

Inhibitory Effect of Aroma on the Bitterness of Branched-Chain Amino Acid Solutions

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Nutritional products for patients with liver failure available on the Japanese market contain many branched-chain amino acids (BCAAs) such as L-leucine, L-isoleucine, and L-valine, which not only have a bitter taste but also strong, unpleasant odours, leading to low palatability. The palatability of these nutritional products can be significantly improved by the addition of flavoured powders containing various kinds of tastants (sucrose, citric acid, etc.) and odourants (fruit, coffee aromas, etc.). The specific effects of the aroma of flavoured powders have not yet been clearly evaluated. In the present article, the inhibitory effect of aroma on the bitterness of BCAA solutions was examined. The bitterness intensity of a BCAA solution at the same concentration as Aminoleban® EN was defined as 3.5 (measured by a previously described gustatory sensation method). The bitterness threshold of a BCAA standard solution without added aroma was estimated to be 1.87, while those of BCAA solutions containing green-tea, coffee, apple, vanilla, or strawberry aromas were 2.02, 1.98, 2.35, 2.40 and 2.87, respectively, when evaluated by the probit method. This shows that the addition of an aroma can elevate the bitterness threshold in human volunteers. The green-tea and coffee aromas predominantly evoked bitterness, while the vanilla aroma predominantly evoked sweetness. Apple and strawberry aromas evoked both sweetness and sourness, with the apple aroma having stronger sourness and the strawberry aroma stronger sweetness. Thus, a 'sweet' aroma suppresses the bitterness of BCAA, with coexisting sourness also participating in the bitterness inhibition.

Key words nutritional product; sweetness; branched-chain amino acid; bitterness threshold; quinine; aroma

Change in amino acid metabolism is a trait of liver disease, and concentration decreases of the branched-chain amino acids (BCAAs: L-leucine, L-isoleucine, L-valine) and/or concentration increases of aromatic amino acids (AAAs: phenylalanine, tyrosine, tryptophan) and methionine in circulating blood are common. For the management of nutrition in hepatic encephalopathy and to prevent recurrence, it is useful to provide additional BCAAs and AAAs for the chronic hepatic failure patient.¹⁾ These nutrients are available on the Japanese market as transfusions and nutritive drinks for parenteral and enteral nutrition, respectively. However, BCAAs are extremely bitter and the low palatability of nutritive drinks is a major problem with respect to patient compliance.²⁾

Table 1 shows the main amino acid concentrations of Aminoleban® EN,³⁾ a typical nutrient product for liver failure

on the Japanese market, when suspended in 50 ml of water as suggested on the package insert. The corresponding absolute thresholds of bitterness of various amino acids were derived from Yosida's article.⁴⁾ As shown in the Table 1, the concentration of BCAAs in Aminoleban® EN, when taken according to the package insert, are 10–50-fold higher than the bitterness threshold concentrations of the BCAAs, suggesting that the bitterness of the product must be unpleasant for patients.⁵⁾

In the previous article,⁶⁾ we examined the bitterness intensity of Aminoleban® EN and BCAA standard solutions of the same concentration as in Aminoleban® EN, by gustatory sensation test according to the Katsuragi's paper.^{7,8)} The obtained bitterness for BCAA standard solution and Aminoleban® EN was 3.5 and 1.5, respectively. This reduction of bitterness intensity was expected to be due to the sweeteners or organic acids, such as phthalic or fumaric acids which were incorporated in the various flavored powders. Furthermore, in the previous article, we also found that the addition of some flavoured powder successfully suppressed bitterness of Aminoleban® EN compared with the case of without flavoured powder even though the effect of the various aromas involved in these flavoured powders on the bitterness of BCAAs, the predominant bitter components of Aminoleban® EN, has not yet been fully elucidated.

