Synergistic Effects of Sour Taste and Low Temperature in Suppressing the Bitterness of Aminoleban® EN

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Aminoleban® EN, a nutritional product for patients with liver failure, contains three branched-chain amino acids (BCAAs): L-leucine, L-isoleucine, and L-valine. As BCAAs are extremely bitter, Aminoleban® EN has a low palatability, which is a major cause of patient noncompliance. Nutrients for liver failure often need to be taken for long periods, and poor medication compliance can cause serious problems, such as encephalopathy. Therefore it is important to suppress the bitter taste of Aminoleban® EN and thereby improve patient compliance. There are already six different flavoured powders (coffee, green-tea, apple, fruit, plum and pineapple) which can be added to Aminoleban® EN to reduce its unpleasant taste and smell, but it is possible that other factors, such as temperature, may also improve the palatability of Aminoleban® EN. In this study, flavours alone significantly decreased the bitterness intensity of Aminoleban® EN. It was thought that the sweetness and sourness of the flavoured powder would be the main factors involved in decreasing the bitterness. However, low temperature (0—5 °C) decreased the bitterness intensity of Aminoleban® EN, with or without the flavoured powders, compared with normal room temperature (25—30 °C). The sourness intensity of flavoured powders was not decreased at low temperatures, but the sweetness intensity of some flavoured powders did decrease. These results suggest that sourness can be tasted even at low temperatures. As not only the addition of flavoured powders but also low temperatures can reduce the bitterness of Aminioleban® EN, the combination of a sour-flavoured powder and a low temperature will improve the palatability of Aminoleban® EN the most.

Key words Aminoleban® EN; bitterness; temperature; sour taste; branched-chain amino acid; flavoured powder

The palatability of a medicine is an important factor in determining compliance. Aminoleban® EN is a bitter nutrient product on the Japanese market which is used in cases of liver failure. Patients with liver failure typically take 50 g of Aminoleban® EN powder suspended in 180 ml of water (final volume approximately 200 ml) up to three times in a day for long periods, in order to control protein metabolism, and poor medication compliance can cause serious problems, such as encephalopathy. As Aminoleban® EN has a highly unpleasant taste and smell, patients are recommended to mix it with flavoured powders to improve palatability. Six different flavoured powders (coffee, green tea, apple, fruit, plum and pineapple) are available, and Miyanaga *et al.* have shown that it is predominantly the sourness and sweetness of these flavours which mask the bitterness of Aminoleban® EN, both in gustatory sensation tests and using an artificial taste sensor.[1] Mukai *et al.* have reported that the aroma of the flavoured powders elevates the bitterness threshold of branched-chain amino acids (BCAAs).[2] Although flavoured powders are known to diminish the bitterness of Aminoleban® EN, additional methods of improving palatability are still required. In order to investigate this further, the effect of the temperature of the Aminoleban® EN suspension on bitterness was investigated.

Kadohisa *et al.* have reported that oral temperature can influence the palatability of foods.[3] They discovered neurons in the orbitofrontal cortex, a brain region important in evaluating the palatability of food, that are sensitive to the temperature of whatever is in the mouth, independent of taste, smell and texture. This suggests that the bitterness of Aminoleban® EN may be influenced by temperature, by affecting taste intensity and/or threshold.

The aim of this study was to evaluate the influence of temperature on the bitterness of Aminoleban® EN. Firstly, the effects of the six different flavoured powders on the unpleasant taste (bitterness) of Aminoleban® EN and its component BCAAs were confirmed, and secondly, the influence of temperature on these effects was investigated.

Experimental

Experimental Procedure. Subjects Four to twelve healthy female subjects, 26±4 years old, participated in the gustatory sensation tests. 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. All subjects were non-smokers and signed an informed consent before the experiments. The experimental protocol was approved by the ethics committee of Mukogawa Women's University.

Materials Aminoleban® EN and six flavoured powders (coffee, green-tea, apple, fruit, plum and pineapple) were gifts from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). 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).

Preparation of Samples Solutions of BCAAs were prepared, at the same concentration as in Aminoleban® EN (bitterness intensity defined as 3.5) and at various dilutions thereof, 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. This scale expresses the relationship between taste intensity and taste substance concentration, such that the numerical values represent differences in the intensities of the taste. The regression lines of the logarithm of the concentration of the four fundamental tastes and their tau scale values show similar slopes. The BCAA solution (tau 3.5) used as bitterness standard[2] contained 0.961 g/dl L-isoleucine, 1.019 g/dl L-leucine and 0.801 g/dl valine. The concentrations of the BCAA standards were calculated using the slope (2.2809) of the regression of quinine sulfate (Table 1). The samples of Aminoleban® EN were suspended in water (50 mg/180 ml), with or without 3% of each flavoured powder (coffee, green-tea, apple, fruit, plum, pineapple).

