; injection temperature, 250°C; oven temperature program, 40°C initial temperature (held for 5 min), increased by 4°C/min to 230°C.

Statistical analyses

All values are expressed as mean $\pm$ SEM. The effects of fluid intake on the numbers of mice were examined by paired *t*-test (Prism 4.0; GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Preference for commercial olive oil oxidized at room temperature over non-oxidized oil

The first two-bottle choice test was performed to examine the preference of mice for non-oxidized commercial olive oil *versus* commercial olive oil oxidized at room temperature for 1, 2, and 3 weeks. As shown in Fig. 1, the mice significantly preferred commercial olive oil oxidized for 3 weeks over non-oxidized commercial olive oil when both oils were presented at concentration of 1%.

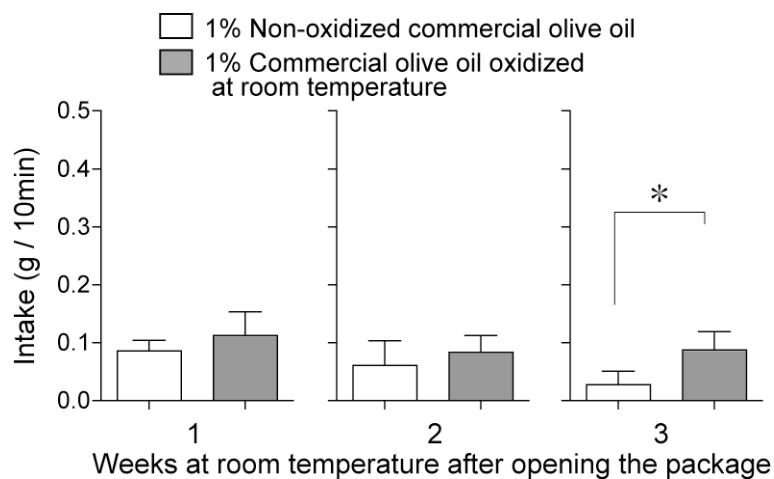


Fig. 1. Two-Bottle Choice Test of Non-Oxidized Commercial Olive Oil *versus* Commercial Olive Oil Oxidized at Room Temperature for 1, 2, and 3 Weeks after Opening the Package.

Mice were offered a pair of test fluids in different orders.

Values represent the means $\pm$ SEM ($n = 9$). * $p < 0.05$ (paired t -test).

Preference for commercial olive oil oxidized by heat treatment over non-oxidized commercial olive oil

The second two-bottle choice test was performed to examine the preference of mice for non-oxidized commercial olive oil *versus* commercial olive oil oxidized by heat treatment. As shown in Fig. 2, the mice significantly preferred oxidized commercial olive oil over non-oxidized commercial olive oil when both oils were presented at concentration of 0.1%.

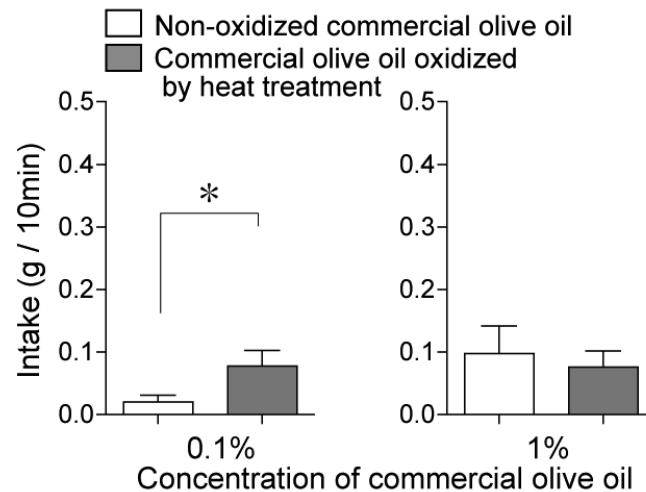


Fig. 2. Two-Bottle Choice Test of Non-Oxidized Commercial Olive Oil *versus* Commercial Olive Oil at Concentrations of 0.1% and 1%, Oxidized by Heat Treatment.