There has been some odor–taste interaction studies.^{9–12)} But studies just focused on fundamental odor–taste interactions themselves, and did not focus on bitterness suppression. Therefore, the purpose of the present study was to examine the bitterness inhibitory effect of five different tasteless aromas (green-tea, coffee, vanilla, apple and strawberry) on BCAA solutions. Firstly, changes of the bitterness threshold of BCAA solutions caused by the odour of the aroma

Table 1. The Amino Acid Component Contained in Aminoleban® EN and Bitterness Threshold Concentration of Amino Acids Reported by Yoshida as Comparison

	Concentration contained in Aminoleban® EN (g/dl) ^{a)}	Bitterness threshold concentration (g/dl) ^{b)}
L-Ile	0.961	0.09
L-Leu	1.019	0.19
L-Val	0.801	0.04
L-Lys·HCl	0.121	0.03
L-Thr	0.067	0.26
L-Arg·HCl	0.151	0.03
L-His·HCl·H ₂ O	0.094	0.005
L-Trp	0.037	0.09

^{a)} Aminoleban® EN, Package Insert Information (2005).³⁾ Aminoleban® EN was prepared as described in the package insert of the product, to a concentration of 1 kcal/ml. ^{b)} Yoshida M. *et al.*, *J. Agric. Chem. Soc. Jpn.*, **40**, 295–299 (1966).⁴⁾

were measured using the probit method¹³⁾ in human volunteers. Secondly, the taste which the odour of each aroma evoked was examined in human volunteers. Finally, the bitterness inhibitory effect of the odour of each aroma is discussed.

Experimental

Subjects Twelve healthy subjects (24±8 years old, 10 females and 2 males) participated in these experiments. No subject reported having a cold or other respiratory tract infection in the week prior to testing. The subjects were asked to refrain from eating, drinking, or chewing gum for at least 1 h prior to testing. Moreover, all subjects participated in this experiment were non-smokers. All subjects received an informed consent before experiments, and the experimental protocol was approved by the ethics committee of Mukogawa Women's University.

Materials The three BCAAs used in the experiment, L-leucine (L-Leu), L-isoleucine (L-Ile), and L-valine (L-Val), were gifts from Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). Five odourants were used: green-tea, coffee, apple, vanilla, and strawberry aromas (Ogawa & Co., Ltd., Tokyo, Japan). None of the aromas had any taste, as confirmed in a pilot study (data not presented). All stimuli were presented at room temperature.

Preparation of Samples Solutions of BCAAs were prepared, at the same concentration as they are found in Aminoleban® EN (bitterness intensity defined as 3.5), and at various dilutions so as to provide solutions with bitterness intensities of 3.0, 2.5, 2.0, 1.5, 1.0, and 0.5 on the tau scale, as shown in Table 2. The tau scale is "equi-distance scale" which adjusted the concentration stage that the intensity of the taste becomes at equal intervals.⁸⁾ Therefore, the difference of each numerical value is given as the same grade as intensity of the taste. The regression lines of the logarithm of the concentration of the four fundamental tastes and their tau scales were reported to show similar slopes.⁸⁾ Therefore, the concentration of BCAA was calculated using the slope (2.2809) of the regression of quinine sulfate.⁵⁾ The BCAA solution without aroma was the control, and BCAA solutions containing 0.2% of each aroma (green-tea, coffee, apple, vanilla, and strawberry) were the samples.

Measuring Method The sample size was 5 ml, and all samples were kept in the mouth for 10 s. The subjects were asked to discriminate between two test solutions: BCAA solution and BCAA solution containing aroma, on the basis of taste alone, recording which sample was the bitterer. After tasting, subjects gargled well before tasting the next samples.

Evaluation of Intensity of Four Basic Tastes Evoked by Smelling Odour Samples After the gustatory sensation test described above, 100 ml of each test sample, with or without 0.2% aroma, were poured into steep-top opaque plastic squeeze bottles (300 ml volume). The subjects evaluated the smell of these samples on a 5-point rating scale, from no sensation (0) to very strong sensation (4) for the four fundamental tastes (sweetness, sourness, saltiness, bitterness).

