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Flavour Improvement of Water Solutions Comprising Bitter Amino Acids

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Abstract

The aim of this project was to improve taste of a liquid food product, comprising extremely bitter and unpalatable amino acids and one unrevealed ingredient. The amino acids were L-leucine, L-isoleucine, L-valine, L-threonine and L-lysine monohydrochloride. Experimental design was set up as a screening of water solutions comprising these ingredients (called *BCAA solutions*) and added ingredients with potential to improve flavour. BCAA solution with ingredients that were potential bitterness suppressors were evaluated in flavour, bitterness and/or palatability. Solutions of separate amino acids were also studied. Sensory panels comprised untrained assessors with varying capability of detecting bitterness. Palatability and bitterness were rated on hedonic scales in sensory analysis. Sensitivity to bitterness varies greatly between individuals, which together with varying preferences of bitterness and flavours caused significantly differing answers. This resulted in difficulties to obtain significant results using Tukey's pair-wise comparison. A decreased content of leucine in the BCAA solution was indicated favourable, as well as replacing lysine monohydrochloride with lysine. Ingredients that indicated moderate bitterness reduction were aspartic acid and glutamic acid, both giving sour solutions but the latter being regarded as unpalatable. Ingredients indicated to reduce bitterness a little were carbonic acid, isomaltulose and some flavours. Opinions of bitterness reducing effect of flavours differed greatly, perhaps because of individual preferences or differences in bitterness sensitivity. The choice of water, more specifically the composition of minerals and salts, might considerably affect flavour qualities and bitterness of BCAA solution. The amino acid concentrations in the BCAA solution were probably too high for successful bitterness reduction using added ingredients in concentrations that would not alter flavour characteristics in any other undesired way. A promising alternative, that was not studied, would be to modify the branched chain amino acids (BCAAs) Leu, Ile and Val by acetylation of the N-terminal.

Key words: Taste masking, bitterness suppression, reduction, branched-chain amino acids, BCAA, off-taste, acidic amino acids, designed peptides, synthesized peptides, sensory analysis

Sammanfattning

Projektets syfte var att förbättra smaken på en flytande livsmedelsprodukt innehållande extremt beska och osmakliga aminosyror samt en hemlig ingrediens. Aminosyrorna var L-leucin, L-isoleucin, L-valin, L-treonin och L-lysin monohydroklorid. Experimenten var en screening av ingredienserna i vattenlösning (kallade *BCAA-lösningar*) tillsammans med ingredienser med potential att förbättra smak. BCAA-lösningar med tillsatta ingredienser som bedömdes ha potential att minska beskan utvärderades i smak, beska och/eller smaklighet. Även lösningar av de separata aminosyrorna studerades. Sensorikpanelerna bestod av otränade deltagare med varierande förmåga att känna besk smak. I sensorisk analys bedömdes smaklighet och beska på hedonistiska skalor. Individuella skillnader i känslighet för beska är stora, vilket tillsammans med skilda preferenser för beska och smak gav signifikanta skillnader i deltagarnas bedömningar. Detta gav svårigheter att finna signifikanta resultat i analys med Tukey's parvis jämförelse. En minskad koncentration av leucin i BCAA-lösningen indikerades lovande, liksom att ersätta lysin monohydroklorid med lysin. Resultaten indikerade att asparaginsyra och glutaminsyra minskade beskan måttligt, men båda gav sura lösningar där glutaminsyra även ansågs osmaklig. Kolsyra, isomaltulos och vissa smaker indikerades att sänka beskan lite. Utlåtanden om den besksänkande effekten av olika smaker varierade i hög grad, kanske på grund av individuella preferenser eller skillnader i känslighet för beska. Sammansättningen av mineraler och salter i det vatten som ingredienserna löses i har troligen stor påverkan på BCAA-lösningens smak och beska, vilket innebär att valet av vatten är viktigt. Koncentrationen av aminosyror i BCAA-lösningen var troligen för hög för att kunna sänka beskan genom tillsatser i koncentrationer som inte ger oönskad smak. En lovande alternativ metod, som inte studerades, kan vara att modifiera de grenade aminosyrorna Leu, Ile och Val genom att acetylera N-terminalen.

Contents

1. Introduction

Consumption of healthy food and beverages is increasing, which motivates the industry to find a solution to the problem of removing bitterness in these products. Many healthy food components, such as polyphenols in chocolate and phytonutrients in nutritional products, are bitter (McGregor 2004). Furthermore, bitterness of many oral pharmaceuticals such as nutrition for patients with liver failure results in poor compliance to treatments (Miyanaga *et al*. 2004). In order for a commercial health food to succeed, it needs not only to have acceptable flavour, texture and appearance, but also an ingredient list comprising natural ingredients and a minimum of additives.

The ingredients in the liquid food product being developed by Aventure AB are extremely bitter and unpalatable. The composition comprises the amino acids L-leucine (Leu), L-isoleucine (Ile), L-valine (Val), L-threonine (Thr) and L-lysine (Lys) in the form of L-lysine monohydrochloride (Lys HCL) as well as one secret ingredient. Leu, Ile and Val are extremely bitter branched-chain amino acids (BCAAs). The undesirable flavour characteristics of the ingredients need to be masked in order to result in a palatable commercial product. This project focused on the improvement of flavour and reduction of bitter taste in water solutions containing the ingredients mentioned.

All amino acids in this report are L-enantiomers.

2. Objectives

The objective was to reduce bitterness of a water solution comprising three or five amino acids and one other ingredient (IngredientW, IngW). IngW will remain unidentified in this report. The composition in itself is extremely bitter and unpalatable.

Solutions comprising Leu, Ile, Val and IngW or Leu, Ile, Val, Thr, Lys HCl and IngW were to be studied and improved. Concentrations of the amino acids in these solutions were 53.4 mM Leu, 21.37 mM Ile, 23.9 mM Val, 49.4 mM Thr and 26.8 mM Lys.

The objectives were to be achieved by literature studies, by contacting distributors for consultations, by ordering ingredients and by sensory analyses using an untrained panel.

3. Literature review

3.1. The Sense of Taste

Many functional food components and active substances have aversive taste qualities. Very few food or beverage products with extremely bitter taste are appreciated. For example coffee, grapefruit, red wine and tea are commonly appreciated bitter foods (Ley 2008).

Flavour is the combined impression of taste and aroma, see figure 1. Taste, gustation, includes impressions of sweet, salty, sour, bitter and umami (delicious or savoury), which are perceived by stimuli of taste buds (clusters of taste cells) on different areas of the tongue. Aroma, or odour, is perceived when volatile molecules, odorants, stimulate the olfactory epithelium via the retronasal (passing through the back of the throat) or via the orthonasal (smelling through the nose) (Roudot-Algaron 1996; Hummel 2008; Pszczola 2004).

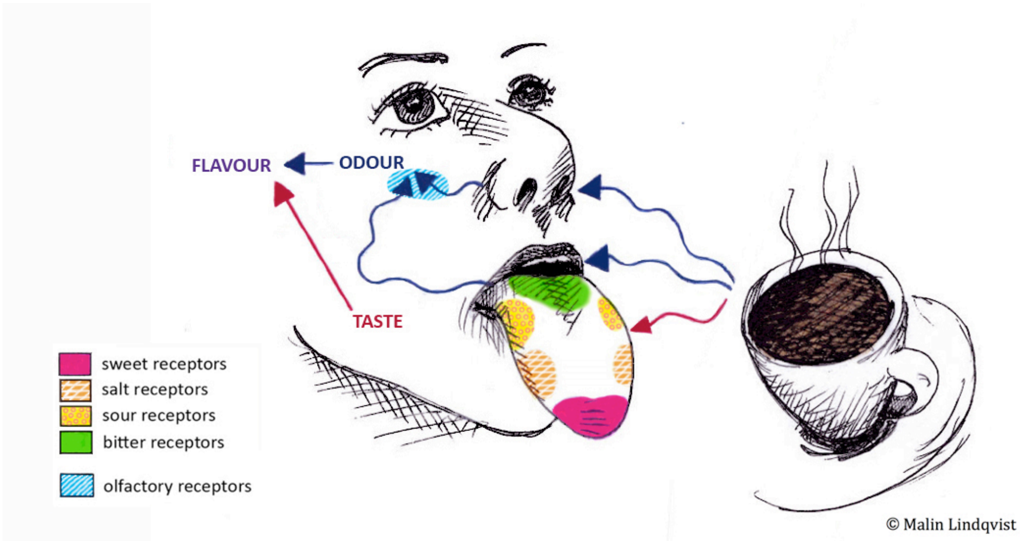

Figure 1. Senses of taste and aroma, together perceived as flavour. Areas with receptors for different tastes as well as the area with olfactory receptors are coloured for identification (Roudot-Algaron 1996; Hummel 2008).

Recognition of these basic tastes allows us to determine the quality of a foodstuff, which a long time ago was vital information for survival. Sour and bitter tastes characteristically generate warning signals for ingesting harmful substances. Sweet, salty or umami, on the other hand, typically evoke associations of nutritious food (McGregor 2004).

3.2. Perception of Bitterness

Some persons, so-called tasters, are sensitive to certain bitter compounds while others, so-called non-tasters, are more or less insensitive. The ability of perceiving bitterness and sweetness differ, for reasons that are not entirely clear. Probable reasons include a combination of experience, learning, gender, age and perceptual differences arising from genetic differences (Reed *et al*. 2006; Montmayeur 2002; Binello *et al*. 2004). A genetic reason to these differences in bitterness sensitivity might be that both tasters and non-tasters have been important in evolution, as tasters are more prone to avoid poisonous or deteriorated foods whereas non-tasters are more likely to choose a varied and healthy diet (Reed *et al*. 2006).

Sensitivity to bitterness has been suggested to be a factor in increased risk of diet related diseases such as heart disease, obesity and cancer. The reason would be that many healthy fruits and vegetables are bitter. However, consistent associations have not been found in studies (Reed *et al*. 2006).

The mechanism of bitterness perception starts when a bitter tastant binds to the specialized receptor proteins (G protein coupled receptors, GPCRs) located in cell membranes of the taste bud cell clusters. The GPCRs activate the transduction proteins (G proteins), which in turn activate effector enzymes within the cell. These modulate the internal cell concentration of second messenger molecules. As the concentration of second messengers changes, various ion channels open within the cell and on the cell surface, leading to depolarization of the cell. This leads to release of neurotransmitters into the synaptic cleft between the taste receptor cell and a neighbouring neuron, which depolarizes. Then an electric signal is transmitted to the central nervous system, where the signal is interpreted as bitterness. This mechanism is the same for perception of sweet and umami tastants (McGregor 2004).

Several bitter taste receptors are selective to specific substances or structurally similar compounds. There are also broad bitter taste receptors, which respond to a range of structurally different bitter compounds (Behrens *et al.* 2004). Bitter taste stimuli are transduced by around 25 different bitter receptor genes, known as T2Rs. The G protein gustducin has been found to play a key part in transduction of bitterness, by interacting with the T2Rs (Sainz *et al.* 2007). Many different models describing the mechanism of bitterness perception have been proposed, which all assume existence of a particular arrangement of certain types of chemical groups within the bitter molecule (Roy 1992). One suggestion was that bitter compounds comprise a polar function that probably affects taste quality and a hydrophobic group that probably affects taste intensity (Asao *et al.* 1987).

3.2.2. Taste Interactions

Interactions of all types of bitter compounds and different tastants cannot be expected to be similar, as bitter compounds not only demonstrate different bitterness qualities, but also act through multiple transductive mechanisms or involve transductive pathways directly by suppression and enhancement. Moreover, effects of some tastant interactions will be mechanism specific (Breslin 1996). Sweet perception and umami perception involve taste receptors as does bitter perception, while sourness and saltiness involve ion channels (Hofmann *et al.* 2004).

Depending on type of food stimuli, concentrations and experimental methods, bitter compounds and acids seem to either enhance or suppress each other. Subthreshold concentrations (below those that generate a taste sensation) and suprathreshold concentrations (that generate a taste sensation) of tastants showed varying effects on taste sensations (Breslin 1996). For example, Mukai *et al.* (2007) concluded that mixtures of sour and bitter compounds in moderate concentrations caused increased sourness and decreased bitterness, while high concentrations caused reduced sourness and variable effects on bitterness intensity and low concentrations caused enhancement of both tastes (Mukai *et al.* 2007).

Mixtures of bitter and sweet compounds in moderate or high concentrations caused suppression of both tastes. Mixtures of the same tastants in low concentrations caused variable effects of suppression or enhancement (Mukai *et al.* 2007).

Among a broad range of bitter compounds (not including BCAAs), sodium chloride was shown to suppress bitterness to a varying degree in almost all cases, while saltiness was not always suppressed. Non-sodium chloride salts, with exception of lithium chloride, were shown not to reduce bitterness to a noteworthy degree in any studied compound (Breslin 1996; Frank *et al.* 1993; Stillman *et al.* 1993).

Interactions between bitter stimuli and other tastants are thus very complicated and cannot be foreseen unless molecular mechanisms and interactions are known (Breslin 1996).

3.3. Bitter Amino Acids

Branched-chain amino acids (BCAAs), such as Leu, Ile and Val, are extremely bitter in taste and have unpleasant odours and flavours (Mukai *et al.* 2007). Other examples of bitter amino acids are phenylalanine, tryptophan and tyrosine. Asao *et al.* noted that proline, cysteine and serine lost their bitterness when in solution, compared to crystal form. D-enantiomers of some bitter L-amino acids are sweet (Roudot-Algaron 1996; Asao *et al.* 1987). Taste and taste thresholds for a selection of amino acids, which were studied in the present project, are presented in table 1.

Table 1. Taste of a selection of amino acids and taste thresholds in solutions (Roudot-Algaron 1996)

Amino acid	Taste profile (Roudot-Algaron 1996)	Taste thresholds (mg/mL) (Kato *et al.* 1989[*])
Valine	Flat to bitter, slightly sweet	0.4 (bitter)
Leucine	Flat to bitter	1.9 (bitter)
Isoleucine	Flat to bitter	0.9 (bitter)
Threonine	Flat to sweet, may be bitter, sour or fatty	2.6 (sweet)
Aspartic acid	Flat, sour, slightly bitter	0.03 (sour); 1 (umami)
Glutamic acid	Particular, may be meaty, salt, bitter	0.05 (sour); 0.3 (umami)
Lysine	Flat, complex, mineral	0.5 (sweet and bitter)
Lysine monohydrochloride	Bitter, complex, salt, sweet	–

3.4. Reducing Bitter Taste

The two traditional methods of masking bitter taste are to physically prevent the bitter molecule from coming into contact with the taste buds or to confuse the brain with additives such as sodium ions, sweeteners, flavours or salts of organic acids (McGregor 2004; Binello *et al*. 2004). Methods of physical prevention of contact by for example encapsulation, emulsification or incorporation into a structure can be expensive and technically challenging. Large amounts, which would be required to confuse the brain, of for instance sodium salt or sugar are associated with health issues such as hypertension, diabetes and obesity (McGregor 2004).

Other approaches include use of strong flavours or tastants, congruent flavours such as grapefruit, complexing agents or scavengers or to simply remove the bitter tastant. The latter is not always suitable, as the effect of the bitter substance disappears (McGregor 2004; Ley 2008).

A hope among scientists is to find a universal bitterness inhibitor, which would ideally block the bitter taste receptors and prevent nerve impulses from communicating the bitter signal. Such a substance would be useful in food and pharmaceuticals and would enable addition of higher amounts of bitter functional ingredients (Roy 1992; McGregor 2004).

The molecular era of taste, which according to McGregor (2004) started in the early 1990's when the taste receptor protein gustducin was discovered, opened for new approaches to study taste modification and taste interactions. By identifying molecular bitterness reducing compounds, interfering with the transduction mechanism of bitterness, it would be possible to prevent the taste receptor cells from being activated. Thereby, no nerve impulse producing the sensation of bitterness would be generated (McGregor 2004; Ley 2008; Ming *et al*. 1999).

Despite efforts to find bitter taste blockers, only a few with broad activity are known, none of which with activity against all known bitter compounds (Binello *et al*. 2004). To produce a palatable food or beverage containing bitter compounds, a combination of different technologies such as encapsulation, masking molecules and/or using strong or congruent

[*] Kato, H., Rhue, M.R., Nishimura, T. 1989. Role of free amino acids and peptides in food taste. *Flavor chemistry; trends and development,* chap. 13:159-174, published in Rodout-Algaron 1996.

flavours or tastants most often needs to be used (Ley 2008).

Another challenge in bitterness reduction is the complexity of mixed sensations, for example that a substance might not only be perceived as bitter but also as astringent or sour (Ley 2008).