Mice were offered a pair of test fluids in different orders. Values represent the means $\pm$ SEM (n = 9). * $p < 0.05$ (paired t -test).

Preference for oxidized refined olive oil over non-oxidized refined olive oil lacking volatile compounds

The third two-bottle choice test was performed to examine the preference of mice for non-oxidized refined olive oil *versus* oxidized refined olive oil. As shown in Fig. 3A, the mice significantly preferred oxidized refined olive oil over non-oxidized refined olive oil when both oils were presented at concentration of 0.1%.

We also observed the effect of olfactory blockade on the ingestion behavior of mice for non-oxidized refined olive oil *versus* oxidized refined olive oil. The anosmic mice, which received bilateral olfactory nerve transection surgery, showed a diminished preference for oxidized refined olive oil (Fig. 3B).

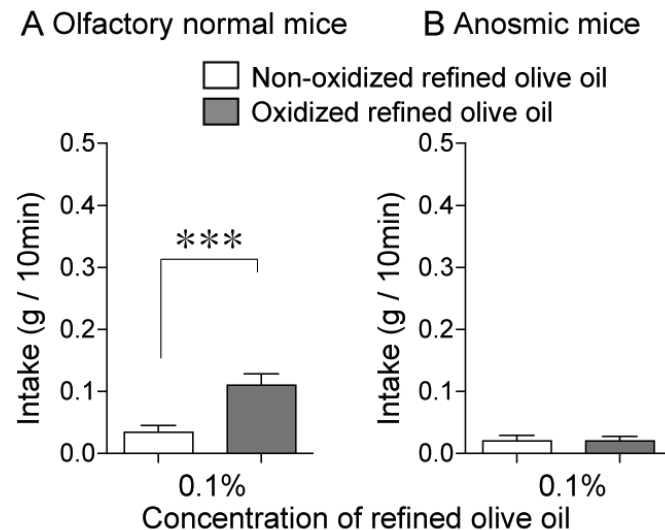


Fig. 3. Two-Bottle Choice Test of Non-Oxidized Refined Olive Oil *versus* Oxidized Refined Olive Oil for Olfactory Normal Mice (n = 9) (A) and Anosmic Mice (n = 10) (B).

Mice were offered a pair of test fluids in different orders.

Values represent the means $\pm$ SEM. *** $p < 0.001$ (paired t -test).

Preference for the major fractions generated by oxidation of refined olive oil

The fourth two-bottle choice test was performed to examine the preference of mice for non-oxidized refined olive oil *versus* non-oxidized refined olive oil with the flavoring preparation. As shown in Fig. 4A, the mice significantly preferred the oil with 0.0005% flavoring preparation, and tended to prefer the oil with 0.00025% flavoring preparation. However the preference was diminished for the oil with the 0.001% flavoring preparation.

We observed the effect of olfactory blockade on the ingestion behavior of mice as to non-oxidized refined olive oil *versus* non-oxidized refined olive oil with the flavoring preparation. The anosmic mice showed no preference for the oil with the 0.0005% flavoring preparation (Fig. 4B).