Analysis of Experiment Data The probit method (maximum likelihood method) was used to calculate the bitterness threshold and its 95% confidence interval, using the bitterness intensity measured in a gustatory sensation test, and the parameter (intercept, slope) of maximum likelihood by repetition of a weighted least-squares method and the regression calculated. A tukey test was performed to analyse the intensity of the evoked fundamental taste within each aroma group; SPSS 11.5.1J was used for analysis.

Table 2. The Rate Scale (Tau Scale) of a Bitterness Intensity, and Corresponding BCAA Concentration (g/dl)

Bitterness intensity (tau scale)	BCAA solution (g/dl)		
	L-Ile	L-Leu	L-Val
3.5	0.961	1.019	0.801
3.0	0.580	0.615	0.483
2.5	0.350	0.371	0.292
2.0	0.211	0.224	0.176
1.5	0.128	0.135	0.106
1.0	0.077	0.082	0.064
0.5	0.047	0.049	0.039

The bold letter 3.5 means control preparation.

Results and Discussion

Elevation of Bitterness Threshold of BCAA by Addition of Aroma The bitterness intensity thresholds of BCAAs and various aromas, translated onto the corresponding tau scale with 95% confidence intervals (CI) as calculated by the probit method are shown in Fig. 1. The bitterness threshold of control BCAA solution (without aroma) was 1.87 (95% CI 1.61 to 2.14). The bitterness threshold for BCAA with green-tea and coffee aromas were only slight changed (2.02, 95% CI 1.72 to 2.31, and 1.98, 95% CI 1.62 to 2.34, respectively), while for BCAA plus apple, vanilla, or strawberry aroma, the bitterness thresholds were considerably higher (2.35, 95% CI 2.02 to 2.71; 2.40, 95% CI 2.18 to 2.63; and 2.87, 95% CI 2.61 to 3.20, respectively). The bitterness inhibitory effect of strawberry aroma was the highest and its CIs did not overlap with those of control.

The probits calculated from the results of a gustatory sensation test and the presumed regression lines drawn by the above-mentioned equation are shown in the logarithmic normal probability graph (Fig. 2). In general, the probits become much bigger and do not have actual values when the probability is 0% or 100%. In the analysis of the gustatory sensa-

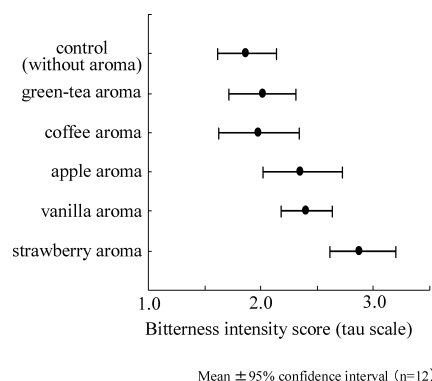


Fig. 1. The Bitterness Threshold of BCAA Solution (with/without Aroma) Measured Using the Probit Method

The addition of green-tea or coffee aroma had no significant effect on the bitterness threshold compared to control. Elevation of the bitterness threshold was observed in the presence of apple and vanilla aroma, and a particularly strong effect was seen with strawberry aroma.

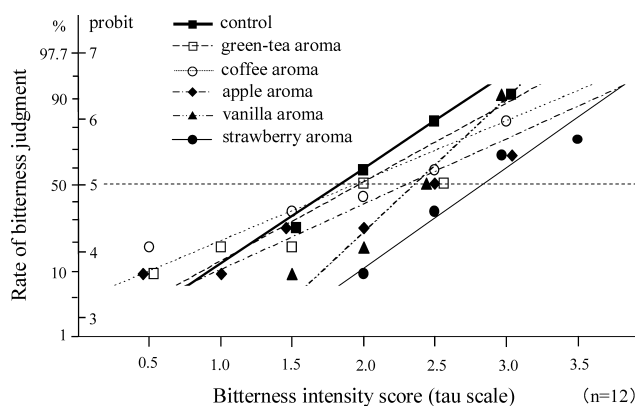


Fig. 2. The Regression Lines Which Show the Relation between Concentration (x) and Bitterness Judgment (y) for Various Samples Calculated from Probit Transformation

The presumed regression line of the BCAA solutions to which aromas might be added, were located below that of control, which suggested that the bitterness threshold rises as increased the smell of an aroma. The bitterness inhibition effect was strongest with apple and vanilla, and especially strawberry aroma.