3.4.1. Examples of Bitterness Reduction

Neodesmin and aryl urea sulfonic acids are examples of patented tasteless inhibitors of bitter and sweet (Roy *et al.* 1991), which are molecules that correspond to a model predicting bitterness inhibitors suggested by Roy (1992). Tasteless molecules with many hydrophobic regions, such as peptides of taurine or aminomethane sulfonic acid, were suggested as starting point in the search for a potent and perhaps more universal bitterness inhibitor (Roy 1992).

Another patented bitter blocker found using molecular knowledge is adenosine 5'-monophosphate (AMP), which blocks activation of gustducin and thereby prevents stimulation of bitter nerve impulses. The substance has GRAS status (Generally Recognized as Safe by the U.S. Food and Drug Administration FDA), is being evaluated by several food and beverage companies and has been shown to reduce bitterness in a number of applications, including beer and diet carbonated soft drinks (McGregor 2004; Ming *et al.* 1999).

Bitter aftertaste in low-alcohol brews can be completely eliminated by addition of the antioxidant ascorbic acid during the production process, described in a patent by Schur and Sauer (1990).

Phosphatidic acid (PA) has hydrophobic character and is known to reduce bitterness (Nakamura *et al.* 2002).

3.5. Reducing Bitterness of Amino Acids

3.5.1. Addition of Flavour or Citric Acid

Palatability of amino acid based pharmaceutical products was significantly increased by addition of flavour. Especially Fruit flavour was successful in reducing bitterness. Also Apple and Pineapple flavours were very effective in suppressing bitterness and increasing overall palatability. An inverse relationship between bitterness intensity and overall palatability score was found, with and without added flavour (Mukai *et al.* 2004; Miyanaga *et al.* 2004).

Mukai *et al.* (2007) studied the bitterness inhibitory effect on BCAA solutions of the tasteless aromas Green tea, Coffee, Vanilla, Apple and Strawberry. Strawberry was most effective in decreasing bitterness. Also Vanilla and Apple decreased bitterness significantly. A gustatory test showed that Strawberry aroma gave an increased sensation of sweetness and sourness, while no bitterness or saltiness was noted. Apple aroma gave increased sensation in sourness and sweetness together with a small sensation of bitterness, with no saltiness. These results indicate that aroma indeed can suppress bitterness intensity of BCAA solutions also in the absence of sweetener. Generated sensations of sourness or sweetness seemed essential for effective bitterness suppression – aromas evoking a taste of sweetness successfully suppressed bitterness and the evoked sourness contributed to the effect. Mukai *et al.* added that the magnitude of bitterness suppressing effect depends on the choice of aroma and of individual preferences (Mukai *et al.* 2007).

The flavours with best bitterness reducing effect and palatability in a BCAA solution contained citric acid in higher concentrations compared to the flavours that were less effective and less palatable. Apple, Pineapple and Fruit flavour were more effective in bitterness reduction, and contained 3.44 to 4.42 mM citric acid. Green tea and Coffee flavours, less effective in bitterness reduction, contained 0.73 mM citric acid or less. A weak concentration of malic acid was also present in Apple flavour (Miyanaga *et al.* 2004; Mukai *et al.* 2004).

A study of a similar BCAA solution (77.65 mM Leu, 73.28 mM Ile, 68.37 mM Val, 1.80 mM tryptophan) with added citric acid in six concentrations from 0.73 to 7.81 mM showed that citric acid significantly reduced bitterness. Addition of 7.81 mM citric acid almost eliminated bitterness. These results thus suggest that the main organic acid in the flavours – citric acid – was a major factor in reducing bitterness and improving overall palatability (Miyanaga *et al.* 2004; Mukai *et al.* 2004).

Sweet and sour compounds in added flavours were concluded responsible for the bitterness reducing effect when adding flavour to a BCAA solution (Miyanaga *et al.* 2004). Sweeteners or organic acids present in the added flavours, such as sugar, citric acid, phtalic acid or fumaric acid, were thought to cause bitterness suppression. Overall palatability was strongly positively correlated to sweetness and sourness but inversely correlated to saltiness and bitterness (Mukai *et al.* 2004; Mukai *et al.* 2007).

3.5.2. Addition of Aspartic Acid, Glutamic Acid or Taurine

Aspartic acid (Asp) and glutamic acid (Glu) are acidic amino acids, producing sour solutions. Taurine (Tau) is not an acidic amino acid and does not produce sourness, although has a sulfonyl group with acidic effect on solutions (Tamura *et al.* 1990). Asp, Glu and Tau were indicated effective in reducing bitterness of solutions comprising bitter amino acids in low concentrations. 200 mM and 300 mM Asp completely removed bitterness of a bitter 300 mM Val solution. Also 200 mM and 300 mM Glu as well as 300 mM Tau completely removed bitterness of the Val solution. 67 mM Asp, 67 mM Glu or 200 mM Tau reduced bitterness of Val effectively (Tamura *et al.* 1990).

3.5.3. Addition of Arginine or Ornithine

Ornithine hydrochloride (Orn HCl) was described to reduce bitterness of Leu, Ile and Val in solution (Tokuyama *et al.* 2006; Kawabe *et al.* 2006). Orn HCl was found to reduce bitterness better than Arginine (Arg). The bitterness of a solution of 12.1 mM Leu, 28.1 mM Ile and 60.7 mM Val was graded 1.33 on a scale from 0 to 2. This bitterness was reduced to 0.50 by the addition of 100 mM Arg and to 0.17 by the addition of 100 mM Orn HCl (Tokuyama *et al.* 2006). Arg was described as bitter in taste with characteristic odour. Ornithine was tasteless according to Kawabe *et al.* (2006), meaning that the product would retain original flavour with less bitterness. However, Asao *et al.* (1987) described Ornithine as sweet.

3.5.4. Modification of Amino Acids or Designed Peptides

The bitter mechanism in peptides was suggested to require a few hydrophobic groups or hydrophobic and basic groups, which should be close to each other in order to produce intense bitterness. Bitterness of bitter amino acids was suggested to derive from hydrophobic side-chains and α-amino groups. Thus, effective reduction of bitterness should be accomplished by blocking one of these. As the N-terminal is one of two bitterness producing features, Tamura *et al.* concluded that the easiest way to reduce bitterness would be to block the N-terminal by modifying amino acid structure (Tamura *et al.* 1990).

Acetylation of hydrophobic amino acids was effective in suppressing bitterness of amino acid solutions of high concentration, as was derivatising with acidic amino acids such as Glu or Asp (Roy 1992; Shinoda *et al*. 1987; Tamura *et al*. 1990). Bitter amino acids with a blocked N-terminal produced sour taste, while sodium salts of the same modified amino acids were salty or umami. The sour dipeptides Glu-Val, Glu-Leu, Glu-Ile, Asp-Val, Asp-Leu and Asp-Ile were difficult to dissolve in water. Sour taste thresholds of the N-terminal acetylated amino acids were 0.47 mM for Ac-Leu, 0.94 mM for Ac-Ile and 0.94 mM for Ac-Val (Tamura *et al*. 1990).

Bitterness of the strongly bitter dipeptide Arg-Pro was completely suppressed by introducing a Gly-Gly residue to both N-terminal and C-terminal. Addition of Gly-Gly only to N-terminal or only to C-terminal did not affect bitterness. Addition of a Gly-Gly residue to the N-terminal of the weakly bitter peptide Val-Val-Val reduced the bitterness by half. This bitterness reducing effect was also shown for other bitter peptides (Shinoda *et al*. 1987).

A small number of designed peptides, some found in nature, each containing one of the bitter amino acids Leu, Ile, Val or Lys have been found acceptable in flavour. These are presented in table 2.

Table 2. Peptides containing one of the bitter and/or unpalatable amino acids, written N-terminal to C-terminal

Peptide	Taste	Taste threshold (mM/L)	Reference
Leu-Gly-Gly	Bitter	75	Ishibashi *et al.* 1987a
Lys-Lys	Flat	-	Ishibashi *et al.* 1988b
Lys-Gly-Asp	None noted	-	Yamasaki and Maekawa 1980
Gly-Val-Gly	Flat	-	Ishibashi *et al.* 1988
Val-Val-Gly	Flat	-	Ishibashi *et al.* 1988
Leu-Asp HCl	Sour	3.0	Ishibashi *et al.* 1987a
Leu-Glu HCl	Sour	1.5	Ishibashi *et al.* 1987a
Glu-Leu HCl	Sour	1.5	Ishibashi *et al.* 1987a

3.6. Mineral Composition of Waters

Subthreshold concentrations, concentrations below those that give taste sensation, of minerals and salts can generate a distinguishable taste by additive effect. Low concentrations of components can also generate suppression or enhancement of different tastes (Breslin 1996). Mineral contents of different waters differ vastly. The mineral contents of waters used in the present project are presented in table 3.

Table 3. Contents of common minerals and salts in different waters (Malmberg homepage 2010; Sydvatten homepage 2010; Carlsberg Sverige homepage 2010; Spendrups homepage 2010)

Water	Sodium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Chloride (mg/L)	Fluoride (mg/L)
Malmberg	4.9	57	4	2.4	7.7	0.24
Lund Municipal	21	46	4.1	2.4	17	less than 0.2
Ramlösa	220	2	0.5	2	20-30	2.7
Loka	150	30	10	90	20	0.1

3.7. Sensory Analysis

3.7.1. Laboratory Acceptance Test

Acceptance tests are beneficial methods for screening product alternatives. A laboratory acceptance test is the most frequently used type of acceptance test. An acceptance test of a product is performed by asking assessors to grade certain characteristics of a sample on a hedonic scale. An example of a nine-point hedonic scale is presented in figure 2 (Stone and Sidel 1993).

The nine-point hedonic scale is generally applicable to measurement of product acceptance–preference. It is also one of the most common sensory analysis methods when measuring preference and acceptance of products. It is a method easy to use and describe, which makes it a useful tool in product development (Stone and Sidel 1993).

The nine-point hedonic scale has been proven remarkably stable and to provide reproducible results on product differences in liking by different groups of respondents. Efforts to modify the hedonic scale, by for example removing the midpoint category or other categories, have generally been unsuccessful (Stone and Sidel 1993).

Code: ____________ Date: ____________

Please circle the term that best reflects your attitude about the product.

Like extremely

Like very much

Like moderately

Like slightly

Neither like nor dislike

Dislike slightly

Dislike moderately

Dislike very much

Dislike extremely

Figure 2. An example of the nine-point hedonic scale, where a term reflecting the assessor's attitude about the product is to be circled (Stone and Sidel 1993).

As acceptance tests use a small number of respondents, it is important that they like the product and that they are capable of using preference rating scales to differentiate products. Employees' preferences and attitudes towards the product should be comparable to other populations. For non-employee consumers, other information such as age and gender is of greater importance. To obtain valid and reliable results, objectivity of respondents is essential. Respondents, especially employees, should probably not be used in more than two or three evaluations per month of the same type of product. Longer periods of participation in sensory analyses would lead to respondents becoming increasingly experienced, which should be avoided. Respondents that are qualified for discrimination tests or descriptive tests should not be used, as their training period would have given them an analytical approach to the evaluation, which would bias the result. For similar reasons, respondents with technical information on the product might be biased. A disadvantage of using employees in laboratory acceptance tests is the familiarity with products (Stone and Sidel 1993).

Stone and Sidel recommended using 25 to 50 responses per sample and at maximum five to six samples per testing occasion. Fewer respondents might give difficulty in obtaining statistically significant differences between samples, but do enable trend identification. The number of products that can be tested in each session depends on for example the amount of time that volunteers or employees can offer, the workload of sample preparation and possible sensory fatigue that products might generate (Stone and Sidel 1993).

3.7.2. Data Analysis

Responses from the nine-point hedonic scale are converted to numerical values, which can be processed to yield means, measures of variance, frequency distributions etcetera. Analysis of variance (AOV, ANOVA) or the t-test can be used to provide information on differences between products. Choice of method depends on the number of products and number of respondents (Stone and Sidel 1993).

ANOVA can be used to determine whether the mean scores among products in a sensory analysis diverge enough to be identified as significantly different at a specified risk level. To identify differing products, a multiple-range test can be used. These tests are not interchangeable and can give different results regarding significance of difference depending on the type of sensory analysis performed. Tukey's test is among the most commonly used multiple-range ANOVA tests in sensory evaluation (Stone and Sidel 1993).

3.7.3. Factors Affecting Outcome of Sensory Analysis

Sample preparation and tasting conditions are important factors with effect on the outcome of sensory analysis (Roudot-Algaron 1996). Instructions can influence assessors to expand or narrow their definitions of a particular sensory attribute, for example sweet taste intensity together with fruitiness or without. The question formulation might bias assessors towards describing subtle differences between stimuli even when these were minimal (Frank *et al.* 1993). Frank *et al.* (1993) noted that grading of sweetness (or bitterness) was highest when only sweet (or bitter) taste was graded, and lowest when total intensity together with sweetness, saltiness, sourness, bitterness, fruitiness and other taste were graded. This result confirms that instructions significantly affect gradings of particular sensory attributes. Also the effect of Strawberry aroma on sweet taste intensity gradings seemed to depend on instructions, while the effect of Lemon aroma did not. Results showed instructions to be of greater importance than "background" sensory stimuli (Frank *et al.* 1993).

Frank *et al.* (1993) published the following hypothesis: "*When an odorant elicits sensations that are perceptually similar to a target taste and when only one rating category is available, subjects will tend to combine the taste and odor dimensions, leading to higher intensity ratings for the target taste. When taste and odor sensations are dissimilar or when appropriate additional attribute scales are provided, odor-induced taste enhancement will not be observed.*" The authors concluded that the number of reference stimuli did not seem to affect grading of bitter taste intensity. A total-intensity rating broken down into categories seemed to restrict the grading of category intensities so that the sum of categories was equal to the total-intensity grading (Frank *et al.* 1993).

4. Experimental

Experimental design was set up as a screening, where promising compositions were studied to evaluate flavour, bitterness and/or palatability. A solution without BCAAs was also studied to get an indication of the affect of experimental design on the accuracy of grading of bitterness and palatability. Studies were divided into *sensory evaluations* and *sensory analyses*. *Sensory analyses* comprised studies of solutions using six to 13 assessors, performed separately using test forms and according to instructions. *Sensory evaluations* comprised studies of solutions using only one to four assessors and/or using verbal comments, including initial sensory evaluations.

Initial sensory evaluations were performed to grasp the general problem of the project and to follow up experiments performed by a colleague at Aventure (unpublished laboratory book no 19, propriety of Aventure AB). The flavour of IngredientW was not studied, as the concentration of this ingredient was predetermined.

Concentrations of Orn HCl, Asp, Glu and Tau to be studied were chosen based on literature. For example, in the approximations of concentrations of Asp, Glu and Tau needed to reduce bitterness in the BCAA solution, literature on a bitter solution of 300 mM Val was used (Tamura *et al*. 1990).

Tukey's pair-wise comparison was used to find significant differences in results because it is a rather conservative method that makes obtaining significant differences relatively difficult.

4.1. Material

4.1.1. Basic Ingredients for BCAA Solution

The amino acids Leu, Ile and Val, Thr and Lys HCl (Fortitech) as well as Lys HCl (Cambridge Commodities Ltd). IngW (provided by Aventure AB).

4.1.2. Waters

The waters Malmberg stilla, Malmberg original, Malmberg citron and Malmberg apelsin (Malmberg Original Waters) as well as Torrhults hälsovatten (Aqua service), Ramlösa original, Ramlösa mango, Ramlösa blåbär, Ramlösa citrus, Loka citron, deionized water and Lund municipal water.

4.1.3. Ingredients to Improve Basic Composition

Ingredients L-ascorbic acid (Sigma-Aldrich), Natural Flavor for Beverages: Resolver (Wild), Orn HCl (Sigma-Aldrich), isomaltulose in the form of Palatinose (Beneo-Palatinit), Asp and Tau (Bröste), 1M sodium hydroxide (NaOH), citric acid (Santa Maria). The flavour concentrates Sparkling Water Feel Good Passion and Acai, Sparkling Water Feel Good Pomegranate Ginseng (AGA). The flavour concentrates Lemon/Lime, Apple+VitC, Orange/Ginger, Pear, Pink Grapefruit and Redberrymix (SodaStream). The flavours Masking flavour (Metarom) and Strawberry flavour (Givaudan). The flavours Pomelo, Naartje, Grapefruit, Scandinavian Berries, Blackcurrant, Lemon Lime, Lemon Perfect, Orange Sweet Perfect, Florida Orange, Strawberry and Sweetness enhancer (*Flavour Company B*[*]).

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[*] The name of the producer can be obtained upon request.

4.1.4. Devices and Other Material

pH-meter (PHM210 Standard pH-meter, MeterLab), pH-meter (Mettler Toledo), magnetic stirrer and heating plate (VMS-C7, VWR), SodaStream G100, wafers (Smörgåsrån, Göteborgs Kex), 5 cl plastic shot glasses, thermometer and sensory test forms.