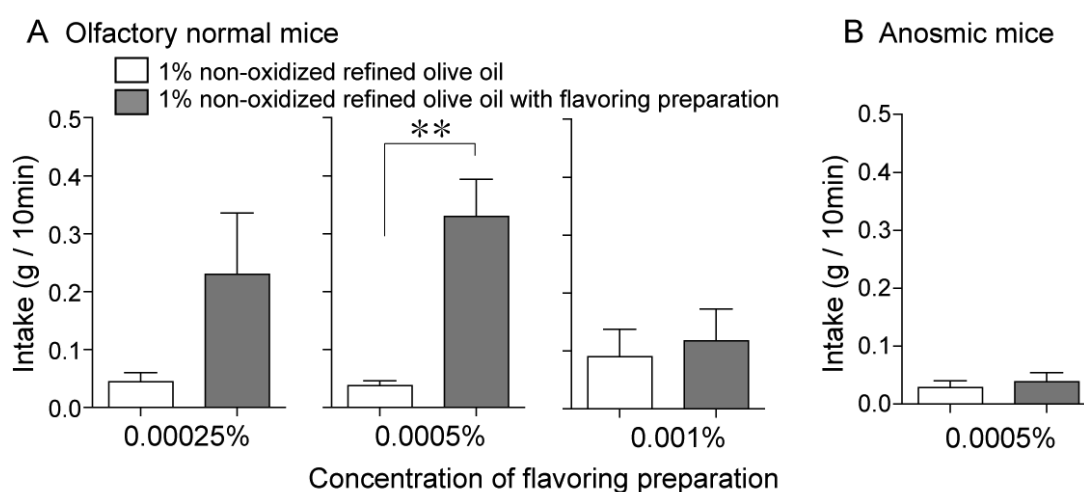


Fig. 4. Effects of Oxidized Refined Olive Oil Flavoring Preparation Added to Vehicle for Olfactory Normal Mice (n = 8) (A) and Anosmic Mice (n = 10) (B), as Assessed by Two-Bottle Choice Test.

Medium-chain triglycerides, used to dissolve the flavoring preparation, were added to the vehicle in another fluid. Mice were provided with a pair of test fluids in different orders.

Values represent the means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ (paired t -test).

GC-MS analysis of the volatile compounds generated by oxidation of refined olive oil

The aroma components of the flavoring preparation were analyzed by GC-MS. The main components of the flavoring preparation detected included (*E*)-2-decenal, (*E*)-2-heptenal, and (*E*)-2-octenal (Fig. 5).

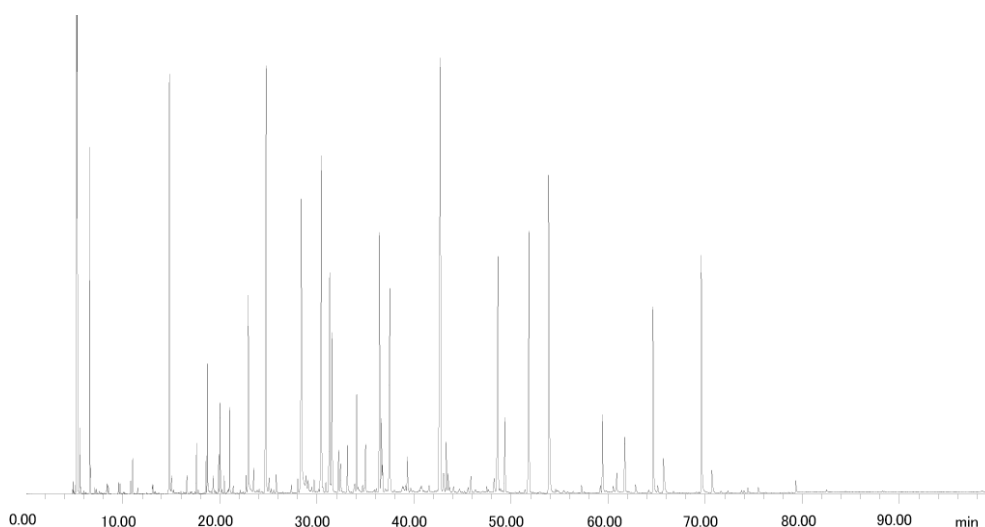


Figure 5. GC chart by GC-MS analysis of oxidized refined olive oil flavoring preparation.

Analysis equipment : GC 6890N (Agilent Technologies) coupled to 1 MS 5975B (Agilent Technologies); column :TC-WAX (60 m Å~ 0.32 mm Å~ 0.25 Pm film thickness); gas: helium; injection temperature: 250°C; oven temperature program: 40°C initial temperature (held for 5 min) increased by 4°C/min to 230°C.