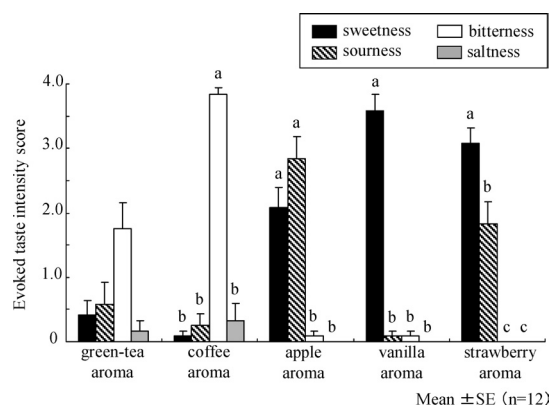


Fig. 3. Evaluation of Intensity of Evoked Image for Four Fundamental Tastes after Smelling Aroma Samples

The subjects evaluated the smell of BCAA solutions containing various aromas on a 5-point rating scale, from no sensation (0) to very strong sensation (4) for the four fundamental tastes (sweetness, sourness, saltiness, bitterness). Differences, indicated by letters (a, b and c) over bars, indicate that the means are statistically different ($p < 0.001$) in intensity scores of evoked taste with and without aromas.

tion test results using the probit method, the judgement of bitterness was estimated to be in the range 3.617 (1/12, 8.3%) to 6.383 (11/12, 91.7%). From such probits, the regression lines showing the relation between concentration (x) and the of judgment of bitterness (y) were calculated as follows (maximum likelihood method): control; $y = 2.611 + 3.265x$ ($r = 0.989$), green-tea aroma; $y = 2.851 + 2.707x$ ($r = 0.938$), coffee aroma; $y = 3.397 + 2.058x$ ($r = 0.967$), apple aroma; $y = 2.903 + 2.234x$ ($r = 0.969$), vanilla aroma; $y = 0.573 + 4.596x$ ($r = 0.974$), and strawberry aroma; $y = 0.975 + 3.445x$ ($r = 0.973$). The presumed regression line of the BCAA solution to which each aroma was added is located below the control line, which suggested that the bitterness threshold is increased by the smell of an aroma. The bitterness inhibitory effect was strongest with the apple and vanilla, and especially strawberry aroma.

These data suggest that aroma can successfully increase the threshold of bitterness of BCAA solutions; in other words, lead to bitterness suppression.

The Bitterness Inhibitory Effect of Aroma on BCAA Solutions The interaction between smell and taste has been studied by several researchers. Taste and odour are integrated senses, and there have been several reports in which odour have been shown to affect the intensity of a taste. The taste-smell illusion has been reported as a tendency to attribute fundamental taste categories (e.g., sweetness) to pure odours.⁹ For example, sweet-smelling odours, such as strawberry, caramel and maracuja, have been shown to enhance the sweetness of sucrose, and the sweetness of aspartame was enhanced in the presence of a vanilla odour.¹⁰ A flavour's effect on taste is, therefore considered to be dependent on both odour and taste.¹¹

Therefore, the addition of a strawberry flavour with a strawberry aroma may suppress the bitterness of Aminoleban® EN or BCAA solutions more than a strawberry flavour without an aroma, as the strawberry aroma enhances the sweetness of sweeteners included in the flavoured powder or Aminoleban® EN. The data of Figs. 1 and 2 suggest that some aromas were successful in suppressing the bitterness intensity of BCAA solutions even in the absence of sweet-

ener.

