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Description

[0001] The present invention relates to a composition comprising free amino acids, oligopeptides, and polypeptides, as well as to the preparation thereof through enzymatic digestion of natural protein sources. The invention is described particularly in the context of its use in the manufacture of foods and beverages, of cosmetics and soaps, and of body care and slimming products. It should be pointed out that the invention is also applicable to other areas, for example the manufacture of animal feed.

[0002] The 20 proteinogenic amino acids are the building blocks of all cellular proteins, including structural proteins, transport proteins, storage proteins, immunoglobins, and enzymes. Most higher organisms depend on food to supply them with individual amino acids (essential amino acids). In humans, these are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. A sufficient supply of amino acids is essential for protein synthesis. Protein synthesis is thus limited quantitatively by the respective limiting amino acid. Protein synthesis is indispensable for all cellular processes. An inadequate supply of amino acids leads to deficiency diseases, marasmus, and a substantial weakening of the immune system, and it has impacts on the vitality and outer appearance of hair, nails, and skin. An adequate supply of amino acids is therefore particularly important for people who play sports, as well as for people who wish to lose weight or people with poor food assimilation/food utilization due to existing health problems, but who wish to be quickly and fully supplied with amino acids and peptides/proteins in order to maintain resistance and musculature or to increase muscle development.

[0003] Amino acids are also constituents of NMF (natural moisturizing factor), a main component of the skin that is responsible for moisture regulation and other skin functions, and collagen, one of the most important structural proteins of connective tissue and skin. Free amino acids also stabilize the protective acid mantle of the skin. For these reasons, amino acids, peptides, and proteins are used in skin and hair cosmetics.

[0004] Amino are acids are ingested with food, mainly in the form of proteins that are then hydrolyzed in the stomach and the small intestine. The resulting amino acids, as well as di- and tripeptides, are reabsorbed in the intestine. It is known that bioavailability can be accelerated substantially through ingestion of already pre-digested, i.e., hydrolyzed, proteins. Free amino acids can be reabsorbed virtually immediately once they reach the intestine. Sports physicians in particular administer amino acids after training and point out that it takes about 30 minutes for the amino acids to be absorbed in the bloodstream. By comparison, consuming eggs would not lead to amino acid absorption until more than five hours later. In the meantime, supply gaps may occur, and the immune system may be weakened.

[0005] DE 196 06 439 A describes a method for the enzymatic degradation of proteins. In the method described therein, preferably protease-producing microorganisms are used rather than purified proteases that are prepared and provided separately.

[0006] WO 2014/130007 A1 describes proteolytic compositions for rapid and extensive digestion of protein supplements. The proteolytic compounds contain subtilisin Carlsberg and, optionally, bromelin, and proteolytic digestion takes place exclusively in the test subjects' bodies.

[0007] It is the object of the invention to provide an alternative method for preparing a composition comprising free amino acids, oligopeptides, and polypeptides.

[0008] This is achieved according to the invention through the teaching of independent claim 1. Preferred refinements of the invention constitute the subject matter of the subclaims.

[0009] According to a first aspect, the invention relates to a method for preparing a composition comprising free amino acids, oligopeptides, and polypeptides.

[0010] A method according to the invention has at least the step of adding at least two different peptidases, which are present in purified form, to a composition having

at least one protein, wherein at least one exopeptidase and at least one endopeptidase are added to the composition having at least one protein, and wherein

(a) the at least two different peptidases are of microbial origin;

(b) the composition comprising at least one protein has a protein concentration of 5 to 30% by weight based on the total weight of the composition comprising at least one protein;

(c) the composition comprising at least one protein has a pH of from 4 to 6 when the at least two different peptidases are added and is incubated at a temperature of 40 °C to 60 °C with the at least two different peptidases; and

(d) the total amount of peptidase added is from 2 to 10 grams in each case per 100 grams of the at least one protein in the composition comprising at least one protein.

[0011] In the context of the present invention, it was found that, with the appropriate design, the yield of free amino acids and/or of oligopeptides with a length of up to 10 amino acids in the enzymatic digestion of at least one protein can be improved if, instead of adding peptide-producing bacteria, at least two peptidases in purified form are added to the at least one protein. In particular, it was found that, through appropriate design of the method of preparation, compositions with all eight amino acids that are essential for humans or even with all 20 proteinogenic amino acids can be obtained by using two different purified peptidases. Compositions prepared by means of the method of preparation according to the invention are therefore suitable for filling supply gaps of individual amino acids and can thus prevent deficiency diseases or deficiency symptoms such as those of the skin or hair, marasmus, and weakening of the immune system.