4.2. Preparation of Solutions

BCAA Solutions: Amino acid solutions (BCAA solutions) were prepared in compositions according to table 4. Amino acids were dissolved using magnetic stirrer. Malmberg stilla and Malmberg original were used to dilute all BCAA solutions except when otherwise mentioned. Magnetic stirrer was used to remove carbonic acid in certain solutions where carbonated water had been used to prepare solutions.

Table 4. Compositions of BCAA solutions per 1000 ml water solution. Lys HCl from Fortitech were used in all solutions except in 5aa'-lys and 5aa-lys, where Lys HCl from Cambridge Commodities was used

Ingredient	3aa''	3aa	5aa	5aa'	5aa''	5aa'-lys	5aa-lys
Leu	7.076 g	7.076 g	7.076 g	5.606 g	5.606 g	5.606 g	5.606 g
Ile	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g
Val	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g	2.803 g
Thr	-	-	5.886 g	5.886 g	5.886 g	5.886 g	5.886 g
Lys HCl	-	-	4.869 g	4.869 g	4.869 g	3.924 g	3.924 g
IngW	-	6.06 ml	6.06 ml	6.06 ml	-	6.06 ml	6.06 ml

Waters with and without 3aa'': 200 ml solutions of 3aa'' were prepared according to table 4 and diluted using Torrhults hälsovatten (3aaTH), Ramlösa original (3aaRO) or Loka citron (3aaLC) using magnetic stirrer. Torrhults hälsovatten (TH), Ramlösa original (RO) and Loka citron (LC) were stirred in order to decrease carbonic acid to be comparable to the uncarbonated solutions.

3aa and 5aa in Lund Municipal Water: The municipal water tap was left open for fifteen minutes to obtain pure water. 1320 ml 3aa solution was prepared with municipal water according to table 4 (3aa municipal water solution). Thr and Lys HCl in amounts according to 5aa solution in table 4 were dissolved in 660 ml of 3aa municipal water solution (5aa municipal water solution). IngW was added to the solutions in concentrations according to table 4.

Table 5. Preparation of dilution series of ascorbic acid in 3aa solution, diluted to 20 ml

Solution	3aaAsk (ml)	Ascorbic acid concentration (g/L)
3aaAsk0.748	1.33	0.748
3aaAsk0.940	1.67	0.940
3aaAsk1.125	2.00	1.125
3aaAsk1.311	2.33	1.311
3aaAsk1.497	2.66	1.497

3aa Solutions with Ascorbic Acid: 7.20 g ascorbic acid was dissolved in 3aa municipal water solution and diluted to 160 ml (3aaAskAcid). After one day's storage, 6.67 ml of 3aaAskAcid was diluted to 20 ml using 3aa municipal water solution (3aaAsk). Solutions were prepared according to table 5 using 3aaAsk, each solution diluted to 20 ml using 3aa municipal water solution.

3aa and 5aa Solutions with Resolver: 71 mg Resolver was dissolved in 40 ml 3aa municipal water solution (3aaRes). 71 mg Resolver was dissolved in 40 ml 5aa municipal water solution (5aaRes). After one day's storage, solutions were prepared according to table 6 using 3aaRes or 5aaRes. Each solution diluted to 20 ml using 3aa municipal water solution or 5aa municipal water solution.

Table 6. Preparation of dilution series of Resolver in 3aa or 5aa municipal water solutions

Solution	3aaRes (ml)	5aaRes (ml)	Resolver concentration (g/L)
3aaRes0.2	2.25	-	0.200
3aaRes0.3	3.38	-	0.300
3aaRes0.4	4.51	-	0.400
3aaRes0.5	5.63	-	0.500
5aaRes0.2	-	2.25	0.200
5aaRes0.3	-	3.38	0.300
5aaRes0.4	-	4.51	0.400
5aaRes0.5	-	5.63	0.500

5aa Solutions with Isomaltulose: Solutions with isomaltulose were prepared according to table 7, each solution diluted to 30 ml using 5aa municipal water solution that had been stored for eight days. 0.100 g Palatinose was prepared with municipal water that had been stored for eight days, and was diluted to 30 ml (wIM3.33).

Table 7. Preparation of 5aa solutions with isomaltulose, diluted to 30 ml using 5aa solution

Solution	Palatinose (g)	Palatinose concentration (g/L)
5aaIM1.73	0.052	1.73
5aaIM3.43	0.103	3.43
5aaIM20	0.600	20
5aaIM49.87	1.496	49.87

5aa Solutions with Ornithine: 100 ml 5aa-a solution was prepared according to table 4. 0.0652 g ornithine HCl was dissolved in 5aa-a solution using magnetic stirrer and diluted to 30 ml (5aaOrn).

5aa'-, Lemon Flavoured 5aa'- and Carbonated Lemon Flavoured 5aa' Solutions: 500 ml 5aa' solution was prepared according to table 4 (Mb5aa'). 500 ml carbonated lemon flavoured 5aa' (MbLem5aa'Carb) was prepared with Malmberg citron according to table 4 and carbonated using SodaStream. 250 ml of carbonated lemon flavoured 5aa' was shaken to remove carbonic acid (MbLem5aa').

Citrus or Berry Flavoured 5aa' Solutions: 1000 ml Mb5aa' were prepared according to 5aa' in table 4. 120 μl of the following flavours were diluted to 100 ml using Mb5aa': Pomelo, Naartje, Grapefruit, Scandinavian Berries, Blackcurrant, Lemon Lime, Lemon Perfect and Orange Sweet Perfect. 90 μl of the following flavours were diluted to 75 ml using Mb5aa': Florida Orange and Strawberry. 45 μ Sweetness enhancer was diluted to 75 ml using Mb5aa'.

Separate Amino Acids: Solutions of the separate amino acids Leu, Ile, Val, Thr and Lys HCl were prepared according to table 8.

Table 8. Composition of each amino acid solution, total volumes 400 ml

Leu	Ile	Val	Thr	Lys HCl
28.304 g	11.212 g	11.212 g	7.772 g	19.584 g

Different Compositions of BCAA Solutions: 5aa solution was prepared according to table 4, frozen for some days and thawed for sensory analysis (Mb5aaFrozen). The solutions Mb5aa' and Mb5aa-lys of the different compositions 5aa', 5aa-lys and reference solution 5aa were prepared according to table 4.

Carbonated 5aa'-lys Flavoured with Lemon, Orange, Apple and Lemon/lime: The solution AppleMb5aa'-lysCarb was prepared by diluting 0.15 ml Apple+VitC to 550 ml using 5aa'-lys solution prepared according to table 4. The solution LemonLimeMb5aa'-lysCarb was prepared by diluting 0.65 ml Lemon/lime to 550 ml using 5aa'-lys solution. Mb5aa'-lysLemCarb and Mb5aa'-lysOraCarb were prepared by diluting basic ingredients to 550 ml using Malmberg citron and Malmberg apelsin, respectively. 5aa'-lys solution for reference and dilution solutions were prepared according to table 4. 500 ml of all solutions were carbonated using SodaStream and remixed with remaining 50 ml.

Carbonated 5aa'' Flavoured with Pomegranate/ginseng and Passion/acai: 500 ml 5aa' was prepared as reference solution according to table 4. MbLem5aa''Carb was prepared according to 5aa'' composition in table 4 and diluted to 1100 ml using Malmberg citron. PGLem5aa''Carb was prepared by diluting 1.8 ml of pomegranate/ginseng to 540 ml using MbLem5aa''. PALem5aa''Carb was prepared by diluting 1.8 ml of passion/acai to 540 ml using MbLem5aa''. The solutions were carbonated using SodaStream. 5aa''PGLemCarb was prepared by cautiously mixing 110 ml of PGLem5aa''Carb with 130 ml of Mb5aa''Carb solution. 5aa''PALemCarb was prepared by cautiously mixing 110 ml of PALem5aa''Carb with 130 ml of Mb5aa''Carb solution. Solutions were stored in refrigerator over night.

Table 9. Composition of Strawberry flavoured 5aa' with aspartic acid or citric acid, total volumes 400 ml

Ingredient	Mb5aa'StrawCit	Mb5aa'StrawAsp	Mb5aa'StrawMaskAsp	Mb5aa'StrawMaskCit
5aaS4	12 ml	12 ml	12 ml	12 ml
5aaMF1	-	-	8 μl	8 μl
Aspartic acid	-	1.3317 g	1.3317 g	-
Citric acid	0.664 g	-	-	0.665 g

Strawberry Flavoured 5aa' with Asp or Citric Acid: 5aaS4 was prepared by diluting Strawberry flavour (Givaudan) with 5aa solution. 5aaMF1 was prepared by diluting Masking flavour with 5aa solution. Mb5aa'StrawCit, Mb5aa'StrawAsp, Mb5aa'StrawMaskAsp and

Mb5aa'StrawMaskCit were prepared according to table 9 and diluted using 5aa' solution. 5aa' solution for reference and dilution solution was prepared according to table 4. Aspartic acid was dissolved using magnetic stirrer and heating plate at about 55°C for approximately 10 minutes.

5aa' with Tau or Asp and Pomelo Flavoured Solutions: 2000 ml Mb5aa' was prepared according to 5aa' solution in table 4. 1.907 g Tau was dissolved in 500 ml of Mb5aa' solution and some carbonic acid remained after preparation (Mb5aa'Tau). 1.912 g Asp was dissolved in 500 ml of Mb5aa' solution (Mb5aa'AsparticAcid), and 13 ml NaOH was stepwise added (Mb5aa'AspNa) in order to achieve a pH comparable to the other solutions. Solutions were stored in refrigerator for three days. Mb5aa'Pom was prepared by adding 1.50 ml Pomelo flavour to 750 ml of Mb5aa'. Mb5aa'TauPom was prepared by adding 0.80 ml Pomelo flavour to 400 ml of Mb5aa'Tau. Mb5aa'AspNaPom was prepared by adding 0.82 ml Pomelo flavour to 410 ml of Mb5aa'AspNa. RamOrPom was prepared by stirring 410 ml Ramlösa original energetically using magnetic stirrer to remove carbonic acid and adding 0.82 ml Pomelo flavour. Aspartic acid was dissolved using magnetic stirrer and heating plate at about 55°C for approximately 10 minutes. Taurine was dissolved using magnetic stirrer.

Pomelo Flavoured 5aa' Mineral Water: 1.50 ml of Pomelo was added to freshly prepared 750 ml of Mb5aa', prepared according to table 4 (Mb5aa'Pom).

4.3. Sensory Evaluations

Waters with and without 3aa'': One assessor evaluated bitterness in the mineral waters Torrhults hälsokälla, Ramlösa original and Loka citron with and without added 3aa'' at 17°C. Carbonic acid had been reduced but was still present. Bitterness was compared and graded on a scale of 0–5. Freshly prepared solutions were studied.

Sensory Comparison of Mineral Waters: Flavours of carbonated Malmberg original and Ramlösa original were compared by two assessors at refrigerated temperature. The same assessors also compared flavours of carbonated and citrus or lemon flavoured Ramlösa citrus, Loka citron and Malmberg citron.

3aa Solutions with Ascorbic Acid: Bitterness and general flavour of 3aa municipal water solution with ascorbic acid in different concentrations were evaluated by one assessor at 17-18°C and repeated once. Samples were coded and served in randomized order together with Resolver samples, using 5aa and 3aa municipal water solutions as references. Bitterness was evaluated and graded on a scale of 0–5. Solutions were studied after over night storage in refrigerator.

3aa and 5aa Solutions with Resolver: Bitterness and general flavour of 3aa and 5aa municipal water solutions with Resolver in different concentrations were evaluated by one assessor at 17-18°C and repeated once. Samples were coded and served in randomized order together with ascorbic acid samples, using 5aa and 3aa municipal water solutions as references. Bitterness was evaluated and graded on a scale of 0–5. Solutions were studied after over night storage in refrigerator.

5aa Solutions with Isomaltulose: A straightforward evaluation of bitterness and general flavour of different concentrations of isomaltulose in 5aa solution was performed by one assessor at 17°C. Samples were coded and served in randomized order, using 5aa solution as reference. Bitterness was evaluated and graded on a scale of 0–5. Solutions were studied after over night storage in refrigerator.

5aa Solutions with Ornithine: A straightforward evaluation of bitterness and general flavour of coded 5aaOrn and 5aa solution was performed by four assessors at refrigerated temperature, using 5aa solution as bitter reference. Bitterness was graded on a scale of 0–5. Freshly prepared solutions were studied.

5aa'-, Lemon Flavoured 5aa'- and Carbonated Lemon Flavoured 5aa' Solutions: One assessor from *Flavour Company B* tasted and described flavour of each solution at refrigerated temperature, after over night storage in refrigerator.

Citrus or Berry Flavoured 5aa' Solutions: An untrained panel of volunteers, both females and males, mixed ages from under 25 up to 55 years, participated. Six volunteers evaluated Pomelo, Naartje, Grapefruit and Scandinavian Berries, Blackcurrant, Lemon Lime, Lemon Perfect and Orange Sweet Perfect. Four volunteers evaluated Florida Orange, Strawberry and Sweetness enhancer. The samples were divided in three groups and evaluated at three different occasions for each participant. Pomelo, Naartje, Grapefruit and Scandinavian Berries were evaluated together. Blackcurrant, Lemon Lime, Lemon Perfect and Orange Sweet Perfect were evaluated together. Florida Orange, Strawberry and Sweetness enhancer were evaluated together. Approximately 20 ml of the freshly prepared flavoured 5aa solutions were served in a randomized order at refrigerated temperature, in coded shot glasses. Torrhults hälsokälla was used as rinsing water and wafers were an optional choice for neutralizing the taste buds in the mouth. Sensory qualities were described verbally and notes were taken by the test executor. One participant tasted a mix of the Lemon Perfect sample and Lemon Lime sample as well as a stronger concentration of Sweetness enhancer. Three participants tasted a mix of the Strawberry sample and the Florida Orange sample. Two assessors compared flavour qualities of Sweetness enhancer sample and 5aa solution.

5aa' with Aspartic Acid or Taurine: Four participants, two female and two male, evaluated flavour and bitterness in coded Mb5aa', Mb5aa'Tau and Mb5aa'Asp served in randomized order at refrigerated temperature. Freshly prepared solutions were studied.

Pomelo Flavoured 5aa' Mineral Water: An untrained panel of eight volunteers, four female and four male, mixed ages from under 25 up to over 55 years, participated. The test comprised one unlabelled sample of 40 ml Mb5aa'Pom, presented at approximately 17°C. The solution was studied after one day's storage in refrigerator. Information given was that this was a lightly flavoured mineral water. Questions asked were: "*What do you think of the flavour compared to other mineral waters, on a scale of 0-10, where 0 means Dislike very much, 5 means Neither like nor dislike and 10 means Like very much?*", "*Would you buy this mineral water?*" and on the back of the form the following question was asked: "*Would you buy this mineral water if it was very healthy and prevented certain diseases?*" The water Torrhults hälsokälla was served to rinse and neutralize the mouth before tasting the sample.

4.4. Sensory Analyses - Laboratory Acceptance Tests

4.4.1. Method Details

Subjects: Six to eleven untrained participants, both female and male, mixed ages from under 25 up to over 55 years, participated. Some assessors in the sensory panel were employees with knowledge of project details and aim. Others were volunteers, found by advertisement, uninformed about project details.

Performance: Assessors were seated far apart at separate tables, facing the wall. The tests consisted of three to five coded 30-40 ml samples, presented in a randomized order. Palatability and bitterness were rated on hedonic scales and the possibility to comment was given. The water Torrhults hälsokälla was served for rinsing between samples. Wafers were served for neutralizing between samples in all analyses except analysis of separate amino acids and analysis of different compositions of amino acid solutions. Samples were compared to a bitter reference consisting of 5aa solution and rinsing water (Torrhults hälsokälla) as a palatability reference and a not bitter reference (used as defined in section 4.4.2. Study of Solutions). Samples were to be tasted in the predetermined order, swirled around in the whole mouth and kept for a few seconds before swallowing. Samples could be compared to one another.

4.4.2. Study of Solutions

Rinsing water was used as a reference for palatability (predetermined at score 50, corresponding to *Neither like nor dislike*) and as reference for bitterness (predetermined at score 0, corresponding to *Not bitter*) in all analyses except analysis of *Pomelo flavoured 5aa' with Tau or Asp*.

Separate Amino Acids: solutions of Leu, Ile, Val, Thr and Lys HCl were studied at refrigerated temperature without use of reference, after over night storage in refrigerator. Bitterness was graded on a 4-point hedonic scale with boxes (*Not bitter; Slightly bitter; Bitter; Very bitter*) and palatability was graded on a 7-point hedonic scale with boxes (*Like very much; Like moderately; Like slightly; Neither like nor dislike; Dislike slightly; Dislike moderately; Dislike very much*).