Gustatory Sensation Tests The protocol and experimental design for all gustatory sensation tests was given prior approval by the ethical committee of Mukogawa Women's University. A previously described method was used in this study.[4] The sample size was 2 ml, and samples were kept in the mouth to evaluate the taste for 5 s before rinsing out. In testing for the bitterness threshold, subjects were asked whether each sample was bitter or not. In testing for the bitterness intensity, subjects rated the tau score of each sample.

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Table 1. The tau Scale of Bitterness Intensity, and Corresponding BCAA Concentrations (g/dl)[2)]

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

Table 2. The tau Scale of Sweetness and Sourness Intensity, and Corresponding Concentrations of Sucrose and Tartaric Acid (g/100 ml)[5)]

Tau	Sweetness Sucrose (g/100 ml)	Sourness Tartaric acid (g/100 ml)
1	1.0	0.0025
2	3.0	0.0090
3	6.4	0.0260
4	14.0	0.0700
5	34.0	0.1800

Experimental Design. The Influence of Flavoured Powders on the Bitterness Intensity of Aminoleban® EN The bitterness intensities of Aminoleban® EN suspended in water (tau 0—3.5) with or without each flavoured powder (coffee, green-tea, apple, fruit, plum and pineapple) were investigated at two different temperatures (0—5, 25—30 °C). The bitterness intensities of BCAA solutions mixed with three sucrose solutions (tau 1, 2 or 3) or four tartaric acid solutions (tau 1, 2, 3 or 4) were also determined.

The Influence of Temperature on the Bitterness Intensity of Aminoleban® EN Firstly, the perceived bitterness of BCAA solutions (tau 0—3.5) was investigated at three different temperatures (0—5, 25—30, 45—50 °C). Secondly, the bitterness thresholds of the BCAA solutions with/without flavoured powder were measured at two different temperatures (0—5, 25—30 °C) using the Probit method. Thirdly, the bitterness intensities of the BCAA solutions, with or without sucrose solution (tau 3), were investigated at two different temperatures (0—5, 25—30 °C).

The Influence of Tartaric Acid and Sucrose on the Bitterness and Sweetness Intensities of BCAA Solutions at Different Temperatures The bitterness and sweetness intensities of BCAA solutions (tau 0—3.5) were evaluated at two different temperatures (0—5, 25—30 °C) with or without tartaric acid solution (tau 4) or sucrose (tau 3)[5)] (Table 2).

The Influence of Temperature on the Sweetness and Sourness of Flavoured Powders The sweetness and sourness intensities of the six flavoured powders were investigated at two different temperatures (0—5, 25—30 °C).

Statistical Analysis Results are expressed as mean±S.E.M. except in Fig. 4, where they are expressed as mean±95% confidence interval (CI). Multiple comparisons were evaluated by Sheffe's test after one-way analysis of variance (ANOVA). Two group comparisons were analyzed using the Mann Whitney *U*-test. $p<0.05$ was considered to be statistically significant.

Results and Discussion

Influence of Flavoured Powders on the Bitterness Intensity of Aminoleban® EN In gustatory sensation tests, the bitterness of Aminoleban® EN was significantly decreased by all the flavoured powders except green tea (Fig. 1). The bitter taste of green tea itself probably accounts for its lack of ability to suppress the bitterness of Aminoleban® EN. It was postulated that the coffee-, apple-, fruit-, plum- and pineapple-flavoured powders may have some common features to account for their ability to suppress bitterness.