[0012] The composition can optionally also comprise varying quantities of the uncleaved source protein, depending upon the duration and efficiency of the fermentation and the quantity of peptidases used.

[0013] Furthermore, it was found that, with the appropriate design, the process duration of the method according to the invention can be shortened in comparison to the bacterial fermentation of proteins, i.e., the enzymatic digestion of proteins through

the addition of peptidase-producing bacteria. This enables production cycles to be designed more efficiently and makes the use of large fermentation tanks unnecessary.

[0014] Amino acid-containing compositions that are obtained by conventional bacterial fermentation, i.e., through the addition of peptidase-producing bacteria, typically have a distinct, bitter taste. In addition, a strong foul taste is frequently reported, which can render such compositions virtually unsuitable for human consumption. Surprisingly, both foul and bitter tastes can be reduced or even eliminated completely by the method according to the invention with the appropriate design. The amino acid-containing compositions prepared using the method according to the invention can, with the appropriate design of the method, be free of off-flavors and cheesy aftertastes.

[0015] In the context of the invention, the term "protein" is understood to mean organic molecules that are composed of amino acids that are interconnected by peptide bonds. In the context of the invention, proteins are undigested, meaning that they do not originate from the hydrolysis of a larger protein. In the context of the invention, the size of proteins is not limited, although proteins preferably comprise at least 100 amino acids. In the context of the invention, proteins particularly comprise animal, vegetable, and fungal proteins, although the use of proteins of bacterial or other microbial origin as a starting substrate for enzymatic hydrolysis is not excluded. In the context of the invention, proteins are not in any way limited in terms of their secondary, tertiary, and quaternary structure and include globular and fibrillary proteins. Nor are proteins limited in the context of the invention in terms of their function and therefore include structural proteins, transport proteins, storage proteins, immunoglobulins, enzymes, and others.

[0016] The term "peptidase" is understood in the context of the invention to mean enzymes that are capable of cleaving proteins or peptides. Peptidases catalyze the hydrolysis of peptide bonds in peptides and proteins. The expressions “protein or peptide cleavage,” “protein or peptide digestion,” and “hydrolysis of peptide bonds” are used synonymously here. The expression “fermentation of proteins, peptides, or protein sources" is understood in the context of the invention to mean the enzymatic cleavage of proteins and peptides. The expression "bacterial fermentation" is

understood to mean fermentation through the addition of peptidase-producing bacteria. The expression "enzymatic fermentation" is understood to mean fermentation through the addition of purified peptidases. The term "peptidase" is used synonymously with the terms "protease" and "proteinase" here. In the context of the invention, the term "peptidase" is understood to mean the enzyme in purified form, unless expressly mentioned otherwise. The expression "in purified form" is understood to mean the presence of the enzyme in free form in an enzyme preparation. Such enzyme preparations can be produced in any manner, for example through isolation and purification of the enzyme after homologous or heterologous expression in microorganisms. The use of purified peptidases offers the advantage over the use of peptidase-producing bacteria of better controllability and reproducibility of the method. Furthermore, the risk of contamination with bacteriophages can be avoided.

[0017] In the context of the present invention, the expression "free amino acids" is understood to mean amino acids that do not have peptide bonds to other amino acids. Free amino acids arise in the context of the invention through the enzymatic digestion of proteins or peptides, including oligopeptides, as a result of the enzymatic hydrolysis of peptide bonds in these proteins or peptides.

[0018] The term "peptide" is understood in the context of the invention to mean a cleavage product of a protein that arises through enzymatic hydrolysis of one or two of the peptide bonds in the protein.

[0019] The term "oligopeptide" is understood in the context of the invention to mean a peptide, i.e., a cleavage product of a protein produced by enzymatic digestion, which consists of 2 to 10 amino acids. The term “oligopeptide” therefore encompasses the terms “dipeptide” (peptide consisting of two amino acids), “tripeptide” (peptide consisting of three amino acids), “tetrapeptide” (peptide consisting of four amino acids), and “pentapeptide” (peptide consisting of five amino acids).

[0020] The term "polypeptide" is understood in the context of the invention to mean a peptide, i.e., a cleavage product of a protein produced by enzymatic digestion, which consists of at least 11 amino acids. The only restriction on the upper limit of the length of a polypeptide in the context of the invention is that the polypeptide must have at

least one fewer amino acid than the source protein, the cleavage of which gave rise to the polypeptide.