As shown in Fig. 3, the aromas evoked various kinds of fundamental tastes in all subjects. The green-tea and coffee aromas strongly evoked bitterness, while the vanilla aroma evoked sweetness. Apple and strawberry aromas evoked both sweetness and sourness; the sourness was stronger with the apple aroma and the sweetness was stronger with a strawberry aroma.

Thus, the aromas which successfully elevated the bitterness threshold strongly evoked sweetness and/or sourness (apple, vanilla and strawberry), while the green-tea and coffee aromas which did not elevate the bitterness threshold, did not. In other words, aromas which evoke a taste of sweetness successfully suppress the bitterness of BCAA solutions, with evoked sourness also contributing to this suppression.

Changes in odour perception in gustation are central effects.¹² The orbitofrontal cortex of primates is known to respond to olfactory and taste stimuli. Integration of information on taste and odour is based on associative learning.¹⁴ Small *et al.* used functional magnetic resonance imaging to show that in a congruent taste-odour pair (vanilla/sweet) compared with an incongruent taste-odour pair (vanilla/salt), activation was observed in the insula, orbitofrontal cortex, and anterior cingulate cortex.¹⁵

Keast and Breslin have reviewed interactions between tastes in detail.¹⁶ Mixtures of bitter and sweet tastes were variably affected at low intensity/concentration, while mixtures at moderate and high intensity/concentrations were mutually suppressive. Mixtures of sour and bitter compounds enhanced each other at low intensity/concentration; at moderate intensity bitterness was suppressed and sourness enhanced, while at high intensity/concentrations sourness was suppressed and the effect on bitterness was variable. This suggests that the interaction between tastes is not a fixed action depending on the intensity/concentration of each taste, but rather an enhancing or inhibitory effect, changing with the combined pattern of intensity and concentration. It is possible that the interaction between smell and taste may be similar.

The bitterness inhibitory effect of strawberry aroma was stronger than that of either vanilla, which evoked only sweetness, or apple, which evoked both sweetness and sourness, although less sourness than strawberry (Figs. 1, 3). The combination of sweetness and sourness was therefore most successful in inhibiting bitterness; the balance between these two tastes seemed to be critical in determining the magnitude of the inhibitory effect.

The bitterness suppression of BCAA is critical in improving the palatability of many products which contain BCAAs. There are many different approaches to this problem, such as taste modification by the addition of various sweeteners. Another approach is to decrease the surface area by increasing the mean diameter of BCAA crystals, which achieves bitterness suppression by reducing the release rate of the BCAAs.⁵ It is intriguing that aroma alone can change the bitterness threshold of BCAAs, as demonstrated in the present study. It is a non-caloric method of bitterness suppression and merits further study. The magnitude of the effect is dependent on the specific aroma, which will also be affected by individual preferences. Moreover, the bitterness inhibitory effect of aroma demonstrated in this study may applicable

to the drug with strong bitterness, such as quinine. In the following study, we will try to examine the above inhibitory effect on drugs, and it might contribute to the theoretical design of taste masking.

Conclusion

In the present study, strawberry, apple, and vanilla aromas were shown to evoke an image of sweetness, which contributed to the bitterness inhibition of BCAA solutions. The strawberry aroma, which evoked an image of both sweetness and sourness, was particularly successful in inhibiting bitterness, more so than either vanilla (which also evoked both sweetness and sourness although the sourness intensity was lower) or apple (which did not evoke sourness at all). This suggests that the evocation of both sweetness and sourness by an aroma is necessary for effective bitterness suppression.

Nutrients for liver failure often need to be taken for long periods, and poor medication compliance may cause serious problems, such as hepatic encephalopathy. Knowledge of the interaction of taste and odour provides useful information, not only for the manufacture of various types of nutritional product but also in the theoretical design of taste-masking for commercial medicines.

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