First, the sweetness and sourness of the flavoured powders

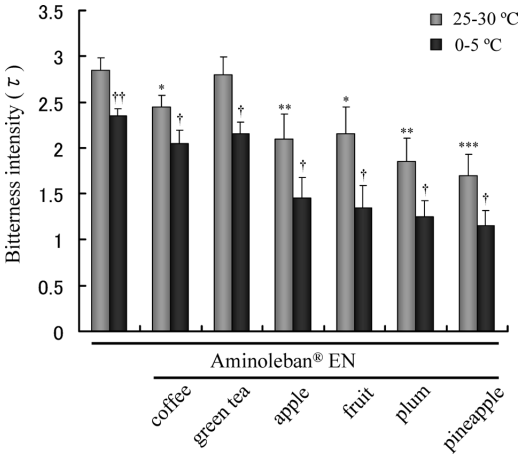

Fig. 1. The Bitterness Intensity of Aminoleban® EN with or without Flavoured Powder

Apple-, fruit-, plum- and pineapple-flavoured powders decreased the bitterness intensity of Aminoleban® EN. $*p<0.05$, $**p<0.01$, $***p<0.001$ *versus* Aminoleban® EN (Sheffe's test). Temperatures of 0—5 °C decreased the bitterness intensity of Aminoleban® EN compared with temperatures of 25—30 °C. $\dagger p<0.05$, $\dagger\dagger p<0.01$ *versus* 0—5 °C (Mann Whitney *U* test).

was examined, using sucrose and tartaric acid as standards, to see whether and to what extent these characteristics were responsible for the suppression of the bitterness of BCAA. BCAA solutions were used as bitterness standards.[2,4)] Both sucrose and tartaric acid were found to suppress the bitterness intensity of BCAA in a dose-dependent manner. A tau 3 sucrose solution significantly decreased the bitterness intensity of BCAA tau 1.5—3.5 solutions (Fig. 2A) and tartaric acid tau 4 significantly decreased the bitterness intensity of BCAA tau 2 and 2.5 solutions (Fig. 2B). These results confirm that both sweetness and sourness can suppress the bitterness intensity of BCAA.

Second, the intensities of sweetness or sourness of the six flavoured powders were investigated (Table 3). Apple-, fruit-, plum- and pineapple-flavoured powders had a high intensity of sourness. As suggested in Fig. 1, these four powders are able to significantly suppress the bitterness of Aminoleban® EN. From these results, it seemed likely that suppression of the bitterness intensity of Aminoleban® EN was due, at least partly, to the sourness of the flavoured powders. Keast and Breslin reported that mixtures of bitter and sweet tastes at moderate and high concentrations were mutually suppressive, while bitterness was suppressed and sourness enhanced in the mixtures of sour and bitter compounds at moderate intensity.[6)] Our results agreed with their report in that sweetness and sourness both suppressed bitterness. While it has been reported that bitter and sweet things stimulate the Type II receptor cells, which have G-protein-coupled taste receptors and similar signaling pathways,[7)] it has also been proposed that this type of receptor cell may not participate in the recognition of sour taste.[8)] In fact, Type III taste cells are necessary for the recognition of sour taste.[9,10)] From our results, it can be postulated that sour stimuli, which have a different pathway from bitter or sweet stimuli, may be important for bitterness suppression.

Influence of Temperature on the Bitterness Intensity of Aminoleban® EN The bitterness intensity of Aminoleban® EN with or without flavoured powder was significantly lower

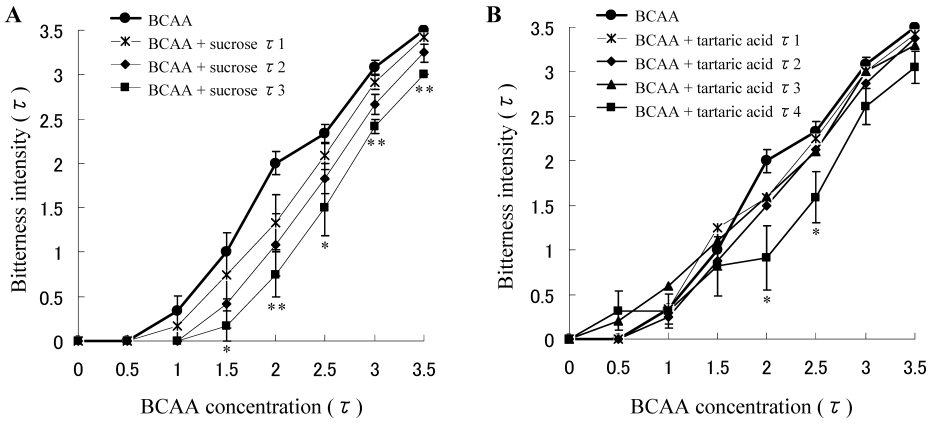

Fig. 2. Influence of Sweetness or Sourness on the Bitterness of BCAA Solution

(A) Sucrose (tau 3) significantly decreased the bitterness intensity of BCAA solutions (tau 1.5—3.5). (B) Tartaric acid (tau 4) significantly decreased the bitterness intensity of BCAA solutions (tau 2, 2.5). $*p<0.05$, $**p<0.01$ *versus* BCAA (Mann Whitney *U* test). Figures are mean±S.E. ($n=6$—11).