[0021] Compositions that are produced by means of the method of preparation according to the invention offer the advantage over conventional dietary protein that amino acids and oligopeptides can be reabsorbed substantially faster in the body than can uncleaved proteins. Free amino acids are reabsorbed virtually immediately upon reaching the small intestine. Depending upon the quantity of the amino acids consumed, reabsorption may take as long as an hour, for example. Oligopeptides must be cleaved into free amino acids before they can be reabsorbed, thus delaying reabsorption by approximately half an hour. In the case of polypeptides, cleavage into free amino acids in the small intestine takes about one to two hours, depending upon the length of the polypeptide. The method of preparation according to the invention thus provides compositions that ensure a rapid, almost immediate, progressive supply of amino acids. A continuous supply of amino acids of virtually immediate onset and several hours in duration is made possible by the simultaneous presence of free amino acids, short oligopeptides, longer polypeptides, and optionally still uncleaved proteins in the composition.

[0022] In body care products, the amino acids and oligopeptides produced by means of the method according to the invention can serve as a protein source for the formation of collagen and elastin, which occur naturally in the skin. Collagens possess a high molecular weight and are therefore unable to penetrate the skin. They have a skin toning effect and are highly moisture-binding. Soluble collagen accelerates wound healing and spontaneous re-epithelization of the skin. In doing so, it stimulates tissue reconstruction and macromolecule formation.

[0023] Peptides are also part of the connective tissue, hair, and nails. They act as messenger substances, hormones, or coenzymes and play key roles in nearly all biological processes. They thus accelerate healing processes and cell renewal. Peptides often have regulatory functions. For example, insulin is a peptide that controls sugar metabolism in the body.

[0024] Peptides, particularly oligopeptides, are partially broken down on the skin surface into free amino acids and thus enhance the natural moisture content of the skin. As constituents of NMF (natural moisturizing factor), amino acids play an important role in moisture regulation and other functions of the skin. Free amino acids also stabilize the protective acid mantle of the skin.

[0025] In the method according to the invention, at least one exopeptidase and at least one endopeptidase are added to the composition comprising at least one protein. This offers the advantage over the exclusive use of endo- or exopeptidases that, for the same total amount of peptidases used and for the same process duration, the content of free amino acids and/or of oligopeptides can be increased at the expense of the polypeptides and/or of the at least one source protein, i.e., of the at least one protein in the composition comprising at least one protein. Specifically, this offers the advantage that, with the appropriate design, an ideal proportion of free amino acids, oligopeptides, and polypeptides can be achieved.

[0026] In the context of the invention, the term "endopeptidase" is understood to mean a peptidase that is capable of catalyzing the hydrolytic cleavage of peptide bonds within a peptide chain. The cleavage products each have at least two amino acids, typically more amino acids.

[0027] In the context of the invention, the term "exopeptidase" is understood to mean a peptidase that is capable of catalyzing the cleavage of individual amino acids at the ends of a peptide chain.

[0028] An ideal composition is characterized by a high proportion of free amino acids and oligopeptides. The composition produced by means of the method of preparation preferably contains 0.5 to 25% by weight of free amino acids, 25 to 65% by weight of oligopeptides, and at most 40% by weight of uncleaved source protein based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases. Especially preferably, the composition produced by means of the method of preparation contains 2 to 20% by weight of free amino acids and at most 10% by weight of uncleaved source protein based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the

added peptidases. By comparison, depending upon the duration of fermentation, which typically ranges from a few days to several weeks or even years, such as in the traditional KOSO process from Japan, for example, only about 1 to at most 12% by weight of free amino acids based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins are obtained in conventional fermentation of protein sources by peptide-producing bacteria.

[0029] The content of free amino acids and oligopeptides in the prepared composition is determined by the method described below. After incubation with the at least two peptidases is complete, samples are taken and homogenized. 0.5 g - 2.5 g of the homogenized sample are weighed to the nearest 0.1 mg into a Falcon tube. 1 ml norleucine solution (0.1312 g/200 ml) are added as an internal standard (IS) and topped up to 25 ml with 3% 5-sulfosalicylic acid. The tubes are placed in a shaker and shaken vigorously for 20 minutes. They are then centrifuged for 5 minutes at 5000 rpm. The supernatant is filtered through a syringe filter (0.45 µm). 0.5 ml of the filtrate and 0.5 ml sample buffer for chromatography are pipetted into a reaction vessel, which is then sealed. The solution prepared in this manner is used for chromatographic analysis on an amino acid analyzer.