Different Compositions of BCAA Solutions: solutions of 5aa', 5aa-lys and previously frozen 5aa were studied at 14.5-16.5°C with 5aa solution as reference predetermined at bitterness score 100. Solutions were studied after over night storage in refrigerator. Bitterness was graded on a 4-point linear hedonic scale (*Not bitter; Slightly bitter; Bitter; Very bitter*) and palatability was graded on a 5-point linear hedonic scale with definitions only at end-points and centre-point (*Like very much*; *Neither like nor dislike; Dislike very much*).

Flavoured and Carbonated 5aa'-lys: Mb5aa'-lysLemCarb, Mb5aa'-lysOraCarb, AppleMb5aa'-lysCarb and LemonLimeMb5aa'-lysCarb were studied at 13.5-18°C with carbonated 5aa' solution as reference predetermined at bitterness score 83.3. Solutions were studied after over night storage in refrigerator at occasions spread over three days. Bitterness was graded on a 7-point linear hedonic scale with definitions only at four points (*Not bitter; Slightly bitter; Bitter; Very very bitter*) and palatability was graded on a 9-point linear hedonic scale with definitions only at end-points and centre-point (*Like very much*; *Neither like nor dislike; Dislike very much*).

5aa'' Flavoured with Pomegranate/Ginseng and Passion/Acai: PGLem5aa'', 5aa''PGLem, PALem5aa'' and 5aa''LemPA were studied at refrigerated temperature with 5aa'' solution as reference predetermined at bitterness score 83.3. Solutions were studied after over night storage in refrigerator at occasions spread over two days. Bitterness was graded on a 7-point linear hedonic scale with definitions only at four points (*Not bitter; Slightly bitter; Bitter; Very very bitter*) and palatability was graded on a 9-point linear hedonic scale with definitions only at end-points and centre-point (*Like very much*; *Neither like nor dislike; Dislike very much*).

Strawberry Flavoured 5aa' with Aspartic Acid or Citric Acid: Mb5aa'StrawCit, Mb5aa'StrawAsp, Mb5aa'StrawMaskAsp and Mb5aa'StrawMaskCit were studied at 11-16°C with 5aa' solution as reference predetermined at bitterness score 83.3. Solutions were studied after over night storage in refrigerator at occasions spread over two days. Bitterness and palatability were graded on hedonic linear scales with definitions only at end-points (*Not bitter; Very very bitter* and *Like very much; Dislike very* much).

Pomelo Flavoured 5aa' with Taurine or Aspartic Acid: Freshly prepared solutions of Mb5aa'Pom, Mb5aa'TauPom, Mb5aa'AspPom and RamOrPom were studied at 11-16°C without use of reference. Bitterness was graded on a 5-point hedonic scale with boxes (*Not bitter; Slightly bitter; Bitter; Very bitter; Extremely bitter*) and palatability was graded on a 9-point hedonic scale with boxes (*Like extremely; Like very much; Like moderately; Like slightly; Neither like nor dislike; Dislike slightly; Dislike moderately; Dislike very much; Dislike extremely*).

4.4.3. Analysis of Experimental Data

Significant differences of results were found using Tukey's pair-wise comparison in MiniTab. Significance level was set to 0.05. Means and standard deviations were calculated using Microsoft Excel 2008 for Mac.

4.5. Measurement of pH

pH-meter Mettler Toledo was used for measurement of pH in solutions, unless otherwise stated. When Mettler Toledo could not be calibrated, pH in calibration buffers was measured to assure reliability.

Mineral Waters: After measuring pH in calibration buffers, pH was measured in the carbonated waters Ramlösa original, Ramlösa mango, Ramlösa blåbär, Ramlösa citrus, Loka citron, Malmberg original, Malmberg citron and Malmberg apelsin. The waters were stirred until most of the carbonic acid had been removed, and pH was measured again. pH was measured also in Malmberg stilla for comparison. pH was again measured in calibration buffers.

3aa and 5aa Solutions with Ascorbic Acid or Resolver: pH in 3aaAsk1, 3aaAsk3, 3aaRes2 and 5aaRes3 were measured three days after preparation of 3aaAskAcid, 3aaRes and 5aaRes. pH in 3aa municipal water solution and 5aa municipal water solution were measured one day after preparation. pH was measured in freshly taken deionized water and in municipal water four days after preparation.

5aa Solutions with Isomaltulose: pH in solutions with isomaltulose and the water solution with isomaltulose were measured as well as 5aa municipal water solution after eight days storage.

Separate Amino Acids and Different Compositions of BCAA Solutions: pH was measured in the separate amino acid solutions Leu, Ile, Val, Thr and Lys HCl five days after preparation. pH as well as in Mb5aaFrozen, Mb5aa' and Mb 5aa-lys solutions one day after preparation.

Lemon Flavoured 5aa'- and Carbonated Lemon Flavoured 5aa' Solutions; 5aa'' Flavoured with Pomegranate/Ginseng and Passion/Acai; Flavoured and Carbonated 5aa'-lys: pH in pH buffers were measured. pH in the solutions MbLem5aa' and MbLem5aa'Carb were measured one day after preparation using pH-meter MeterLab and after 14 days of storage using Mettler Toledo. Using the same procedure, pH in the solutions PGLem5aa''Carb and PALem5aa''Carb were measured after 7 days and 19 days of storage. After 19 days of storage, pH in Lem5aa''PGCarb, Lem5aa''PACarb and reference Mb5aa'' were measured. pH in the solutions Mb5aa'-lysLemCarb, Mb5aa'-lysOraCarb, LemonLimeMb5aa'-lysCarb, AppleMb5aa'-lysCarb and the carbonated reference were measured after 10 days using MeterLab and after 22 days of storage using Mettler Toledo.

Strawberry Flavoured 5aa' with Aspartic Acid or Citric Acid: pH of strawberry flavoured 5aa' solutions with aspartic acid or citric acid was measured, as well as pH in calibration buffers.

Pomelo Flavoured 5aa' with Taurine or Aspartic Acid: pH in Mb5aa', Mb5aa'Tau and Mb5aa'AsparticAcid were measured. pH was also measured in Mb5aa'AsparticAcid while NaOH was added. pH was measured in Mb5aa'AspNa.

5. Results

A summary of indicated bitterness reducing effects of the added ingredients is presented in table 10.

Assessors were randomly numbered, meaning that assessor 7 in experimental 5.2.2. was not necessarily the same individual as assessor 7 in experimental 5.2.3.

Significant differences of results were calculated with a two-way ANOVA with two factors; sample and assessor. A 5 % risk of false positive ($p = 0.05$) was used. The test corrected for assessors' different usages of the hedonic scale.

5.1. Sensory Evaluations

5.1.1. Waters with and without 3aa''

The outcome of the bitterness evaluation of different waters with and without addition of 3aa'', performed by one assessor, is presented in table 11. The taste of 3aa''TH (Torrhults hälsovatten) was very bitter and not palatable, with a pure but very astringent and bitter aftertaste. Remains of carbonic acid in lemon flavoured Loka citron (LC) and Ramlösa original (RO) gave a tingling sensation, obstructing the evaluation of bitterness. Bitterness was considered slightly lower in 3aa''LC, while the aftertaste of 3aa''RO was preferred before the aftertaste of 3aaLC.

5.1.2. Comparison of Mineral Waters

Two assessors compared flavour of unflavoured carbonated mineral waters. Malmberg original was considered to be slightly reminiscent of sour in taste or reminding of bitter almond, while Ramlösa original was considered to be slightly bitter or tasty.

The same two assessors compared flavour of lemon flavoured carbonated mineral waters. Ramlösa citrus was considered to have a fresh lemon flavour and Loka citron to have a somewhat synthetic or acceptable lemon flavour, while Malmberg citron was considered to have a very weak lemon flavour and/or a still noticeable flavour of bitter almond.

Table 10. Summary of indicated effects of added ingredients to BCAA solutions and comments from experiments and preliminary studies, most without reference (NR) or in comparison to a labelled reference (LR) and few with unlabelled reference (UR). Results indicated moderate bitterness reduction (+++), a little bitterness reduction (++), slight bitterness reduction (+) or no bitterness reduction (−). No method used resulted in effective (++++) or total (+++++) bitterness reduction

	Bitterness		
Ingredient	**5aa-solutions**	**3aa-solutions**	**Other characteristics**
Glu (NR)	+++		Sour, unpalatable: umami
Asp (NR)	+++		Sour
Asp + strawberry (NR)	+++		Sour
Asp + NaOH + pomelo (UR)	+++		Unpalatable
Asp + strawberry + masking (LR)	++		Sour
Carbonic acid	++		
Isomaltulose (LR)	++		Sweet
Lemon lime (NR)	++		Diverging preferences
Orange sweet perfect (NR)	++		
Florida orange (NR)	++		
Blackcurrant (NR)	++		Disliked, sweet
Ascorbic acid (LR)		++	Sour
Loka citron (NR)		+	Some carbonic acid remained
Ramlösa original (NR)		+	Some carbonic acid remained
Malmberg citron (LR)	+		Weak flavour of lemon
Malmberg apelsin (LR)	+		Weak flavour of orange
Lemon Perfect (NR)	+		
Pomelo (NR)	+		Diverging preferences, turbid/cloudy
Passion acai conc. (LR)	+		Sweet, pale colour
Pomegranate ginseng conc. (LR)	+		Sweet, pale colour
Citric acid + strawberry (LR)	+		Sour
Citric acid + strawberry + masking (LR)	+		Sour
Orn (LR)	+		May be unpalatable
Resolver (LR)	+	−	
Tau + pomelo (UR)	−		
Sweetness enhancer (NR)	−		No flavour
Apple conc. (LR)	−		Sweet, pale colour
Lemon lime conc. (LR)	−		Sweet
Grapefruit (NR)	−		Diverging preferences
Naartje (NR)	−		Diverging preferences
Scandinavian Berries (NR)	−		Disliked, sweet
Strawberry (NR)	−		Disliked, sweet

Table 11. Outcome of bitterness evaluation of 3aa solutions by one assessor

	Bitterness					
Solution	**0**	**1**	**2**	**3**	**4**	**5**
Torrhults hälsovatten	X					
RO			X			
LC		X				
3aa''TH						X
3aa''RO				X		
3aa''LC				X		

5.1.3. Ascorbic Acid or Resolver in 3aa and 5aa Solutions

Bitterness intensity gradings from the two evaluations, by one assessor, were similar for most 3aa and 5aa municipal water solutions. Gradings deviated slightly for the samples 3aa municipal water solution, 3aaAsk0.748, 3aaAsk1.311 and 5aaRes0.3. The labelled 5aa and 3aa reference solutions were graded slightly more bitter compared to the gradings of coded 5aa and 3aa samples. 3aaAsk0.748 was the only ascorbic acid solution not to be described as sour and was also indicated as least bitter. 5aaRes0.3, 5aaRes0.4 and 5aaRes0.5 were indicated slightly less bitter than other Resolver solutions but were commented to have slight amino acid off-taste, as did most Resolver samples as well as labelled and coded reference solutions. 3aaRes0.4 and 3aaRes0.5 were instead indicated slightly more bitter compared to other samples and references.

Generally, the 5aa municipal water solutions were described as somewhat less bitter and smoother in flavour and bitterness compared to the 3aa municipal water solutions.

5.1.4. Isomaltulose in 5aa Solution

One assessor evaluated bitterness in 5aa municipal water solutions with isomaltulose. 5aa municipal water solution 5aaIM49.87, with the strongest concentration of isomaltulose, was considered the least bitter of 5aa solutions, but was very sweet. Weaker concentrations of isomaltulose in 5aa municipal water solution were considered more bitter or slightly more bitter than 5aa municipal water solution. The water solution wIM3.33 and the strongest concentration in 5aa solution 5aaIM49.87 were the only ones not commented to have amino acid aftertaste.

5.1.5. Ornithine in 5aa Solution

Table 12. Outcome of bitterness evaluation of 5aa-solutions with Orn HCl. Reference (5aa solution) was predetermined at bitterness score 4. The symbols indicate answers from different assessors

	Bitterness						Other flavour
Solution	**0**	**1**	**2**	**3**	**4**	**5**	
5aa solution			o	∇ Δ		×	Also sour. A little sweet. Not bitter, something else.
5aaOrn			Δ	o× ∇			Somewhat sour to certain sweetness. Somewhat delayed bitterness. Not bitter, something else.

A prestudy of 3aa municipal water solutions with 2.1 to 20.0 g/L Orn HCl, evaluated by one assessor, did not indicate Orn HCl to reduce bitterness. Solutions with Orn HCl were considered less palatable than 3aa municipal water solution.

Outcome of bitterness evaluation, performed by four assessors, of 5aa solution with 2.17 g/L Orn HCl and comments on flavour are presented in table 12. Panel average bitterness grading of 5aaOrn was slightly lower than 5aa solution, but two assessors disagreed extremely in judgement of bitterness in 5aa solution.

5.1.6. Aspartic Acid or Taurine in 5aa'

Results from sensory evaluation of Mb5aa', Mb5aa'Tau and Mb5aa'AspNa performed by four assessors are presented in table 13. One female liked Mb5aa'Tau best and strongly disliked Mb5aa'AspNa. The other female also strongly disliked Mb5aa'Asp. One male preferred Mb5aa' and did not like Mb5aa'Tau at all. The other male preferred Mb5aa'AspNa. Three of the participants thought that Mb5aa'AspNa was least bitter, while one male thought Mb5aa' was least bitter.

Table 13. Summarized comments from sensory evaluation of flavoured Mb5aa'-solutions

Solution	Flavour	Bitterness
Mb5aa'	Like strong mineral water, not tasty, slight acidity, kind of ok, somewhat in between the other two.	Bitter, almost no bitterness, almost not bitter at all.
Mb5aa'Tau	Not tasty, slight acidity.	Bitter, not very bitter, more bitter than Mb5aa'AspNa.
Mb5aa'AspNa	Extremely unpalatable, slight sweetness and acidity, salt, not very good.	Not bitter at all, slightly bitter, almost no bitterness, no bitterness noticed.

5.1.7. Lemon Flavoured 5aa'- and Carbonated Lemon Flavoured 5aa' Solutions

Comments by a representative from *Flavour Company B*, on Mb5aa' solution, lemon flavoured MbLem5aa' and carbonated lemon flavoured MbLem5aa'Carb are presented in table 14. Flavour in the lemon flavoured Mb5aa' solution was considered noticeably improved compared to the unflavoured solution. Flavour in the carbonated lemon flavoured Mb5aa' solution was considered to be even more improved.

Table 14. Comments by a representative from *Flavour Company B* on the Mb5aa'-solutions

Solution	Comments
Mb5aa'	No smell. Pleasant first flavour, a little sweet. Aftertaste lingers and the consistency/viscosity makes the solution linger on the tongue. Bitterness.
MbLem5aa'	Lemon certainly masks unwanted flavour. A more fresh lemon flavour would be desired. This lemon flavour is rather weak compared to commercial waters.
MbLem5aa'Carb	The unwanted flavours are even better masked; the carbonic acid helps. A noticeable improvement.

5.1.8. Citrus or Berry Flavoured 5aa' Solutions

Table 15. Summarized comments from sensory evaluation of flavoured Mb5aa'-solutions

Flavoured solution	Sensory comments		
	Aroma/Odour	**Flavour**	**Bitterness**
Pomelo	Nice, grapefruit, orange, similar to Grapefruit-sample but lighter.	Tasty, too weak, unpleasant, too much of everything.	Slight bitterness, noticeable bitterness, too bitter.
Naartje	Slight sweet, mandarin, similar to Grapefruit-sample but sweeter, like bitter clementin peel.	Tasty, not as fresh as Pomelo-sample, the sweetness give bitterness, old schnapps, correlation between taste and odour, not as tasty as Grapefruit-sample, unpleasant, like clementin peel.	Noticeable bitterness but ok, too bitter, more bitter than Pomelo- and Grapefruit-samples.
Grapefruit	Difficult to determine, fake-grapefruit.	Carbonated, tasty, lime, not as tasty as Pomelo-sample, lighter feeling, too weak, like when you add too much orange-peel in a cake.	More bitter than Pomelo-sample, noticeable bitterness, too bitter.
Lemon Lime	Citrus, fresh, nothing, lemon.	Unpleasant, neutral, lemon, citrus, tasty, healthy, pine needle, fresh, potential together with other flavours, unpleasant, salty but bitter.	Slight bitterness, not bitter, some bitterness, too bitter.
Lemon Perfect	Lemon, familiar, concentrated, citrus, slight lemon balm, less smell, reminds of Orange Sweet Perfect-sample but more lemon.	Tasty, lime, fresh, pine needle, better balance.	Too bitter, not that bitter.
Orange Sweet Perfect	Clementin, citrus - orange, mix of grapefruit and cough-medicine, grapefruit but more sour.	Ok, orange, sweeter than other orange-samples, not sweet, weak flavour, quite ok.	Ok bitterness, hardly bitter, some bitterness, bitter.
Florida Orange	Orange, citrus, weak orange.	Tasty, like flat Fanta, fresh, cough-medicine, weak orange flavour.	No bitterness, some bitterness.
Strawberry	Strawberry, a little synthetic, tasty, bubblegum, wild strawberry/berry, butterscotch.	Unpleasant, too sweet, synthetic strawberry, a little sweet butterscotch.	Bitter, a little more bitter than Sweetness enhancer-sample.
Scandinavian Berries	Sweet, synthetic raspberry, nice, fruity, raspberry candy,	Sweet-bitter, no correlation between taste and odour, diluted candy, unpleasant.	Bitter, some bitterness, too bitter, more bitter than Pomelo- and Grapefruit-samples.
Blackcurrant	Berry, sweet, wine gum, fruity, strawberry, unpleasant, artificial.	Berry, unpleasant, too sweet, tasty, much flavour, candy but not correlating to odour.	Too bitter, not that bitter, noticeable but reduced.
Sweetness enhancer	Nothing.	Nothing according to most assessors, 5aa-flavour, more natural than the flavoured samples and best of them all.	Bitter.