GC: gas chromatography; MS: mass spectrometry

The present study investigated the contributions of aroma components produced by oxidation of olive oil to palatability.

The aroma of oxidized fat arises from volatile compounds generated from fats that develop through multiple stages (preparation, storage, processing, and cooking).(14, 18) These volatile compounds contribute to the combined sensation of smell and taste, commonly called flavor,(13) which strongly affects sensory receptors and influences food palatability and consumer preferences.(11) Many studies have

proposed that food manufacturers should develop methods of preventing or at least reducing the oxidation of fats in foods, because oxidation can lead to the development of objectionable flavors, with obvious detrimental consequences to food quality and consumer satisfaction.(19, 20)

We have reported that the gustation of rodents contributes to oil preferences, and we concluded that a preference for oils at concentrations higher than 0.5% might be affected by gustation of rats because no suppression was observed under olfactory blockade at these concentrations. However, anosmic rats diminished preference for oils at concentrations lower than 0.2%.(4) Moreover, anosmic mice showed little preference for oils at concentrations lower than 5%.(17) The results of these previous studies suggest that olfactory sensation might contribute to a preference of oils at low concentrations. Due to the lack of studies, the mechanisms underlying the contribution of olfactory sensation to oil preference remain unclear. The results of the present study confirm that olfactory sensation plays an important role in the preference for oils at low concentration, given that we examined the effects of olfaction on preference of oils at concentrations lower than previously reported.

A recent study indicated that olfaction might interact closely with gustation, suggesting that trace oil might affect olfaction *via* intervening gustation, given that protein-coupled receptors have been reported to be expressed in taste cells to sense dietary fat.(21) Accordingly, there is a possibility that olfaction interacts with gustation *via* signaling in taste cells.

In this study, we found that mice highly preferred commercial olive oil oxidized at room temperature for 3 weeks after opening the package. We also found that mice significantly preferred commercial olive oil oxidized by heat treatment over non-oxidized commercial olive oil provided at the same concentration. These results suggest that the oxidation of commercial olive oil plays a role in enhancing preference.

Our results indicated that the addition of aroma components produced by oxidation of dietary oils to refined olive oil had a preference enhancing effect, suggesting that these aroma components play significant roles in enhancing preference and palatability. The aroma components produced by oxidation of dietary oils were identified as (*E*)-2-decenal, (*E*)-2-heptenal, and (*E*)-2-octenal based on the

results of GC-MS analysis of the flavoring preparation. Therefore, it is possible for these common aroma components, which are present in oxidized general oils as well as in olive oil,(14) to contribute to enhanced palatability. Further studies are needed to analyze other minor components produced by oxidation.

Our analysis of anosmic mice indicates that olfaction is the primary sensory modality involved in enhancing the preference for oxidized oil. Since normal mice significantly preferred a 0.0005% concentration of the flavoring preparation from the major fractions generated by oxidation of refined olive oil, it is possible that olfaction participates in enhancing the preference for oxidized oil. These results are further supported by data showing that the appropriate concentrations of the aroma of oxidized refined olive oil added to refined olive oil can enhance preference *via* olfaction.

Although the mice not exposed to oxidized refined olive oil in preliminary training, the oxidized refined olive oil and its aroma were significantly preferred over non-oxidized refined olive oil. This suggests that the mice had an already existent preference for oxidized olive oil before the study. One possibility is the presence of aroma components produced by oxidation of fats in breast milk. It has been reported that acceptance of and preference for aromas may be affected by being subjected to aromas during the embryonic and infant stages. For instance, infants with no exposure to garlic volatiles in their mothers' milk during the experimental period reportedly spent significantly more time breast-feeding after their mothers ingested garlic capsules compared to infants whose mothers repeatedly consumed garlic during the experimental period. These studies support the effect of prior experience with aroma in mother's milk on subsequent acceptance and preference.(22)

It has been suggested that recognition of the aromas of oxidized oils develops in mice from the weaning period onward. Mizushige reported that cephalic and physiological information derived from high calorie oil intake is integrated into the brain during repeated presentation of corn oil to rats, since a strong appetite for oil was formed after 5 days of oil presentation.(23) The aroma components produced by the oxidation of oils might function as a learned cephalic signal from laboratory chow.