[0030] The amino acid composition of the fraction containing free amino acids and oligopeptides (water-soluble NPN fraction of the composition) is determined in parallel to this. To this end, 0.5 g - 2.5 g of the homogenized sample are again weighed to the nearest 0.1 mg into a Falcon tube. 2.5 ml norleucine solution as an internal standard (IS) are pipetted in and topped up to 25 ml with 3% 5-sulfosalicylic acid. The tubes are shaken and centrifuged as described above; the water-insoluble peptides and proteins contained in the precipitate are discarded. 1 ml of the supernatant is pipetted into a 50 ml glass digestion vessel and mixed with 9 ml 6N hydrochloric acid, and the vessels are tightly sealed with a screw cap. The vessels are placed in the heating block, and the sample solution is digested at 105 °C for 8 h. 2 ml of the disrupted solution are evaporated to dryness. The residue is taken up in 1 ml sample buffer for chromatography and transferred to a reaction vessel, which is sealed. The solution prepared in this manner is used for chromatographic analysis on an amino acid analyzer. In addition, the content of oligopeptides in the composition can be calculated by subtracting the content of free amino acids determined in step 1.

[0031] Preferably about 40 to 80% by weight, especially preferably about 50 to 70% by weight, of the free amino acids are essential amino acids, namely isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

[0032] Preferably all 20 proteinogenic amino acids, namely the amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, aspartic acid, asparagine, cysteine, glutamine, glutamic acid, glycine, histidine, proline, serine, and tyrosine, are contained in the composition produced by the method of preparation according to the invention.

[0033] The simultaneous presence of free amino acids, oligopeptides, and polypeptides in said concentrations and in the resulting ratios to each other in the produced composition offers the advantage of a progressive, continuous supply of amino acids with practically immediate onset after ingestion of the composition over several hours.

[0034] According to the invention, the at least two different peptidases are of microbial origin. According to a preferred refinement, the at least two different peptidases are of microbial origin, preferably of bacterial or fungal origin. Microbial peptidases are considerably more diverse in comparison to human or mammalian peptidases. This offers the advantage that even protein sources that are not readily metabolizable by human beings can be efficiently digested.

[0035] The expression "of microbial origin" is understood to mean peptidases with amino acid sequence corresponding to that of a microbial peptidase — i.e., a peptidase that is expressed endogenously in a microorganism. In the context of the invention, the organism in which the peptidase is produced (for example by heterologous expression) is irrelevant to the designation. Peptidases of microbial origin are thus expressed endogenously in microorganisms. In the context of the invention, “microorganisms” are understood to mean the totality of all organisms that are not visible to the naked eye. This includes viruses, bacteria, archaea, protozoa, fungi, and microalgae.

[0036] According to a preferred refinement, the composition comprising at least one protein has a protein concentration of 10 to 20% by weight based on the total weight of the composition comprising at least one protein; It was found that the aforementioned substrate concentrations are advantageous for enzyme activity, since the enzyme and the substrate are able to come into contact comparatively quickly. This refinement thus offers the advantage of good yields of free amino acids and/or oligopeptides and/or a short process duration.

[0037] According to a preferred refinement, the composition comprising at least one protein is incubated with at least one of the at least two different peptidases for at least 1 h, preferably for a period of 1 h to 24 h, especially preferably for a period of 4 h to 12 h, most preferably for a period of 6 h to 10 h. This refinement offers the advantage that both the demand for efficient cleavage of the at least one source protein and the demand for a time-efficient process are fulfilled. An ideal proportion of free amino acids, oligopeptides, and polypeptides can be obtained with the aforementioned incubation times.

[0038] According to a preferred refinement, the composition comprising at least one protein is incubated at a temperature of 45 °C to 55 °C with the at least two different peptidases. These relatively high temperatures offer the advantage that they enable contamination with bacteria and thus also with bacteriophages to be avoided. Health risks are thus minimized, and decay processes, which can negatively impact the taste and odor of the final product, are thus prevented. Because enzyme producers typically recommend incubation temperatures of about 37 °C for peptidases in order to prevent enzyme denaturation at higher temperatures, the finding that an enzymatic hydrolysis of proteins is feasible at such relatively high temperatures was completely surprising. It was found that, at temperatures of about 50 °C, the peptides used exhibit particularly high activities and that the method of preparation can thus be shortened. In order to obtain optimal yields of free amino acids and oligopeptides, it can be expedient to use the at least two peptidases in higher amounts than is usually recommended for enzymatic digestion at 37 °C. In addition, the repeated addition of the peptidases during the method of preparation can have a positive effect on the yield of free amino acids and oligopeptides.