Odour, flavour and bitterness of 5aa' solutions with different flavours were evaluated by four to six assessors.

Comments from sensory evaluation of the flavoured 5aa samples are presented summarized in table 15. The Pomelo flavoured sample was commented to have potential if carbonated. The Orange Sweet Perfect flavoured sample and the Florida Orange flavoured sample both were commented to have potential if more sour. When naming favourites, the Pomelo flavoured sample and the Grapefruit flavoured sample were chosen three times each, while the Naartje flavoured sample was chosen once. One participant liked the Sweetness enhancer sample or the citrus flavoured samples best. The participant who tried Lemon Lime and Lemon Perfect flavours together in a solution chose this combination as a favourite, while in second hand liked the flavour of the Lemon Perfect flavoured sample best and thought that the Lemon Lime flavoured sample was least bitter.

Flavour of Lemon Lime and Lemon Perfect samples mixed together, evaluated by one assessor, was considered tasty. The stronger concentration of Sweetness enhancer, evaluated by the same assessor, tasted a little like table-sweetener.

The flavour of Strawberry and Florida Orange samples mixed together, evaluated by three assessors, was described as strawberry, citrus, penicillin, and bitter.

There was no difference in flavour of sweetness enhancer sample compared to 5aa solution, evaluated by two assessors.

5.1.9. Pomelo flavoured 5aa' Mineral Water

Eight assessors participated in the evaluation of Pomelo flavoured 5aa solutions.

Panel average likeness of the Pomelo flavoured 5aa' mineral water was 3.86 on a scale of 0–10, which can be interpreted as dislike or dislike slightly. Standard deviation was 1.77. Five volunteers answered that they would not buy this water, while three answered that they would buy it. Three volunteers would buy the water if it was very healthy, while two would not. The other three answers were "Very possible as I like the grapefruit flavour", "Yes, if I considered myself being in the risk group" and "Difficult to answer without further scientific evidence for the statement". Comments are presented in table 16.

Table 16. Comments on Mb5aa'Pom from sensory evaluation. No comments were given by assessors 1 and 8

Assessor	Comments on Mb5aa'Pom
2	Disliked the flavour.
3	I do not mind the flavour, but would perhaps buy another water in the long run. Mix of flavours: first citrus and then maybe mango.
4	Tasty grapefruit flavour.
5	If the water were flavoured with something, it would probably be very tasty. The first gulp was not so tasty but thereafter the taste was neutral and nice, perhaps the mouth was a little anaesthetized.
6	Too much flavour, I prefer more neutral flavours in water.
7	Sour and a bit flat taste.

5.2. Sensory Analyses

5.2.1. Separate Amino Acids in Solutions

Eight assessors participated in the analysis of bitterness and palatability of solutions containing separate amino acids in concentrations corresponding to 5aa solution. Panel average and standard error of the mean (SEM) of palatability and bitterness of separate amino acids in water solutions are presented in figures 3 and 4.

Leu (mean score 54.2, corresponding to Bitter) was significantly more bitter than both Val (P = 0.0072, mean score 8.3) and Thr (P = 0.0030). Lys HCl (mean score 77.1 corresponding to Dislike moderately) was significantly less palatable than both Val (P = 0.0208, mean score 39.6 corresponding to Like slightly) and Thr (P = 0.0133, mean score 37.5).

Assessor 7 was the only assessor to consider Thr bitter, while others found it not bitter. Assessor 7 was also one of only two assessors to consider Val bitter.

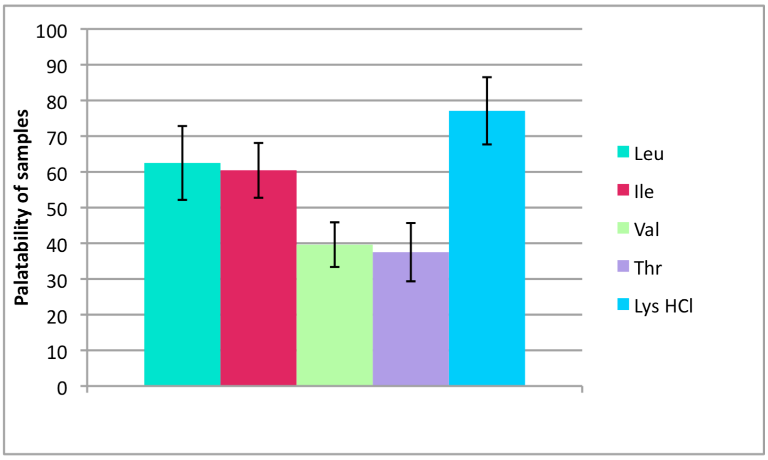

Figure 3. Panel mean and SEM of palatability of amino acids in water solution. 8 assessors participated. Score 0 equals "Like very much", 16.7 equals "Like moderately", 33.3 equals "Like slightly", 50 equals "Neither like nor dislike", 66.7 equals "Dislike slightly", 83.3 equals "Dislike moderately" and 100 equals "Dislike very much". The water reference was predetermined at 50.

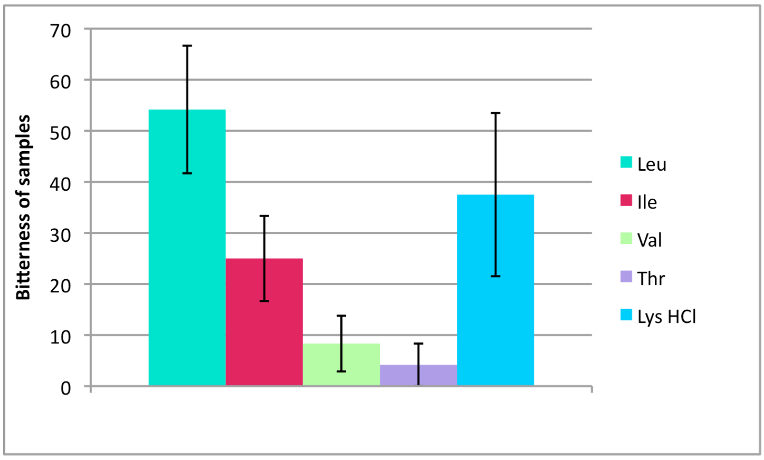

Figure 4. Panel mean and SEM of bitterness of amino acids in water solution. 8 assessors participated. Score 0 equals "Not bitter", 33.3 equals "Slightly bitter", 66.7 equals "Bitter" and 100 equals "Very bitter".

5.2.2. Solutions of 5aa, 5aa' and 5aa-lys

Eight assessors participated in the analysis of bitterness and palatability of the different compositions of 5aa solutions. Panel average and SEM of palatability and bitterness of 5aa', 5aa-lys and previously frozen 5aa solution are presented in figures 5 and 6. Comments from sensory analysis are summarized in table 17. No significantly differing results were found neither in sample palatability nor bitterness using Tukey's pair-wise comparison.

Answers of assessor 1 on bitterness were significantly different from assessor 2 (P = 0.0545), assessor 5 (P = 0.0352) and assessor 7 (P = 0.0104). Answers of assessor 2 were significantly different from assessor 3 (P = 0.0211) on bitterness. Answers of assessor 3 on bitterness differed significantly from assessor 5 (P = 0.0135) and assessor 7 (P = 0.0040). Answers of assessor 7 were almost significantly different from assessor 8 (P = 0.0549) on bitterness. Answers of assessor 2 on palatability were significantly different from assessor 3 (P = 0.0381). Answers of assessor 3 on palatability differed significantly from answers of assessor 4, assessor 5, assessor 6, assessor 7 and assessor 8 (P = 0.0297; 0.0004; 0.0034; 0.0054; 0.0762, respectively).

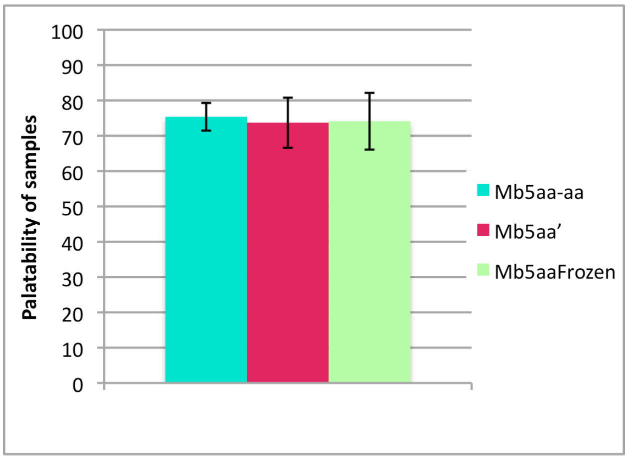

Figure 5. Panel mean and SEM of palatability of 5aa samples. 8 assessors participated. Score 0 equals "Like very much", 50 equals "Neither like nor dislike" and 100 equals "Dislike very much". The water reference was predetermined at 50.

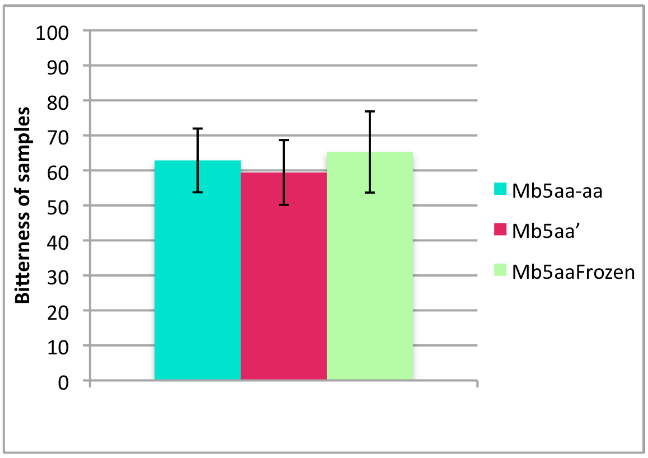

Figure 6. Panel mean and SEM of bitterness of 5aa samples. 8 assessors participated. Score 0 equals "Not bitter", 33.6 equals "Slightly bitter", 66.8 equals "Bitter" and 100 equals "Very bitter". Bitter reference was predetermined at 100.

Table 17. Summary of comments from the sensory analysis

Comments
All were too bitter. The samples all tasted the same. Tasted terrible. Mb5aaFrozen and Mb5aa' were slightly sweet/sour and Mb5aaFrozen definitely tasted best. Mb5aa-lys was fresher than Reference, reminiscent of citrus. Mb5aaFrozen was not as fresh as Mb5aa-lys, but had a fresher aftertaste. Reference was not the most bitter of samples, more "Bitter" than "Very bitter".

5.2.3. Carbonated 5aa'-lys Solutions Flavoured with Lemon, Orange, Apple and Lemon/Lime

Preliminary studies showed that diluted Lemon/Lime and Apple were acceptable in flavour with slight sweetness. Recommended concentrations of flavour concentrates from SodaStream were very sweet and most were strong in colour, with exception of Pear and Apple that were pale yellowish and Lemon/Lime that was transparent.

Eleven assessors participated in the analysis of bitterness and palatability of carbonated flavoured solutions. Panel average and SEM of palatability and bitterness of carbonated Mb5aa'-lys solutions prepared with orange flavoured water (Mb5aa'-lysOraCarb), citrus flavoured water (Mb5aa'-lysLemCarb) or plain water flavoured with lemon/lime (LemonLimeMb5aa'-lysCarb) or apple (AppleMb5aa'-lysCarb) are presented in figures 7 and 8. Mb5aa'-lysLemCarb was nearly significantly more palatable than AppleMb5aa'-lysCarb (P = 0.0658, mean score of 69.7). Comments from the sensory analysis are summarized in table 18. One assessor commented that carbonic acid covered bitterness better compared to when it had disappeared.

The answers of assessor 10 were significantly different from assessor 2 (P = 0.0174), assessor 3 (P = 0.0184), assessor 5 (P = 0.0077) and assessor 6 (P = 0.0184) in palatability. The answers of assessor 11 on palatability were significantly different from assessor 2 (P = 0.0284), assessor 3 (P = 0.0299), assessor 5 (P = 0.0129) and assessor 6 (P = 0.0299).

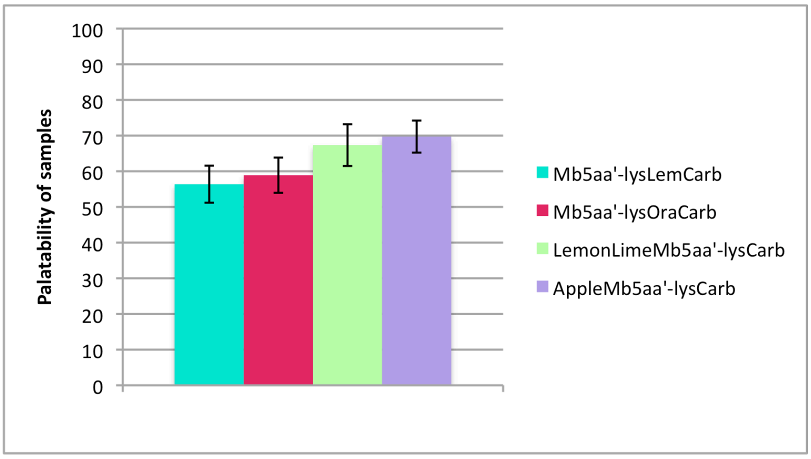

Figure 7. Panel mean and SEM of palatability of carbonated and flavoured Mb5aa'-lys samples. 11 assessors participated. Score 0 equals "Like very much", 100 equals "Dislike very much". The water reference was predetermined at 50.

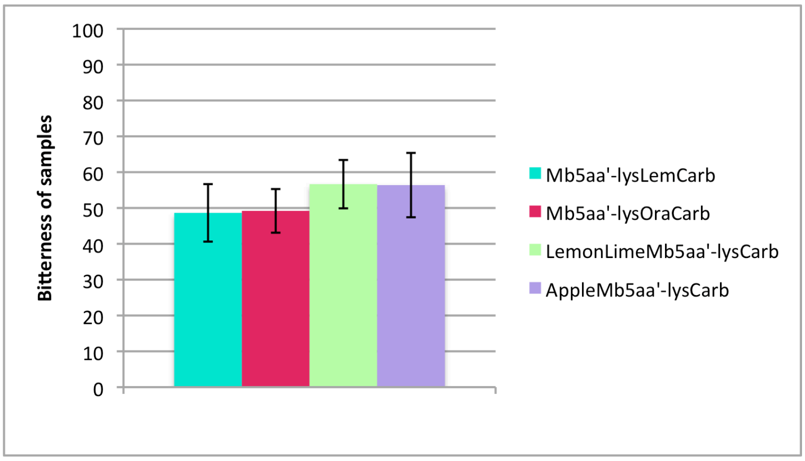

Figure 8. Panel mean and SEM of bitterness of carbonated and flavoured Mb5aa'-lys samples. 11 assessors participated. Score 0 equals "Not bitter" and 100 equals "Very very bitter". The carbonated bitter reference was predetermined at 83.3.