Fats are exposed to the phenomenon of oxidation to varying degrees in the natural environment. These fats, many of which contain aromas of oxidized fats, are ingested by animals. Because animals, including humans, ingest foods containing the aroma of oxidized fats, they may learn that the aroma of oxidized fats indicates the presence of fats. While there is a possibility that the preference for oxidized fats is congenital, few studies support this idea. Further studies are required to investigate the mechanisms underlying this preference.

On the other hand, the mice tended to not prefer refined olive oil containing a high concentration of the flavoring preparation, suggesting that excess oxidized oil may be unfavorable. Although the mechanism is unclear, it is possible that animals, especially humans, feel gastrointestinal discomfort from the aroma of excess oxidized oil, as it acts as an aversive olfactory and gustatory stimulus. It is also possible that laboratory mice might have neophobia for the aroma of excess oxidized oil, or might have a congenital palatability response to repel strong bitterness and invasive aroma generated by toxic products in excess oxidized oil.

We performed GC-MS analysis of the flavoring preparation used in this study and detected (*E*)-2-decenal, (*E*)-2-heptenal, and (*E*)-2-octenal as the main components, in accord with volatile compounds possibly responsible for oxidized virgin olive oil flavor (18) and volatile aldehydes in heated olive oil. (*E*)-2-decenal is a compound derived from oleic acid hydroperoxide, and (*E*)-2-heptenal and (*E*)-2-octenal are derived from linoleic acid hydroperoxide.(14) (*E*)-2-heptenal is a major rancidity indicator.(13)

It has been reported that oxidation of fats generates relatively stable radicals that abstract H-atoms from activated methylene groups, forming an olefinic compound. Unsaturated fatty acid olefinic compounds, such as oleic acid, linoleic acid, and linolenic acid, appear with shorter induction periods and at higher oxidation rates.(24) Because the main components of the flavoring preparation were volatile compounds from fatty acids, it is possible that the aroma of oxidized oil acted as a signal of a source of fatty acids, including essential fatty acids such as linoleic acid and linolenic acid.

In conclusion, we found that the aroma components of oxidized olive oil enhanced palatability. Because mouse preference for olive oil was enhanced by the addition of a certain concentration of the flavoring preparation, these results support the idea that oxidized olive oil aroma can act as a signal or can play an important role in the detection of the presence of oils, fats, and sources of fatty acids.

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SUMMARY

CHAPTER 1

This study explored the possibility of generating a novel sensory evaluation instrument for describing comprehensive food palatability via its subdomains (rewarding, cultural, and informational) while keeping physiological factors constant. Seventy-five Japanese participants were asked to taste cheese samples and to respond to a questionnaire that was developed to dissect the distinct subdomains of palatability. The subsequent factor analyses revealed that three major factors may serve as distinct subdomains of palatability: rewarding, cultural, and informational, although the informational factor was not sufficiently robust. Multivariate regression analysis on cheese samples with exactly the same ingredients but sold in different packages led to different comprehensive palatability ratings due to the contribution of the cultural, but not the rewarding factor. This study presents the first experimental demonstration that palatability could be dissociated to its subdomains.

CHAPTER 2

This study explored the possibility to quantitatively illustrate differences in palatability perception across generations or gender by the application of multivariate model. Cheese samples with the same ingredients led to different comprehensive palatability ratings due to different contributions of the cultural factor of palatability. This striking result clarifies that palatability is not merely based on the physical and chemical properties that are intrinsic to a food product itself, but also depends on psychological properties that can arise through the interaction between humans and the food product. Most importantly, the author demonstrated that such psychological properties could be quantified if physiological factors, predominant determinants of palatability, are adequately regulated to contrast out the important but less influential

effects of the rewarding and cultural factors.