[0039] According to a preferred refinement, upon addition of the at least two different peptidases, the composition comprising at least one protein has a pH of from 4 to 6, preferably from 4.5 to 5.5, the pH being optionally adjusted by adding at least one organic acid particularly selected from among lactic acid, citric acid, and malic acid. It is preferable that no bacteria or other microorganisms such as yeasts, for example, be added during the preparation process. The aforementioned pH ranges are optimal for the enzyme activity of peptidases. In contrast, the pH must be lowered to about 3 to 4 in the case of conventional fermentation with peptidase-producing bacteria. The fermented product produced has a very sour taste as a result. By using purified peptidases in a pH range of greater than 4, the taste of the produced product can be substantially improved. The compositions produced have a slightly acidic to neutral taste.

[0040] According to a preferred refinement, the method also comprises the step of adding calcium carbonate and/or magnesium carbonate, the addition preferably taking place after the addition of the at least two different peptidases, especially preferably upon completion of the incubation of the composition comprising at least one protein with the at least two peptidases. This refinement offers the advantage that the ratio of calcium to magnesium in the final product can be adjusted to a desired value. The weight ratio of magnesium to calcium in the prepared composition is preferably about 1.25:1 to about 3:1, especially preferably about 1.5:1 to about 2.5:1, and most preferably about 2:1. An additional advantage of this refinement is that the pH of the final product can be raised after enzymatic hydrolysis is complete, thus making it possible to improve the taste of the final product.

[0041] According to a preferred refinement, the composition comprising at least one protein comprises at least one protein source selected from among whey, whey protein concentrate, milk protein concentrate, slurry from food production such as distillery slurry or brewery slurry, peas, lentils, beans, soybeans, and a combination of two or more thereof. The use of natural protein sources such as the ones mentioned above has the advantage that the naturally occurring ingredients in these protein sources such as coenzymes, vitamins, and trace elements are maintained in the prepared composition and can effect a particularly rapid and/or efficient reabsorption in the intestine. In principle, all types of protein sources are conceivable as substrates for the

method of preparation according to the invention. Depending upon the use of the final product, particularly high-quality protein sources such as whey and milk protein concentrate, vegetable protein sources such as soybean, peas, lentils, beans, brewery and distillery slurries for the preparation of vegetarian or vegan products, or relatively inexpensive protein sources such as brewery and distillery slurries can be selected and optionally combined. In addition to its high quality, whey as a protein source also has the advantage of a high content of additional ingredients of value to human beings such as vitamins, coenzymes, minerals, and others. This "whey matrix" also increases the bioavailability of amino acids, oligopeptides, and polypeptides by accelerating reabsorption. Supplying amino acids and oligopeptides that are present in such a whey matrix is considered to be more efficient than supplying chemically synthesized amino acids that are added to a product. Whey protein concentrate has the additional advantage of a high protein content of about 80% and a low carbohydrate and fat content. Whey protein concentrate is thus comparatively low in calories. Furthermore, it has a very low lactose content. Regardless of the protein source used, the lactose remaining in the final product can be optionally removed without any problem, for example through the addition of purified lactase or lactase-producing lactobacilli.

[0042] In the context of the invention, the term "protein source" is understood to mean a composition comprising at least one protein, preferably a protein mixture. The term "protein" as used in this context encompasses animal, vegetable, and microbial proteins.

[0043] According to a preferred refinement, the total amount of each added peptidase is from 500 to 20,000, preferably from 1,000 to 15,000, especially preferably from 2,000 to 10,000 U per 100 grams of the at least one protein in the composition comprising at least one protein.

[0044] In the context of the invention, a U is understood to mean the quantity of enzyme that cleaves one micromole of substrate protein per minute at 37 °C.

[0045] According to the invention, the total amount of peptidase added is from 2 to 10 grams per 100 grams of the at least one protein in the composition comprising at least one protein.

[0046] The specified amounts of peptidase added are higher than the manufacturers' recommendations for enzymatic digestion at 37 °C. These refinements offer the advantage of enabling the efficiency of the enzymatic digestion of the at least one source protein to be increased, thereby enabling the yield of free amino acids and/or oligopeptides to be increased and/or the duration of the method of preparation to be decreased. It was surprisingly found that the use of peptidases in excess, for example in the specified quantity ranges, permits the incubation temperature to be increased to over 40 °C up to about 60 °C without enzymatic hydrolysis coming prematurely to a standstill as a result of enzyme degradation. Increasing the incubation temperature to over 40 °C, in turn, reduces or completely eliminates the risk of contamination with bacteria, including bacterial pathogens, and thereby simultaneously reduces or completely eliminates the risk of contamination with bacteriophages. The method of preparation is preferably carried out at about 45 °C to about 55 °C, especially preferably at about 50 °C, since it is possible in that temperature range to prevent a contamination with bacteria and/or bacteriophages while simultaneously minimizing the degradation of the added peptidases.