Table 18. Summary of comments from the sensory analysis of flavoured Mb5aa'-lys samples

Solution	Comments
Mb5aa'-lysLemCarb	Citrus or weak citrus, orange, lemon, sour or a little sour, fresh, fruity and quite ok, bitter aftertaste, less carbonic acid compared to other samples, ok to drink more.
Mb5aa'-lysOraCarb	Weak citrus, fresh or tasty lemon, lemon with unpalatable and/or bitter aftertaste, weak orange, fruity and quite ok, a little sweet, like cough-medicine, ok to drink more.
LemonLimeMb5aa'-lysCarb	Too bitter, unpalatable stronger taste, no flavour, a little sweet or sweet aftertaste, not ok to drink more.
AppleMb5aa'-lysCarb	Too sweet or slightly sweet and sour, bitter aftertaste or too bitter, flat flavour, not ok to drink more.
Reference	A little sweet.

5.2.4. 5aa' Solutions Flavoured with Pomegranate/Ginseng and Passion/Acai

Preliminary studies showed that diluted Pomegranate/ginseng and Passion/acai flavour concentrates were almost transparent and tasty with only little sweetness. Concentrations recommended on product packages were considered too sweet.

Solutions evaluated in sensory analysis were Pomegranate/ginseng or Passion/acai flavoured carbonated 5aa'' solutions diluted with lemon flavoured Malmberg citron. Flavour concentrations PGLem5aa''Carb and PALem5aa''Carb were medium strong, whereas flavour concentration of 5aa''PGLemCarb and 5aa''LemPACarb were weaker. The colour of 540 ml PGLem5aa''Carb was slightly pale pink, but the colour was hardly detectable in 40 ml. The colour of 540 ml PALem5aa''Carb was slightly brownish, but the colour was hardly detectable in 40 ml. Colours of 5aa''PGLemCarb and 5aa''PALemCarb were pale but noticeable in 240 ml, however not visible in 40 ml volumes.

Six assessors participated in the analysis of bitterness and palatability of flavoured 5aa' solutions. Panel average and SEM of palatability and bitterness of pomegranate/ginseng and passion/acai flavoured solutions are presented in figures 9 and 10. Comments from sensory analysis are summarized in table 19. No statistical differences were found between samples in neither bitterness nor palatability using Tukey's pair-wise comparison.

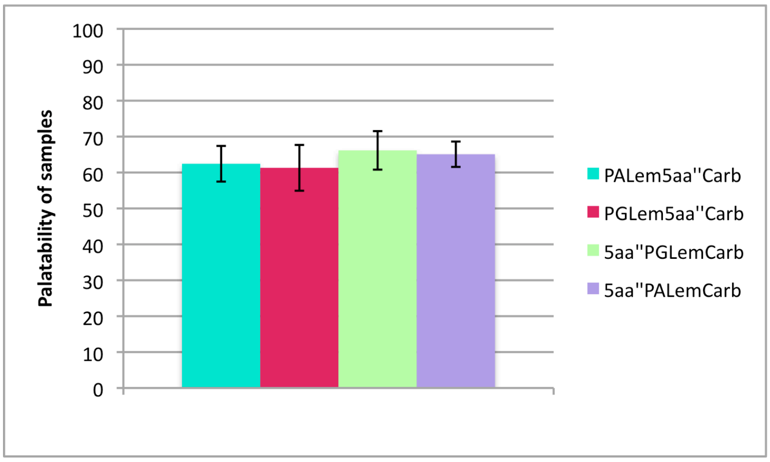

Figure 9. Panel mean and SEM of palatability of carbonated and flavoured Mb5aa'' samples. 6 assessors participated. Score 0 equals "Like very much", 100 equals "Dislike very much". The water reference was predetermined at 50.

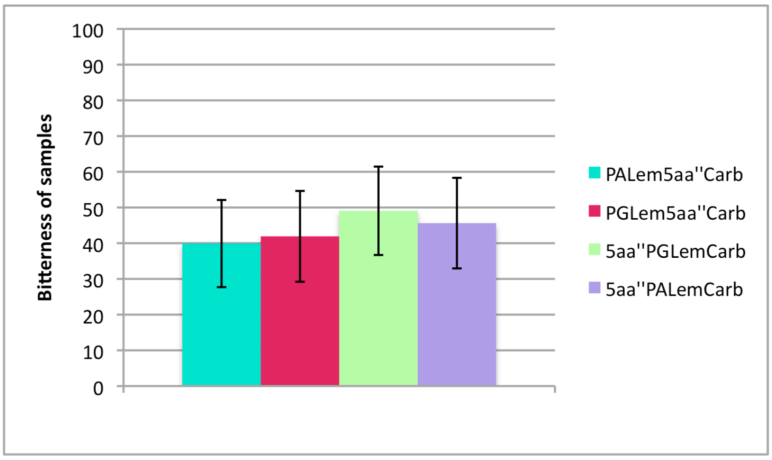

Figure 10. Panel mean and SEM of bitterness of carbonated and flavoured Mb5aa'' samples. 6 assessors participated. Score 0 equals "Not bitter" and 100 equals "Very very bitter". Bitter non-carbonated reference was predetermined at 83.3.

Table 19. Summary of comments from the sensory analysis of carbonated flavoured Mb5aa'' solutions

Solution	**Comments**
PALem5aa''Carb	Somewhat sweet.
PGLem5aa''Carb	Somewhat sweet.
5aa''PALemCarb	Unpalatable off-taste, fresh, citrus. Was one of the best in taste the first time, but was one of the most unpalatable in the second tasting.
5aa''PGLemCarb	Somewhat sweet, unpalatable off-taste. Was one of the best in taste the first time, but was one of the most unpalatable in the second tasting.

Answers from assessor 5 on palatability differed significantly from the answers of assessor 1 ($P = 0.0421$) and assessor 4 ($P = 0.0503$). Answers from assessor 5 on bitterness differed significantly from assessor 1 ($P = 0.0000$), assessor 2 ($P = 0.0001$), assessor 3 ($P = 0.0231$) and assessor 4 ($P = 0.0000$). Answers from assessor 6 on bitterness were significantly different from assessor 1 ($P = 0.0000$), assessor 4 ($P = 0.0005$) and assessor 5 ($P = 0.0001$). Answers from assessor 4 on bitterness were significantly different from assessor 2 (P =

0.0005) and assessor 3 (P = 0.0000). Answers from assessor 1 of bitterness of samples were significantly different from assessor 2 (P = 0.0000) and assessor 3 (P = 0.0000).

5.2.5. Strawberry Flavoured 5aa' Solutions with Aspartic Acid or Citric Acid

Preliminary studies showed that strawberry flavour in 5aa solution was bitter and sometimes sweet with an "empty" strawberry flavour, which was improved by adding a little citric acid or sugar. A satisfactory concentration of strawberry flavoured 5aa solution (5aaS4) in 5aa'solution was found to give tasty strawberry flavour but bitter taste. Addition of 9.16 g/L Glu to this strawberry flavoured solution was rather difficult to dissolve and resulted in a very sour and overwhelmingly food-like tasting solution with reduced bitterness (pH 3.41). Addition of the same amount of Asp in strawberry flavoured solution was rather difficult to dissolve and resulted in a very sour, tasty and faintly food-like tasting solution with reduced bitterness (pH 3.43). Addition of masking flavour 5aa solution (5aaMF1) to 5aa' solution was a little sweet and bitter.

Eight assessors participated in the analysis of bitterness and palatability of strawberry flavoured solutions with citric acid or Asp (a slightly weaker concentration compared to the preliminary study). Panel average and SEM of palatability and bitterness of samples are presented in figures 11 and 12. Comments from the sensory analysis are summarized in table 20. No significant differences in bitterness or palatability were found using Tukey's pair-wise comparison.

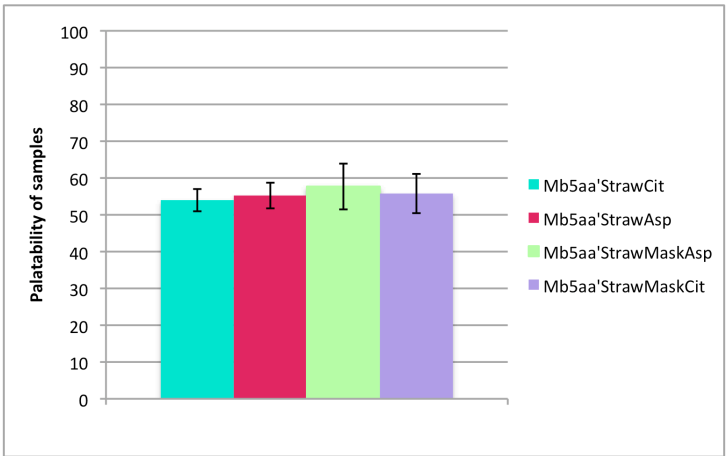

Figure 11. Panel mean and SEM of palatability of strawberry flavoured Mb5aa' samples. 8 assessors participated. Score 0 equals "Like very much", 100 equals "Dislike very much". The water reference was predetermined at 50.

Answers from assessor 7 on palatability of samples were significantly different from assessor 1 (P = 0.0003), assessor 2 (P = 0.0020), assessor 3 (P = 0.0003), assessor 4 (P = 0.0000), assessor 5 (P = 0.0031), assessor 6 (P = 0.0009) and assessor 8 (P = 0.0028). Answers from assessor 7 on bitterness of samples were significantly different from assessor 1 (P = 0.0035), assessor 2 (P = 0.0008), assessor 3 (P = 0.0003) and assessor 5 (P = 0.0064). Answers of assessor 4 on palatability differed significantly from assessor 1 (P = 0.0239), assessor 2 (P = 0.0042), assessor 3 (P = 0.0331), assessor 5 (P = 0.0027), assessor 6 (P = 0.0088) and assessor 8 (P = 0.0030). Answers from assessor 6 on bitterness differed significantly from assessor 1 (P = 0.0006), assessor 2 (P = 0.0001), assessor 3 (P = 0.0001) and assessor 5 (P = 0.0011). Answers from assessors 3 and 4 on bitterness were also significantly different (P = 0.0317).

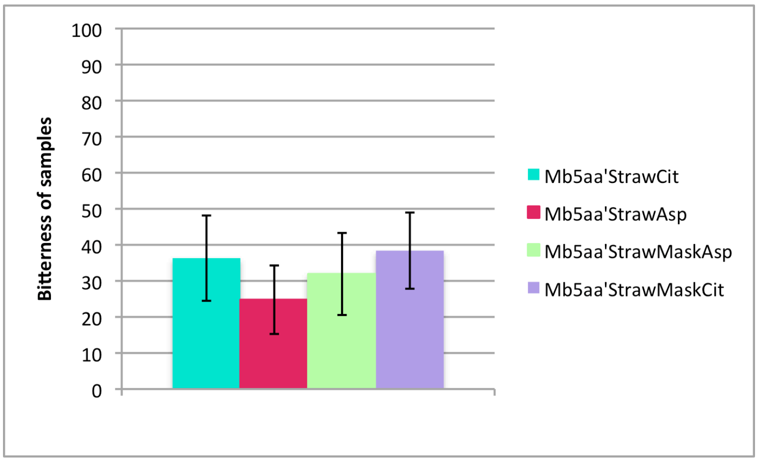

Figure 12. Panel mean and SEM of bitterness of strawberry flavoured Mb5aa' samples. 8 assessors participated. Score 0 equals "Not bitter" and 100 equals "Very very bitter". Bitter reference was predetermined at 83.3.

Table 20. Summary of comments from the sensory analysis

Solution	Comments
Mb5aa'StrawCit	Slight bitter aftertaste, a little more bitter than Mb5aa'StrawMaskAsp but ok, too sour or the least sour, citrusy, first raspberry but then bitter.
Mb5aa'StrawAsp	Sour but not bitter, too sour or less sour than Mb5aa'StrawMaskAsp, the least bitter, lime-flavour with bitter aftertaste, ok to have some more.
Mb5aa'StrawMaskAsp	Sour but not bitter, sour or very sour, a little bitter but covered well by lemon-taste, strawberry, like Dragster-candy, fresh lemon with very little bitterness, not at all as bitter as Reference, could drink more.
Mb5aa'StrawMaskCit	Sour but bitter aftertaste, a little bitter but ok, tasty and distasteful at once, reminds of some candy, sour or less sour than Mb5aa'StrawAsp, raspberry/lime, tasty at first but then quite bitter.
Reference	Extremely bitter.

5.2.6. Pomelo Flavoured 5aa'-solutions with Taurine or Aspartic Acid

Preliminary studies showed that citrus flavoured 5aa' solutions were more intense in bitterness than unflavoured 5aa' solution, with exception of Pomelo flavour. Pomelo flavoured solutions were turbid (cloudy).

Eleven assessors participated in sensory analysis of bitterness and palatability of Pomelo flavoured solutions with Tau or Asp/NaOH. Panel average and SEM of palatability and bitterness of Mb5aa'Pom, Mb5aa'TauPom, Mb5aa'AspNaPom and RamOrPom are presented in figures 13 and 14. Comments from sensory analysis are summarized in table 21. 5aa'AspNaPom (mean score 72.5) was significantly less palatable than RamOrPom (P = 0.0055, mean score 45). RamOrPom (mean score 10) was significantly less bitter than 5aa'Pom (P = 0.003, mean score 42.5) and 5aa'TauPom (P = 0.0014, mean score 45).

Answers of assessor 1 on palatability differed significantly from assessor 2 (P = 0.0219) and assessor 4 (P = 0.0069). Answers of assessor 1 on palatability differed significantly from assessor 3 (P = 0.0280), assessor 5 (P = 0.0149) and assessor 9 (P = 0.0511).

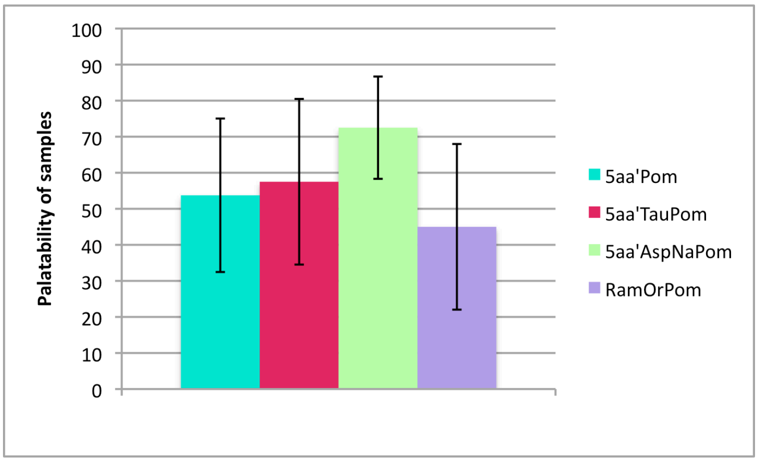

Figure 13. Panel mean and SEM of palatability of Pomelo flavoured Mb5aa' samples and Ramlösa original. 11 assessors participated. Score 0 equals "Like extremely", 12.5 equals "Like very much", 25 equals "Like moderately", 37.5 equals "Like slightly" 50 equals "Neither like nor dislike", 62.5 equals "Dislike slightly", 75 equals "Dislike moderately", 87.5 equals "Dislike very much" and 100 equals "Dislike extremely".

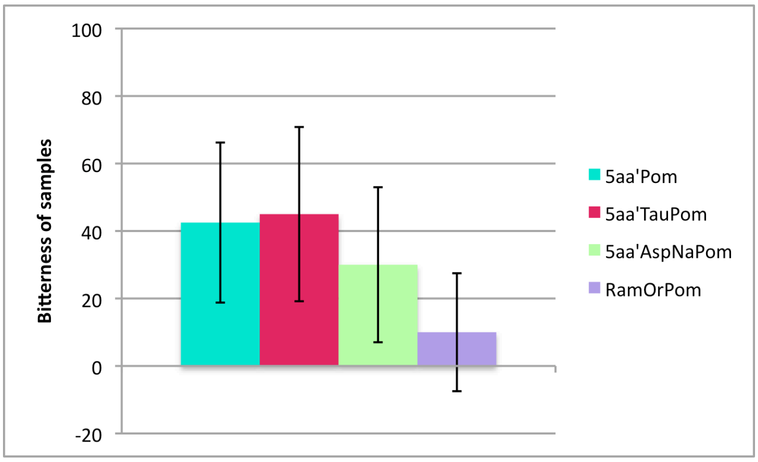

Figure 14. Panel mean and SEM of bitterness of Pomelo flavoured Mb5aa' samples and Ramlösa original. 11 assessors participated. Score 0 equals "Not bitter", 25 equals "Slightly bitter", 50 equals "Bitter", 75 equals "Very bitter" and 100 equals "Extremely bitter".

Table 21. Summary of comments from sensory analysis of Pomelo flavoured solutions

Solution	Comments
Mb5aa'Pom	5aa'Pom was carbonated, which was very interesting. A little too sour, but the bitterness fits very well to the grapefruit flavour. Like flat carbonated water.
Mb5aa'TauPom	A favourite. Too sour.
Mb5aa'AspNaPom	Very unpalatable. Does not taste anything but is not fresh like ordinary water. Salty.
RamOrPom	Mild and tasty, like water with a fresh flavour. The tastiest. Tastes like water.