CHAPTER 3

The author investigated the possibility that aroma components generated by the oxidation of olive oil may enhance the palatability of olive oil. Using a mouse behavioral model, we found that olive oil oxidized at room temperature for 3 weeks after opening the package, and heated olive oil were both significantly preferred over non-oxidized olive oil. Furthermore, this preference was enhanced with an additive of oxidized refined olive oil flavoring preparation at a certain concentration. These results suggest that the aroma of oxidized fat might be present in most fats, and might act as a signal that makes possible the detection of fats or fatty acid sources.

ACKNOWLEDGMENTS

The author is deeply grateful to Dr. Tohru Fushiki, Professor of Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, for his continuous guidance, kind encouragement and generous support throughout this study.

The author would like to express his sincere gratitude to Dr. Kazuo Inoue, Associate Professor of Laboratory of Nutrition Chemistry, for his valuable discussion and advices of this study.

The author would like to express his sincere gratitude to Dr. Satoshi Tsuzuki, Assistant Professor of Laboratory of Nutrition Chemistry, for his valuable discussion and advices of this study.

The author wishes to express his grateful acknowledgement to Dr. Shigenobu Matsumura, Assistant Professor of Laboratory of Nutrition Chemistry, for his kind support and continuous encouragement of this study.

The author would like to thank Dr. Hanae Yamazaki, Associate Professor of Ryukoku University, for her kind support and continuous encouragement of this study.

The author would like to thank Dr. Ai Eguchi, the members of the same research group, for her kind support and continuous encouragement of this study.

The author would like to thank Ms. Minako Sawa, Ms. Haruka Kubo, Mr. Akihiro Oishi, the members of the same research group, for their useful discussion and encouragement.

The author would acknowledge all members of Laboratory of Nutrition Chemistry for their helpful support and warm encouragement.

The author is deeply grateful to Dr. Ippeita Dan, Professor of Chuo University, for his strict or kind guidance and valuable advices of this study.

The author is deeply grateful to Dr. Yasushi Kyutoku, Associate Professor of Chuo University, for his kind support and valuable advices of this study.

The author thanks Meiji Co., Ltd. (Tokyo, Japan) for supplying all cheeses.

The author thanks T. Hasegawa Co. Ltd. (Tokyo, Japan) for supplying flavoring preparation.

The author would like to thank Mr. Tsukasa Saito, T. Hasegawa Co. Ltd., for his kind support and useful advices of this study.

The author would like to thank Mr. Takahiko Amitsuka, visiting researcher from T. Hasegawa Co. Ltd., for his useful advices and discussion of this study.

Finally, the author is deeply grateful to her parents, family, friends for their continuous, warm encouragement and informative advice.

Kumiko Nakano

Jan.2014

LIST OF PUBLICATIONS

- 1) Analyzing comprehensive palatability of cheese products by multivariate regression to its subdomains.

Kumiko Nakano, Yasushi Kyutoku, Minako Sawa, Shigenobu Matsumura,
Ippeita Dan, Tohru Fushiki
Food Science & Nutrition., 1(5):, 369–376, 2013

- 2) Quantitative approach for analyzing palatability using multivariate regression model.

Kumiko Nakano, Tohru Fushiki
(In preparation)

- 3) Effects of aroma components from oxidized olive oil on preference.

Kumiko Nakano, Haruka Kubo, Shigenobu Matsumura, Tsukasa Saito,
Tohru Fushiki.
Biosci. Biotechnol. Biochem., 77 (6), 1166-1170, 2013

Related papers

- 1) おいしさを評価する方法の探索

中野 久美子、伏木 亨
New Food Industry , Vol.53 No.9 p49-55, 2011

- 2) おいしさを数式であらわす

中野 久美子、伏木 亨
食品と開発, Vol.46 No.12 p4-6, 2011