[0047] According to a preferred refinement, at least one of the at least two peptidases is added repeatedly to the composition comprising at least one protein. This refinement offers the advantage that the premature standstill of the process due to degradation of the added peptidases can be prevented, or more specifically that the total quantity of added peptidase can be reduced, particularly at incubation temperatures of over 40 °C, over 45 °C, or at about 50 °C.

[0048] According to a preferred refinement, at least one of the at least two peptidases is added repeatedly in substantially equal amounts and in substantially equal time intervals to the composition comprising at least one protein. By adding the peptidases repeatedly in substantially equal amounts and in substantially equal time intervals, it was found that the efficiency of the enzymatic hydrolysis can be increased, or that the total quantity of peptidases used can be reduced for a comparable composition of the final product.

[0049] According to a preferred refinement, the at least one endopeptidase is added before adding the at least one exopeptidase. Surprisingly, it was found that, by doing so, the bitter taste of the prepared composition can be reduced or even eliminated entirely. In particular, the amino acids valine, leucine, isoleucine, and arginine have a bitter taste. A high concentration of these free amino acids can render a composition very bitter to inedible. Amino acid-containing compositions that are obtained by bacterial fermentation, i.e., through the addition of peptidase-producing bacteria, typically have a distinct bitter taste, which can make them unsuitable for human consumption. The reduced bitterness of the composition prepared by means of the method according to the invention could be a result of the formation of ornithine during enzymatic digestion. It is known that ornithine can help reduce the bitterness of amino acid-containing compositions.

[0050] According to a preferred refinement, the composition comprising at least one protein is initially incubated with the at least one endopeptidase for 1 to 10 h, preferably for 2 to 6 h, especially preferably for about 5 h, and then incubated with the at least one exopeptidase for 3 to 40 h, preferably for 20 to 40 h, especially preferably for about 30 h, with the incubation with the exopeptidase being preferably performed in the presence of the at least one endopeptidase. The at least one endopeptidase and/or the at least one exopeptidase are/is preferably added repeatedly, preferably twice, three times, four times, five times, or six times, in substantially equal amounts and in substantially equal time intervals. This refinement offers the advantage that a composition with optimum proportions of free amino acids, oligopeptides, and polypeptides is obtained.

[0051] According to a second aspect, the invention relates to a composition comprising free amino acids, oligopeptides, and polypeptides, which can be obtained by at least one of the methods described herein, characterized in that the composition comprises (a) from 0.5% by weight to 25% by weight of free amino acids based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases and (b) from 25 to 65% by weight of oligopeptides based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases.

[0052] A composition containing free amino acids, oligopeptides, polypeptides, coenzymes, vitamins, and trace elements is also described, the composition preferably containing at least the following free amino acids: Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, the composition especially preferably containing the 20 proteinogenic amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, aspartic acid, asparagine, cysteine , glutamine, glutamic acid, glycine, histidine, proline, serine, and tyrosine.

[0053] The free amino acids, oligopeptides, and polypeptides were obtained by enzymatic hydrolysis of at least one protein with at least two different purified peptidases. Compared to the conventional fermentation of protein sources by means of peptidase-producing bacteria or other microorganisms, this offers the advantage of especially high proportions of free amino acids and/or oligopeptides in the composition. In particular, with the appropriate design of the enzymatic hydrolysis with the at least two different purified peptidases, it is possible to obtain a composition with all eight amino acids that are essential for human beings or even with all 20 proteinogenic amino acids. These compositions are thus suitable for filling supply gaps in individual amino acids and can thus prevent deficiency diseases, marasmus, and weakening of the immune system.

[0054] According to the invention, the composition comprises from 0.5% by weight to 25% by weight, preferably from 2% by weight to 20% by weight, especially preferably from 5% by weight to 15% by weight of free amino acids based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases.

[0055] According to another preferred embodiment, the composition comprises from 0.3 to 15% by weight, preferably from 1.2% by weight to 12% by weight, especially preferably from 3 to 9% by weight in total of the free essential amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases.

[0056] According to the invention, the composition comprises from 25 to 65% by weight of oligopeptides based on the total weight of free amino acids, oligopeptides, polypeptides, and proteins without the added peptidases.

[0057] According to another preferred refinement, from 0% by weight to 40% by weight, preferably from 0% by weight to 20% by weight, especially preferably from 0% by weight to 10% by weight of the at least one source protein for the enzymatic hydrolysis is present in the composition as uncleaved protein.