5.3. pH in Solutions

Mineral Waters: Mean pH of carbonated flavoured and unflavoured mineral waters was 5.2 and standard deviation was 0.17. Mean pH of mineral waters with decreased carbonic acid was 6.1 and standard deviation was 0.34. pH of the uncarbonated and unflavoured Malmberg stilla was 7.8.

3aa and 5aa Solutions with Ascorbic Acid or Resolver: pH in BCAA solutions are presented in table 22. pH in municipal water was 6.58 and pH in deionized water was 7.61.

Table 22. pH in a choice of BCAA solutions with ascorbic acid or Resolver

Solution	pH
3aaAsk0.748	5.38
3aaAsk1.125	4.62
3aaRes0.3	7.61
5aaRes0.4	7.36
3aa municipal water	7.44
5aa municipal water	7.08

5aa Solutions with Isomaltulose: pH in 5aaIM1.73, 5aaIM20 and 5aaIM49.87 were around 7.12, while pH in 5aaIM3.43 was 7.19. pH in wIM3.33 was 6.88 and pH in 5aa municipal water solution was 7.20.

Separate Amino Acids and Different Compositions of BCAA Solutions: pH in solutions of separate amino acids and BCAA solutions of different compositions are presented in table 23.

Lemon Flavoured 5aa'- and Carbonated Lemon Flavoured 5aa' Solutions; 5aa'' Flavoured with Pomegranate/Ginseng and Passion/Acai; Flavoured and Carbonated 5aa'-lys: pH of solutions, measured using pH-meters Mettler Toledo and MeterLab, are presented in table 24. pH in solutions using Mettler Toledo were generally slightly higher compared to pH in solutions using MeterLab, with exception of PALem5aa'Carb.

Strawberry Flavoured 5aa' with Aspartic Acid or Citric Acid: pH of strawberry flavoured 5aa' solutions with Asp or citric acid are presented in table 25.

Pomelo Flavoured 5aa' with Taurine or Aspartic Acid: pH of Mb5aa', Mb5aa'Tau, Mb5aa'AsparticAcid and Mb5aa'AspNa are presented in table 26.

Table 23. pH in solutions of separate amino acids as well as different compositions of BCAA solutions

Solution	pH
Leu	7.34
Ile	7.42
Val	7.47
Thr	7.41
Lys HCl	7.44
Reference (Mb5aa)	7.26
Mb5aa'	7.26
Mb5aaFrozen	7.21
Mb5aa-lys	7.53

Table 24. pH in unflavoured and lemon flavoured 5aa' solutions, 5aa'' solutions flavoured with lemon and pomegranate/ginseng or passion/acai and 5aa'-lys solutions flavoured with lemon, apple, lemon/lime or orange

Solution	pH (MeterLab)	pH (Mettler Toledo)
MbLem5aa'	5.57	5.77
MbLem5aa'Carb	5.27	5.48
PGLem5aa''Carb	6.00	5.72
PALem5aa''Carb	5.70	6.13
Lem5aa''PGCarb	-	7.63
Lem5aa''PACarb	-	7.63
Reference (Mb5aa'')	-	7.74
Mb5aa'-lysLemCarb	5.86	6.52
Mb5aa'-lysOraCarb	5.82	6.04
LemonLimeMb5aa'-lysCarb	6.42	7.08
AppleMb5aa'-lysCarb	5.86	6.05
Reference (Mb5aa'-lysCarb)	5.88	6.13

Table 25. pH of strawberry flavoured 5aa'-solutions with aspartic acid or citric acid

Solution	pH
Mb5aa'	7.15
Mb5aa'StrawAsp	3.74
Mb5aa'StrawMaskAsp	3.74
Mb5aa' strawberry	7.23
Mb5aa' strawberry/masking	7.22
Mb5aa' strawberry +cit	4.86
Mb5aa' strawberry/masking +cit	3.70
Mb5aa'StrawCit	3.81
Mb5aa'StrawMaskCit	3.81

Table 26. pH of 5aa'-solutions with Tau or Asp

Solution	pH
Mb5aa'	7.18
Mb5aa'Tau	7.20
Mb5aa'AsparticAcid	3.63
Mb5aa'AspNa	7.48

6. Discussion

Water solutions of the ingredients were used, as these were easy to study in sensory analysis.

Note the different uses of the terms *sensory evaluation* and *sensory analysis*. Studies of solutions using six or more assessors, performed separately using test forms and according to instructions are referred to as sensory analyses. Studies of solutions using one to four assessors or using verbal comments are referred to as sensory evaluations.

Note also that the term *BCAA solution* is used interchangeably for 5aa solutions and/or 3aa solutions.

Because few assessors were used, and especially as bitterness sensitivity varies between individuals, these results can only be used as indications.

6.1. Results and Literature

6.1.1. Early Experiments

The first evaluation of BCAA solution, that was performed by one assessor, showed that 3aa'' solution in a plain and neutral water was considered very bitter and not palatable, with bitter and astringent aftertaste. Bitterness was noticeable also in the two mineral waters Ramlösa original and Loka citron without added amino acids. Carbonic acid made evaluation of bitterness difficult and the lemon aroma in the carbonated water Loka citron seemed to suppress bitterness slightly compared to the 3aa'' solutions in the carbonated water Ramlösa original or in the still water Torrhults hälsovatten. Another explanation might be the differences in mineral composition of Loka water and Ramlösa water. See *6.1.2. Effect of carbonic acid, pH and mineral composition*.

Results from one assessor's evaluation of BCAA solutions with ascorbic acid or Resolver witness of the difficulty in repeating ratings of bitterness, especially when too many samples are evaluated in one occasion (five samples or less is preferable). Samples of 3aa municipal water solution, evaluated by one assessor, were described as sharper in bitterness, while samples of 5aa municipal water solution were described as more smooth in bitterness. Partly based on these observations and partly on the need of narrowing project limitations, focus was thereafter set to only solutions of all five amino acids (5aa).

The 5aa solution containing the strongest concentration of isomaltulose was indicated to reduce bitterness, but was on the other hand very sweet. As Mukai *et al.* (2007) concluded, mixtures of bitter and sweet compounds in different concentrations can either suppress or enhance each other. Therefore, specific concentrations of isomaltulose or other sweet compounds might have suppressive effect without sweetness. Because the same can be said about mixtures of sour and bitter compounds, certain concentrations of ascorbic acid or other acids might suppress bitterness without causing unwanted sourness (Mukai *et al.* 2007).

Not only bitterness was indicated to be a problem in BCAA municipal water solutions, but also overall flavour. Therefore, both bitterness and palatability were studied in sensory analyses.

Literature study (Roudot-Algaron 1996) and sensory analysis on flavour and bitterness of the separate amino acids comprised in the composition indicated that the BCAA solution would be improved if content of Leu was lowered. Leu was significantly more bitter than both Val and Thr. Results pointed also to Lys HCl being an important cause of bitterness and unpalatability in 5aa solution, as Lys HCl was significantly less palatable than both Val and Thr. Lys HCl was indicated, by a review article by Roudot-Algaron (1996), to be less palatable compared to Lys, which leads to the conclusion that replacing Lys HCl with Lys might improve flavour of 5aa solution. However, 5aa' solution (containing less Leu) and 5aa-lys solution (containing less Lys HCl) did not differ significantly from 5aa solution that had been frozen. Assuming that freezing of the BCAA solution did not affect taste properties, the slightly altered versions of 5aa solution were similarly bitter and unpalatable. Subsequent experiments nevertheless studied 5aa' solutions, with a lower content of Leu.

6.1.2. Effect of Carbonic Acid, pH and Mineral Composition

Several assessors in sensory evaluations and analyses commented that they preferred carbonated solutions or that carbonic acid seemed to suppress bitterness or obstruct assessment of bitterness in BCAA solutions.

Carbonation of water lowers pH, which might affect bitterness. The effect of pH and acids on taste such as bitterness is complicated and is dependent upon the type of bitter compound, the type of acid or acids used, pH in the finished product, as well as the type of product or matrix (Sakurai *et al*. 2009).

Miyanaga *et al*. (2004), Mukai *et al*. (2004) and Mukai *et al*. (2007) suggested addition of citric acid to reduce bitterness. pH in solutions were not presented but supposed to have been recorded, indicating that reduction of bitterness might have been due to a combination of low pH and citric acid or mainly to low pH.

Choice of water in the BCAA solution might have great effect on sensory qualities, particularly as low concentrations of minerals and salts can enhance or suppress the bitterness generated by the amino acids (Breslin 1996).

6.1.3. Effect of Flavours

Studied SodaStream flavour concentrates were too sweet and often too coloured to be used in concentrations that generated sufficient flavour strength. Studies by Mukai *et al*. (2007) indicated that strawberry flavour would reduce bitterness. When strawberry flavoured 5aa' solution was evaluated together with other flavoured samples, it was not indicated as having potential to decrease bitterness but was commented to not fit well with the bitterness. A theory by Mukai *et al*. (2007) might be part of an explanation. The authors concluded that strawberry flavour with strawberry aroma might reduce bitterness more than strawberry flavour without aroma, as strawberry aroma seemed to enhance sweetness in the composition.

Results showed that the lemon flavoured Malmberg citron and the orange flavoured Malmberg apelsin might have slightly positive effect of flavour and bitterness of BCAA solution. However, no significantly differing results were found. BCAA solution in Malmberg citron were almost significantly more palatable compared to Malmberg apelsin solution, lemon/lime flavoured solution and apple flavoured solution, all carbonated. The BCAA solution of Malmberg citron was also almost significantly more palatable compared to the apple flavoured solution.

Citrus flavourings were considered to match the bitterness of 5aa' solution best, while berry flavourings such as blackcurrant were commented to not correlate well with the bitterness. Flavourings that were pointed out as favourites by most assessors were grapefruit flavour and pomelo flavour. Lemon flavour was not indicated to suppress bitterness to a noteworthy degree. Another evaluation, by two assessors, indicated that citrus flavoured solutions were more bitter compared to unflavoured BCAA solution, with exception of pomelo flavour.

The pomelo flavoured BCAA solution, evaluated as mineral water by assessors who were first-time tasters, received an average likeness grade of 3.86 (on a scale of 0–10) that corresponds to dislike or dislike slightly. Differences in preferences, of for example flavouring and bitterness, were indicated to be rather large judging from the standard deviation (1.77).

Citric acid and sweeteners comprised in added flavours were concluded responsible for bitterness suppression (Miyanaga *et al*. 2004; Mukai *et al*. 2004), which suggests that flavour in itself does not affect bitterness. Evoked tastes in BCAA solution by added tasteless aromas were suggested to affect bitterness, where evoked sweetness and sourness seemed to suppress bitterness (Mukai *et al*. 2007).

Mukai *et al*. (2007) stated that individual flavour preferences affect the bitterness suppressing effect of chosen flavours. This would complicate studies and conclusions of flavour effects on bitterness.

6.1.4. Effect of Asp, Tau and Na^+

Preliminary studies showed that a strong concentration of Asp effectively reduced bitterness, with strong sourness and perhaps a slight umami taste. Sensory analyses of BCAA solutions containing Asp resulted in comparable mean bitterness scores corresponding to *slightly bitter* or somewhat higher but not as much as *bitter*.

Sensory analysis of sour strawberry flavoured BCAA solutions with Asp or citric acid indicated that Asp suppressed bitterness *moderately* to approximately *slightly bitter*, however no statistical differences were found. Bitterness score of the solution with Asp was somewhat lower than the solution with citric acid, as was the solution with masking flavour and Asp compared to the solution with masking flavour and citric acid. Both Asp and citric acid were indicated by literature to be bitterness suppressors (Tamura *et al*. 1990; Mukai *et al*. 2004). The substantially lowered pH in these solutions might have affected bitterness, and different acids can affect bitterness differently at different pH (Sakurai *et al*. 2009). Therefore, another sensory analysis of solution containing Asp at a normal pH was evaluated.

Bitterness of BCAA solution with Asp and normalized pH using NaOH (Na^+) was indicated reduced, somewhat bitterer than *slightly bitter*. The preliminary evaluation of BCAA solution containing Asp and NaOH at a normalized pH resulted in differing opinions on palatability. Two (female) considered the taste of solution with Asp and NaOH to be strongly unpalatable and/or salty. Two (male) instead rather preferred this solution in comparison to plain BCAA solution. Sensory analysis of pomelo flavoured solutions showed the solution with Asp and NaOH to be significantly less palatable than the Ramlösa water. It was considered unfresh, unpalatable or salty. However, some assessors did not point to anything deviating with this sample. Pomelo flavoured solution containing Tau was not indicated less bitter compared to pomelo flavoured plain BCAA solution, but rather slightly less palatable.

Because bitterness can be affected by low pH, acids and sodium salts (Breslin 1996; Sakurai *et al*. 2009), results cannot be used to conclude if Asp is an effective bitterness suppressor for BCAA solutions. Additive or suppressing effects, or confusion of senses, might have occurred, for example in the solution containing pomelo flavour, Asp and Na^+. Asp is sour and lowered pH, while NaOH gave a salty taste and increased pH. Both these tastes are perceived involving ion channels, while bitterness is perceived involving taste receptors.

6.1.5. BCAA Solutions

The flavours and compositions of waters used to prepare BCAA solutions differed considerably. For example, sodium content of Ramlösa water (220 mg/L) and Loka water (150 mg/L) were high, while content of Malmberg water was low (4.9 mg/L). Fluoride content of all waters were low (around 0.2 or less) except Ramlösa water which had a high fluoride content (2.7 mg/L). Swedish National Food Administration's threshold limit value was 100 mg/L for sodium and 1.5 mg/L for fluoride (Carlsberg Sverige's homepage 2010;

Spendrups homepage 2010; Malmberg homepage 2010; Sydvatten's homepage 2010; SLV 2001). As previously mentioned, low concentrations of minerals and salts can affect bitterness.

Solutions that were flavoured prior to carbonation seldom kept carbonic acid at all or kept it to a small degree. Professional advice by *Flavour Company A* was that products like mineral water are commonly, in industrial manufacturing, carbonated before addition of flavour and other ingredients. Water should thus be carbonated before addition of flavouring in experiments, and if possible before addition of a concentrated BCAA solution.

Normal pH in still mineral water (Malmberg stilla) was 7.8, which corresponded well to the producer's specified pH 7.9 (Malmberg homepage 2010). pH in Lund municipal water was specified to 8.2 (Sydvatten homepage 2010). Normal pH in 5aa' solutions was slightly lower at pH around 7.2 to 7.7.

Normal pH in carbonated mineral water, determined as a mean of pH in several flavoured and natural mineral waters, was concluded to be approximately 5.2. pH of carbonated Malmberg water and Ramlösa water were specified to 5.5 (Spendrups homepage 2010; Malmberg homepage 2010).

As the BCAA solution does not contain buffering molecules, pH is easily affected by added ingredients.

6.2. Parallel Tracks

Literature indicated that certain designed peptides might have a more favourable flavour profile compared to the amino acids with less or no bitterness. An industrial synthesizer of food grade peptides was found and designed peptides were ordered, which unfortunately were not delivered before the end of this project.

Literature and sensory analysis on solutions of the separate amino acids comprised in the composition indicated that the solution would be improved if Lys HCl were replaced by Lys. A manufacturer of Lys in its pure form was found, but the ordered amino acid was not delivered until this project had come to an end.

Two flavour companies were contacted and involved in the improvement of bitterness and flavour. *Flavour Company B* provided advice and flavour samples, which were used in this project. *Flavour Company A*, was provided with 5aa' water solutions and performed improvement studies of their own. In a follow-up meeting with *Flavour Company A*, flavourings similar to the solutions comprising strawberry flavour or lemon flavour in this study were presented. A strawberry flavoured solution and a lemon flavoured solution sweetened with sucrose were presented, which both were regarded rounder in taste with less bitterness but with unwanted sweetness. A solution flavoured with an unrevealed mix of citrus flavourings, reminding of lemon with a touch of pomelo flavour, was regarded best in flavour of the samples presented. The bitterness was however more noticeable in this sample compared to the sweetened lemon flavoured sample.

6.3. Experimental Design and Sources of Error

6.3.1. General Methods

Focusing on water solutions of the ingredients was beneficial as these were easy to study in sensory analyses and evaluations. Deionized water would be beneficial to use in similar studies, as it is neutral and without minerals or salts. Experiments were basically conducted using solutions of Malmberg water or Lund municipal water. These differ in flavour and mineral composition. Malmberg water was quite neutral in flavour, while the municipal water would be described as less neutral and less palatable.