Table 3. The Sweetness and Sourness Intensity of Flavored Powder Measured by Gustatory Sensation Tests

Flavored powder	Taste intensity (τ)	
	Sweetness	Sourness
Coffee	2.4±0.24	0.3±0.21
Green tea	2.2±0.37	3.0±0.45
Apple	2.4±0.24	4.3±0.21
Fruit	2.0±0.32	4.5±0.22
Plum	1.6±0.24	4.8±0.17
Pineapple	2.2±0.37	4.3±0.33

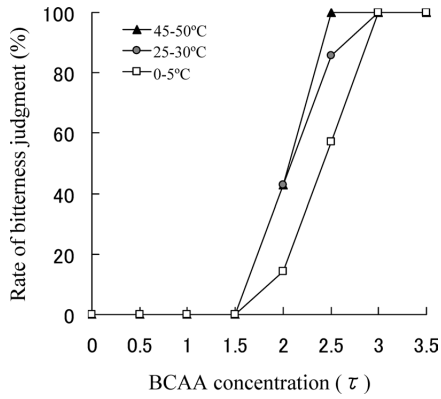

Fig. 3. Bitterness of BCAA Solutions at 0—5 °C, 25—30 °C, and 45—50 °C

The bitterness estimates of BCAA solutions of different concentrations were higher at 0—5 °C than at 25—30 °C and 45—50 °C ($n=7$).

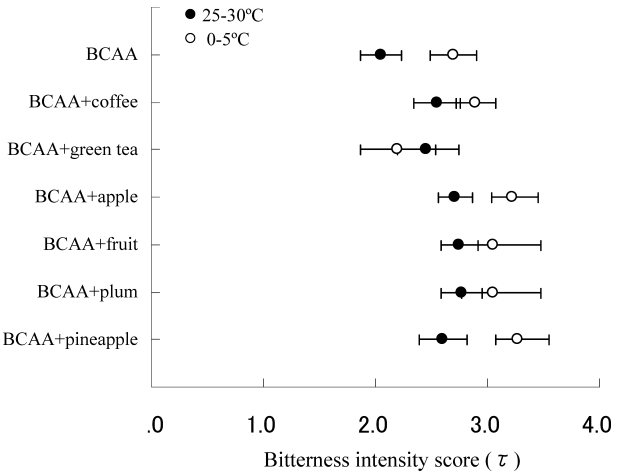

Fig. 4. Bitterness Thresholds of BCAA Solutions (with/without Flavoured Powder) Measured Using the Probit Method

At 25—30 °C, the bitterness thresholds of BCAA solutions with flavoured powders were higher than those without flavoured powders. The bitterness thresholds of BCAA solutions without flavoured powders were higher at 0—5 °C than at 25—30 °C. Figures are mean±95% confidence interval ($n=8$).

when the temperature of the suspension was 0—5 °C compared with 25—30 °C (Fig. 1). As shown in Fig. 3, the bitterness rating was affected by the temperature of the BCAA solution. BCAA solutions of tau 0—1.5 were not rated as bitter, while BCAA solutions of tau 3—3.5 were rated bitter by every subject at every temperature. With BCAA solutions of tau 2.02 at 45—50 °C, tau 2.08 at 25—30 °C, and tau 2.42 at 0—5 °C were rated bitter by 50% of subject. These results suggest that the bitterness thresholds of BCAAs were higher at low temperatures than at room or higher temperatures. In other words, having the solutions at low temperatures made it more difficult for subjects to taste bitterness. As shown in Fig. 4, the bitterness threshold of BCAA solution at 0—5 °C was nearly the same as that of BCAA with added flavoured powders at room temperature (25—30 °C). This suggests that low temperature (0—5 °C) has as great an influence on bitterness suppression as the effective flavoured powders. The bitterness intensity of a BCAA solution with tau 2—3 was significantly decreased, while a solution with tau 3.5 tended to decrease, when the temperature was 0—5 °C, compared with 25—30 °C (Fig. 5A). When the bitterness intensities at temperatures of 25—30 °C and 0—5 °C were compared, they were found to increase as a logarithmic function of the BCAA concentration in a rate-dependent manner, and the two factors (bitterness intensity and logarithm of BCAA concentration) were correlated at both temperatures (25—30 °C: $y=2.4622x+3.445$, $R^2=0.9855$, 0—5 °C: $y=2.0193x+2.5687$, $R^2=0.9607$) (Fig. 5B).