[0058] These refinements offer the advantage of a progressive, continuous supply of amino acids with practically immediate onset after ingestion of the composition over several hours.

[0059] According to a preferred refinement, the composition has a pH of from 4 to 6, especially preferably from 4.5 to 5.5. This refinement offers the advantage that the pH of the composition is similar to that of the skin and can therefore be advantageously used for the preparation of body care products. This refinement also offers the advantage that it is relatively neutral in taste, particularly not too sour, and can therefore be advantageously used for the preparation of food products.

[0060] According to a preferred refinement, the weight ratio of magnesium to calcium in the composition is from 1.25:1 to 3:1, preferably 1.5:1 to 2.5:1, especially preferably about 2:1.

[0061] The composition can be used immediately after enzymatic digestion, without any further purification and/or isolation steps, for the preparation of food products or body care products. It is also conceivable to eliminate individual components from the composition, such as lactose or uncleaved protein and/or water-insoluble polypeptides, for example. The volume of the composition obtained by enzymatic digestion can also be reduced by any method, or a powder comprising amino acids and oligopeptides as well as optionally polypeptides and/or uncleaved protein can be made from the prepared composition.

[0062] According to a third aspect, the invention relates to the use of the composition according to the invention for the preparation of a food product, particularly for the preparation of a fruit juice beverage, a milk beverage, a whey beverage, a yogurt, an ayran, or a kefir.

[0063] According to a preferred refinement, a portion of the water of a food product, particularly of a known food product, is replaced with the composition according to the invention. Food products can thus be particularly easily "refined" and improved in quality by adding the composition according to the invention.

[0064] According to a fourth aspect, the invention relates to the use of the composition according to the invention for the preparation of a body care product, particularly for the preparation of a soap, a shampoo, a hair treatment, a hair mask, a hair pack, a hair tonic, a conditioner, a bath essence, a body lotion, a body gel, a lotion for treating rosacea, a cream for treating acne, a facial mask, or a facial cream.

[0065] According to a preferred refinement, a portion of the water, preferably at least 80% of the water, of a body care product, particularly of a known body care product, preferably of a soap, is replaced with the composition according to the invention. Body care products can thus be "refined" and improved in quality with particular ease by adding the composition according to the invention. In particular, the improved skin tolerance of such compositions should be emphasized. The oligo- and polypeptides in soaps and body care products produced in this manner are capable of promoting collagen formation in skin and hair and thus counteracting skin irritations.

[0066] According to a fifth aspect, the invention relates to a food product comprising the composition according to the invention.

[0067] According to a preferred refinement, the total content of free amino acids, oligopeptides, and polypeptides in the food product is from 2% by weight to 25% by weight, preferably from about 5 to 10% by weight.

[0068] According to a preferred refinement, the total quantity of free amino acids, oligopeptides, and polypeptides in the food product is 5 to 50 grams, preferably about

10 grams. This refinement offers the advantage that a substantial portion of the daily recommended protein dose can be covered by ingesting the food product. It is recommended that an adult consume about 50 to 100 grams of protein per day. With increased physical stress, the recommended amount of protein doubles.

[0069] According to a preferred refinement, the food product is a beverage comprising magnesium and calcium. The ratio of magnesium to calcium in the beverage is preferably 1.25:1 to 3:1, especially preferably about 2:1.

[0070] According to a preferred embodiment of this refinement, the beverage is an ayran. The total amount of free amino acids, oligopeptides, polypeptides, and proteins without peptidases is preferably between 5 and 50 g per portion, especially preferably about 10 g per portion. The ayran beverage preferably has exclusively vegetable amino acids, oligopeptides, and polypeptides, especially preferably amino acids, oligopeptides, and polypeptides originating from soy protein. Preferably, the beverage is substantially free of bacteria.

[0071] According to an alternative embodiment of this refinement, the beverage comprises caffeine and/or taurine. Together with caffeine and/or taurine, and in conjunction with calcium and magnesium, the beverage according to the invention supplies the body with important nutrients and can thus improve the condition of early risers, night owls, or nightshift workers, strengthen the body's defenses, and reduce the risk of infection due to overexertion.

[0072] According to a sixth aspect, the invention relates to a body care product comprising the composition according to the invention.

[0073] According to a preferred refinement, the body care product is a body wash or bath additive. The body wash or bath additive is preferably a whey bath comprising enzymatically fermented whey. The body wash is preferably marketed as a ready-made product in a volume of 1 to 25 liters. The body wash or bath additive preferably comprises neither soap nor shampoo. The body wash or bath additive gently cleanses the skin and prevents it from drying out. It is capable of regenerating the acid mantle of the skin and helps heal skin inflammations and eczemas.