Use of few assessors gives great risk of errors. As sensitivity to bitterness of different bitter compounds varies greatly between individuals, the risk of using few assessors in evaluation of bitterness is further enlarged. Around 10 to 20 assessors might be considered enough if these are healthy, non-smoking tasters of bitterness. In these studies however, assessors were not screened before sensory analysis or evaluation.

Repeatedly asking assessors, especially those who did not recognize bitterness in the BCAA solutions, to evaluate bitterness in solutions might have resulted in a subconscious grading of bitterness that was not actually perceived. An interesting example is results on evaluation of 5aa solution with or without Orn HCl, where one assessor comments that neither of the solutions was bitter but at the same time grades solutions as somewhat bitter.

Tasting methods used will affect the gradings of sensory qualities. A liquid sample will taste differently if it is swallowed immediately compared to if it is sipped to produce contact with taste buds in the whole mouth. Also temperature of the solutions and the speed of flow when swallowing or sipping will have effect (Stone and Sidel 1993). As an untrained panel was used, equality of tasting methods could not be assured.

The context around the experiment as well as the number of qualities to be graded in the analysis test form will affect the intensity of gradings (Breslin 1996). As an example, the weakest concentration of ascorbic acid evaluated was indicated as slightly less bitter than other solutions and references. This might be the case, but the grading might also be a result of only using a scale for bitterness and none for sourness. Frank *et al.* (1993) showed that a narrow number of qualities to be graded increased the risk of adding noted tastes of one type (that were not to be graded) to the scale of a taste that was graded.

At execution of most sensory analyses and evaluations, participants were provided with a *bitter* reference and a *not bitter* reference. Bitter references consisted of BCAA solutions, which were not only bitter but also with more complex taste (and flavour) profiles. The *not bitter* reference was a neutral water, without noticed taste or flavour. Lack of a *not bitter* reference with broader taste or flavour profile (similar to BCAA solution) might have influenced grading of bitterness. Assessors might have been subconsciously inclined to use an extended concept of bitterness, including associated or perceptually similar impressions (Frank *et al.* 1993). An unlabelled reference would be crucial for obtaining results that can be fully evaluated, but was not included in most analyses and evaluations. A labelled reference was not actually needed as the statistical method used for finding significant differences in analysis results eliminated differences in the use of the scales for bitterness and palatability.

6.3.2. Solutions

Differences in solutions might have affected flavour qualities, such as the isomaltulose solutions that were prepared using eight days old BCAA solution. The solutions in sensory analysis of BCAA solutions flavoured with pomegranate/ginseng and passion/acai were prepared without IngW, which might have affected flavour and bitterness.

Studied concentrations of Orn HCl, Asp, Glu and Tau were chosen based on concentrations and proportions between BCAA and bitterness suppressor found in literature. In the approximations of Asp, Glu and Tau needed to reduce bitterness in the BCAA solution, literature on a bitter solution of only 300 mM Val was used (Tamura *et al*. 1990). As Asp might not reduce bitterness of Leu or Ile or that another proportion between bitter compound and suppressant might be optimal, further studies are needed to improve results.

Reduction of pH in solutions to generate comparability to Asp solutions using citric acid restricted possibilities to draw conclusions. This was because both acids and low pH might reduce bitterness, and effects differ between combinations of different acids at varying pH (Breslin 1996; Sakurai *et al*. 2009). pH was not successfully lowered in the strawberry flavoured sample containing citric acid, which might have affected results.

In one or two occasions, plans to study carbonated solutions were changed as carbonic acid escaped completely or to a large extent. Results from studies with carbonates samples might have been affected due to varying levels of carbonic acid. This occurred because of difficulty in preparing different solutions of BCAA (especially when flavoured) containing similar concentrations of carbonic acid. Carbonic acid in solutions prepared from carbonated water was successfully eliminated in almost all cases. The unlabelled reference solution in the sensory analysis of pomelo flavoured BCAA solutions did however still contain some carbonic acid, which might have affected results significantly. This incident contradicts the supposition that flavoured solutions would hold carbonic acid to a lesser degree.

Increase of pH in Asp solution from around 3.4 to around 7.2 required large concentrations of NaOH. The choice of acidity regulator led to unpalatable off-taste, which might have been avoided if another additive had been used to normalize pH.

6.3.3. Method of Sensory Evaluation

Assessors in sensory evaluations, except evaluation of pomelo flavoured mineral water, were employees with knowledge of project details and therefore biased. Assessors in the evaluation of pomelo flavoured mineral water were first-time-tasters.

The evaluation of solutions with ascorbic acid or Resolver comprised 15 samples and two bitter references, which might have affected the quality of bitterness rating. Bitterness was described to linger after tasting. As the sense of taste is adaptable, the sensation of bitterness might have decreased as tasting proceeded. A comment on pomelo flavoured mineral water – that taste was improved after the first gulp – indicated that taste was adapted to bitterness.

Bitterness evaluation of 5aa solution with Orn HCl was performed with quite small samples – merely 7.5 ml per assessor, corresponding to a small or normal sip. As some assessors prefer greater amounts, this might have obstructed evaluation of bitterness. However, considering that only one quality needed to be graded (bitterness), this amount might have been beneficial as only the immediate impression could be rated.

Assessors in evaluation of pomelo flavoured mineral water were seated quite close to one another, which might have affected the outcome.

6.3.4. Method of Sensory Analysis

The sensory panel comprised untrained volunteers of varying capability of detecting bitterness, illustrated in figure 15. This variability of sensitivity to bitterness together with varying preferences of bitterness and flavours caused difficulty in acquiring significant results. As the panel was to reflect society in general, no volunteers were excluded from the sensory panel because of (what seemed to be) lacking ability to detect bitterness in samples.

Because so few assessors were used, results are unfortunately prevented from being used as more than indications to the acceptance of a larger consumer group.

Stone and Sidel (1993) expressed the importance of assessors to like the product and that they are capable of using preference rating scales to differentiate products. These details were not assured in experiments. Due to repetitive use of assessors, making them experienced, and that several were employees, making them biased, objectivity could not be assured. Three to eight of the assessors in each panel were familiar with the product and the aim of the project. Other assessors were volunteers found by advertisement, who were told that the product had positive health effects and that the aim of this project was to improve flavour. These were thus less biased, but could have been affected by the questions of bitterness and palatability ratings in the test form.

An untrained panel was used mainly because no trained panel was accessible and the time frame did not allow screening and training of respondents for a trained panel. Disadvantages with an untrained panel are for example that individual interpretations on definitions used in the test form might differ and that assessors might misinterpret or ignore instructions. A trained panel would have given more reliable results in the study of bitterness, partly because trained assessors would be chosen after screening the capability to detect bitter taste and partly because they would use standard references to compare sample characteristics to. A disadvantage of using a trained panel of respondents would have been that they were unfit for the acceptance-preference analysis.

Several answers of assessors differed significantly in sensory analyses, see the example presented in figure 15. Responses on palatability did not differ to the same extent as responses on bitterness. Choosing another design of sensory analysis might have prevented these disagreements. Further, screening assessors for ability to detect bitterness and differentiate between products using the hedonic scale before selecting assessors to the sensory panel would have been preferable (Stone and Sidel 1993). Another example, from analysis of separate amino acids, showed assessor 7 to be the only one to consider Thr bitter. Reasons might be that assessor 7 was more sensitive than other assessors, that the number of assessors was too low and perhaps that the question on grading bitterness led to a subconscious notion of bitterness. The fact that assessors were unable to volunteer for sensory analyses at the same time resulted in solutions sometimes being tasted at occasions spread over two to three days and at different sample temperatures, which might have contributed to the differing gradings by assessors.

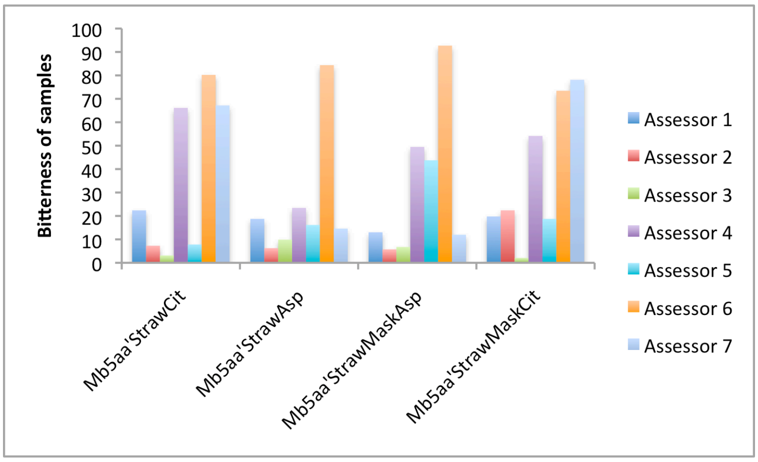

Figure 15. Example of differing bitterness ratings of assessors from an analysis of BCAA solutions.

Surroundings might have disturbed or influenced the performance of sensory analysis, such as sound or presence of company associates sporadically walking by, continuous change in light intensity due to the glass ceiling, changing art exhibitions on the walls in front of the assessors, change of table cloth from a terracotta coloured to a royal blue etcetera.

Sensory analyses and test forms were of similar design but were continuously improved during the project. Due to the varying design of sensory analysis forms, results are less comparable. As an example, maximum scores of bitterness scales varied from *very bitter* to *extremely bitter*. Modified versions of the nine-point hedonic scale were used. According to Stone and Sidel (1993), trials using modified versions have generally been shown unsuccessful.

Particularly design of sensory analyses and test forms used could be improved. Most importantly, nine point hedonic scales should be used without exception, as well as a test form broadened with grading of at least also sweetness, sourness and saltiness. Literature suggested that the design of sensory analyses and test forms might have large influence on the outcome of the analysis (Frank *et al*. 1993). Instructions can influence assessors to expand or narrow their definitions of a particular sensory attribute, for example sweet taste intensity together with fruitiness or without. The question formulation might bias assessors towards subconsciously noting and describing subtle differences between stimuli even when these were minimal (Frank *et al*. 1993). According to the authors, unpleasantness of citric acid might add to unpleasantness of quinine, contributing to an increase in unpleasantness that might be graded together as bitterness. The reason would be that concepts of bitterness and unpleasantness are sometimes related, as when an unpleasant event is referred to as *bitter* (Frank *et al*. 1993). Sourness of citric acid was repeatedly found to be confused with bitterness of quinine. Similarities along a critical dimension such as pleasantness might be important, but not a general similarity (Frank *et al*. 1993). The authors suggested that: "*When an odorant elicits sensations that are perceptually similar to a target taste and when only one rating category is available, subjects will tend to combine the taste and odor dimensions, leading to higher intensity ratings for the target taste. When taste and odor sensations are dissimilar or when appropriate additional attribute scales are provided, odor-induced taste enhancement will not be observed*." On the other hand, Frank *et al*. (1993) thought that people's reactions to a food product might be more accurately predicted from sensory analysis results where assessors had graded only bitterness or sweetness rather than multiple stimuli. The reason was illustrated by the example of the every-day questions asked to a coffee drinker; *Is your coffee too sweet?* or *Is your coffee too bitter?* The authors concluded that the appropriate design for a study depends on the question to be answered. Effects of perceptual and judgemental factors on responses to multiple-quality mixtures need to be further studied, according to Frank *et al*. (1993).

Results from the sensory analysis that comprised a solution without BCAAs (Ramlösa original with decreased carbonic acid), suggest that the sensory analysis design using only one scale for taste characteristics did not generate considerable deviations. Most assessors preferred pomelo flavoured Ramlösa original before pomelo flavoured BCAA solutions although a couple graded the Ramlösa solution more bitter or more unpalatable compared to BCAA solutions, which might be mostly because of individual taste sensitivities or question formulation.

6.4. Suggestions for Continued Studies

Results indicate that Lys HCl added to bitterness and unpalatability of the BCAA solution, and literature indicate Lys to have better flavour and taste profile compared to Lys HCl (Roudot-Algaron 1996). Therefore, replacing Lys HCl with Lys might improve the BCAA solution.

Addition of Asp together with a suitable acidity regulator to normalize pH might improve the sensory qualities of the BCAA solution. Optional acidity regulators to normalize pH without off-taste might be for example potassium carbonates (E 501), magnesium carbonate (E 504), potassium hydroxide (E 525), calcium oxide (E 529) or calcium hydroxide (E 526). However, as large concentrations might be needed to increase pH from around 3.4 to around 7.2, these additives might also generate off-taste. Note that Tamura *et al.* (1990) concluded that addition of Asp or Tau would be effective in BCAA solutions of low concentrations.

N-terminal acetylated amino acids (Ac-Leu, Ac-Ile and Ac-Val) are interesting possibilities if an appropriate method to deal with the sourness is available. N-terminal derivates of the bitter (perhaps also the unpalatable) amino acids and acidic amino acids (such as Asp or Glu) are worthy of note for similar reasons, although might be difficult to solve. Gly-Gly residues attached to the N-terminal and C-terminal of the bitter amino acids is also a possibility, however probably more expensive than acetylation (Roy 1992; Shinoda *et al.* 1987; Tamura *et al.* 1990).

The bitter or unpalatable amino acids might be interesting to substitute with one or more of the peptides Lys-Lys, Leu-Gly-Gly, Lys-Gly-Asp, Gly-Val-Gly, Val-Val-Gly, Leu-Asp HCl, Leu-Glu HCl and Glu-Leu HCl. For example, the bitter taste threshold of Leu-Gly-Gly was defined to 75 mM/L, which is interesting considering that less than 45 mM/L Leu is used in the BCAA solution and that the bitter threshold of Leu is as low as 20 mM/L. However, the solutions would be expensive, if they at all give desired flavour and taste characteristics (see section *3.5.4. Modification of amino acids and designed peptides)*. Other risks might be decreased solubility and unwanted flavour characteristics.

The area of available commercial bitterness (and sweetness) inhibitors could be looked into. Examples are AMP (McGregor 2004; Ming *et al.* 1999) and phosphatidic acid (PA) (Ley 2008; Nakamura *et al.* 2002). Also combinations of PA and α-lactalbumin or β-lactoglobulin have been shown to reduce BCAA bitterness, without effect of sweet, sour or salty tastes (Katsuragi *et al.* 1996a; Katsuragi *et al.* 1996b).

6.5. Reflections

A narrow and specific aim and question formulation is important to allow valuable results to be produced in a project. Related to this aspect, the present project was indeed too large to be carried out during this limited time.

Another reflection is the amount of time spent, that leads to no valuable results whatsoever in research projects. In my case, much time has been used to explore areas outside my academic expertise; particularly sensory analysis (which was also one reason to why I was eager to learn). I did learn a lot from the planning and performance of sensory analyses. Many mistakes might have been avoided if guidance in the design and performance of sensory analysis had been available.

Nevertheless, I value gained experiences high. Being involved with more or less “experts” in areas like flavour improvement and bitterness masking or in development and marketing of products with special biotechnological or nutritional characteristics were exciting peeks into the career of a product developer working with groundbreaking ideas.

7. Conclusions

Reducing bitter taste is a complicated and not fully understood area. Different specific methods are used to suppress bitterness in each bitter compound. No bitterness inhibitor with universal effect had been found as of 2008, according to Ley (2008).

A promising alternative to reduce bitterness in the BCAA solution would be to modify the amino acids by acetylation. Also to replace unpalatable or bitter amino acids with peptides comprising one of the amino acids might be a promising alternative.

The choice of water, more specifically the composition of minerals and salts, might considerably affect flavour qualities of the BCAA solution.

Results and literature indicated that a decreased content of Leu in the BCAA solution would be favourable for bitterness and palatability, as well as replacing Lys HCl with Lys. Results indicated 5aa solution to be less bitter than 3aa solution. The effects of a specific flavour on bitterness seemed to differ greatly depending on individual preferences or differences in bitterness sensitivity. No method used resulted in effective or total bitterness reduction of BCAA solutions. The amino acid concentrations were probably too high to allow successful bitterness reduction using added ingredients in concentrations that would not alter flavour characteristics in any other undesired way. Of the ingredients studied, Asp seemed to have greatest potential to suppress bitterness of the BCAA solution. Carbonation showed potential in reducing bitterness.

Additive or suppressive effects might have occurred in several of the studied solutions, for example in the solution containing pomelo flavour, Asp and NaOH or in the solution containing strawberry flavour and citric acid at a low pH. This screening did not fully study the effects of several added ingredients such as Orn HCl, Tau, Asp, citric acid, isomaltulose or Resolver.

Last but not least, another design of sensory analysis would probably have improved results by reducing differences in assessors' ratings. Important changes would be to use a larger number of assessors and to ask assessors to grade also sweet, sour, saltiness, fruitiness etcetera. Screening assessors for the ability to detect bitterness and differentiate between products using the hedonic scale would probably have improved results.

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- *Malin*

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