Influence of Tartaric Acid and Sucrose on the Bitterness and Sweetness Intensities of BCAA Solutions at Different Temperatures The degree of suppression of BCAA bitterness when the solution temperature was 0—5 °C, was similar to the degree of suppression when the solution contained sucrose, tau 3 (Fig. 5C) or tartaric acid, tau 4 (Fig.

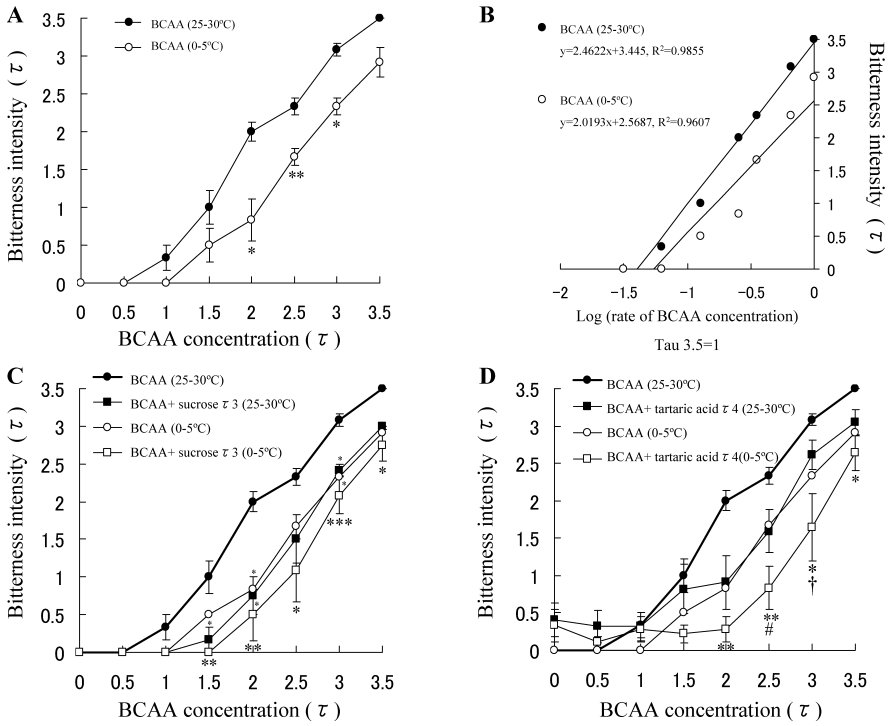

Fig. 5. Effects of Sucrose, Tartaric Acid and Temperature on the Bitterness Intensity of BCAA

(A) Bitterness intensity of BCAA solutions at 0—5 °C and 25—30 °C, $*p<0.05$, $**p<0.01$ *versus* BCAA at 25—30 °C (Mann Whitney *U*-test). (B) Correlation between bitterness intensity and logarithm of BCAA concentration. (C) The influence of sweetness (sucrose) and temperature or (D) sourness (tartaric acid) and temperature on the bitterness intensity of BCAA solutions. $*p<0.05$, $**p<0.01$, $***p<0.001$; *versus* BCAA at 25—30 °C (Sheffe's test), $\dagger p<0.05$, *versus* BCAA+tartaric acid (tau 4) at 25—30 °C; and $\#p<0.05$, *versus* BCAA at 0—5 °C (Mann Whitney *U*-test).

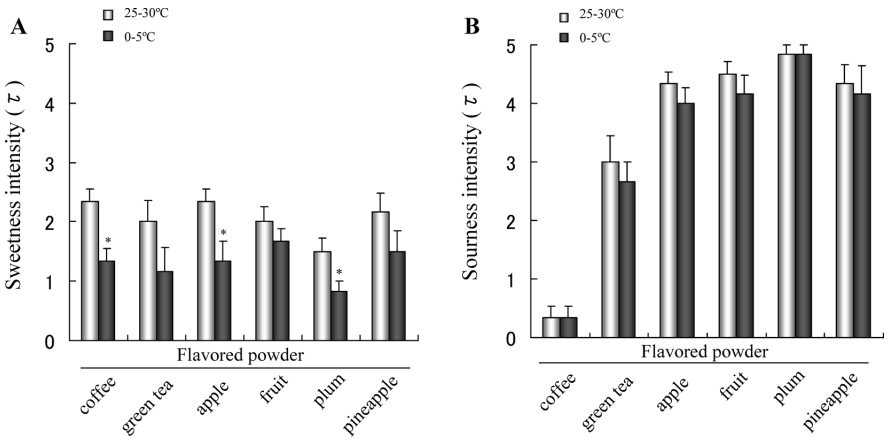

Fig. 6. Influence of Temperature on Sweetness and Sourness Intensity of Flavoured Powders

(A) Sweetness intensity of flavoured powders at 0—5 °C and 25—30 °C, $*p<0.05$ *versus* 25—30 °C (Mann Whitney *U*-test). (B) Sourness intensity of flavoured powders at 0—5 °C and 25—30 °C, no significant differences.

5D). When a BCAA solution containing sucrose, tau 3, was evaluated at low temperatures (0—5 °C), the degree of bitterness suppression achieved was equal to the sum of that achieved by each factor alone (sucrose, tau 3, and low temperature, 0—5 °C). On the other hand, when a BCAA solution containing tartaric acid, tau 4, was evaluated at low temperature (0—5 °C), there was a greater decrease of bitterness intensity than the sum of the two single factors (tartaric acid, tau 4, and low temperature, 0—5 °C). These results suggest that a combination of low temperature and sourness has the greatest bitterness-suppressing effect.

Influence of Temperature on the Sweetness and Sourness Intensities of Flavoured Powders As shown in Fig. 6A, the sweetness intensities of flavoured powders (3% coffee, apple and plum solutions) were significantly decreased at low temperatures, while those of green tea, fruit and pineapple showed a tendency to decrease at low temperatures compared with normal temperatures. However, the sourness intensities of the flavoured powders at low temperature were not decreased compared with those at normal temperatures (Fig. 6B). This suggests that sourness remains, even at low temperatures, and is still capable of suppressing bitterness. The existence of thermosensitive ion channels has been reported, all of which belong to the transient receptor potential (TRP) superfamily.[11] Talavera *et al.* reported that increased temperature activates TRP cation channel, subfamily

M, member 5 (TRPM5) which is expressed in taste buds of the tongue, causing enhanced sweetness.[12] It means that sweetness is influenced by temperature dependent TRPM5. Also in our experiment, there is a possibility that the TRPM5 were related to the decreasing sweetness intensity at low temperature. Polycystic kidney disease 2-like 1 (PKD2L1), which is reported to be a candidate for the sour taste receptor, also belongs to the TRP channel.[9,13] That is to say, compounds with sour taste and those at low temperature may pass through TRPs. There are few reports about relation between PKD2L1 and temperature so the detail is unclear but there is a possibility that this common mechanism, utilising TRPs, might be related to the suppression of bitterness. Cola *et al.* reported that sour taste and cold stimuli, used at the same time, changed swallowing patterns by shortening the pharyngeal transit time, which may have a positive effect in patients with oropharyngeal dysphagia.[14] Taking this into consideration, combination of sourness and temperature might have some relation, as sweetness and temperature do. In order to dissolve the matter, finding relation between PKD2L1 and temperature would be useful, and further investigation is required.

In conclusion, the bitterness intensity of Aminoleban® EN can be suppressed by flavoured powders, particularly apple, fruit, plum and pineapple flavours, which have a strong sour taste. The bitterness intensity of Aminoleban® EN with or without the flavoured powders is also significantly suppressed at low temperatures (0—5 °C) compared with normal temperatures (25—30 °C). The sourness of the flavoured powders is not decreased at low temperatures, while their sweetness is decreased. Therefore, sweetness, sourness, and low temperatures are the most important factors in the inhibition of bitterness. The use of a flavoured powder with strong cold-resistant sourness and a low temperature will offer the best combination for suppressing the bitterness of Aminoleban® EN. There is a problem that our data was obtained from healthy subjects and some patients with liver failure may have taste disorder. It is not clear that this combination effect to healthy control is directly connected to the cirrhotic patients who may have dysgeusia, and further investigation with patients would be required. However, our discovery to improve palatability of Aminoleban® EN will be an effective clue to improve patient compliance.

Acknowledgments This work was supported by Grant-in-Aid for Scientific Research (C) from the Japan Society for Promotion of Science 20590166 (to T.U.).

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