[0074] According to a preferred refinement, the body care product is a soap. The soap preferably has a pH of from 5 to 7, especially preferably about 5.5. This corresponds approximately to the pH of the skin and is therefore particularly gentle on the skin. However, soaps in the alkaline range, for example with a pH of from about 7 to 8.5, are not excluded. It has been found that the peptides contained in the soap prepared by enzymatic fermentation can reduce the negative effect of the soap on the acid mantle of the skin and enhance the regeneration of the skin. Soaps comprising the amino acid-containing composition produced by enzymatic fermentation can alleviate skin dryness and itching.

[0075] Also described herein are soaps comprising amino acids and peptides that are obtained by enzymatic and/or bacterial fermentation.

Examples

Example 1:

[0076] Whey protein concentrate WPC 80 with a dry matter content of 25% and a protein content of 80% in the dry matter was diluted 1:2 with water. The protein content of the solution prepared in this manner was 10% by weight based on the total weight of the prepared solution.

[0077] The pH of this solution was adjusted to 5.5 with citric acid.

[0078] Two test batches of 1 liter each (100 g protein) were prepared. 2 g of an endopeptidase with an activity of about 1000 SAPU/g were added to each of the two batches at the beginning and five more times in one-hour intervals. The total quantity of added endopeptidase was 12 g/100 g source protein in each case.

[0079] The batches were incubated at 50 °C.

[0080] After five hours, a 50 ml sample was taken from each batch (samples I5 and II5 - comparison example). The batches were further incubated overnight at 50 °C.

[0081] The next morning, 2 g of an exopeptidase with about 900 CPGU were added to each of the two batches five times in two-hour intervals.

[0082] The total quantity of added exopeptidase was 10 g/100 g source protein in each case. The batches were further incubated at 50 °C.

[0083] Another 50 ml sample was then taken from each batch (samples I9 and II9), and all four samples were analyzed.

[0084] To this end, the samples were homogenized. 0.5 g - 2.5 g of the homogenized samples were weighed to the nearest 0.1 mg into a Falcon tube. 1 ml norleucine solution (0.1312 g/200 ml) was added as an internal standard (IS) and topped up to 25 ml with 3% 5-sulfosalicylic acid. The tubes were placed in a shaker and shaken vigorously for 20 minutes. They were then centrifuged at 5000 rpm for 5 minutes. The supernatant was filtered through a syringe filter (0.45 µm). 0.5 ml of the filtrate and 0.5 ml sample buffer for chromatography were pipetted into a reaction vessel, which was then sealed. The solution prepared in this manner was used for chromatographic analysis on the amino acid analyzer.

[0085] In parallel, the amino acid composition of the fraction containing free amino acids and oligopeptides was determined. To this end, 0.5 g - 2.5 g of the homogenized sample were again weighed to the nearest 0.1 mg into a Falcon tube. 2.5 ml norleucine solution (0.1312 g/200 ml) were pipetted in as an internal standard (IS) and topped up to 25 ml with 3% 5-sulfosalicylic acid. The tubes were shaken and centrifuged in the manner described above, and the water-insoluble polypeptides and proteins contained in the precipitate were discarded. 1 ml of the supernatant was pipetted into a 50 ml glass digestion vessel, mixed with 9 ml 6N hydrochloric acid, and the vessels were tightly sealed with a screw cap. The vessels were placed in the heating block, and the sample solution was digested at 105 °C for 8 h. 2 ml of the digested solution were evaporated to dryness. The residue was taken up in 1 ml sample buffer for chromatography and transferred to a reaction vessel, which was then sealed. The solution prepared in this manner was used for chromatographic analysis on the amino acid analyzer.

[0086] In addition, the content of oligopeptides in the composition can be calculated by subtracting the content of free amino acids determined in step 1.

[0087] Table 1 shows the composition of the four samples that were analyzed.

Table 1: Sample composition

Results free amino acids:	**Sample I 5**	**Sample II 5**	**Sample I 9**	**Sample II 9**
	mg/kg test batch			
essential amino acids (proteinogenic amino acids):				
Threonine (Thr)	57	56	709	738
Valine (Val)	112	111	977	949
Methionine (Met)	75	85	135	142
Isoleucine (Iso)	49	44	344	361
Leucine (Leu)	947	853	>2800	>2800
Phenylalanine (Phe)	175	143	843	815
Lysine (Lys)	1279	1209	>2800	>2800
Tryptophan (Trp)	not determined			
semi-essential amino acids (proteinogenic amino acids)				
Histidine (His)	74	68	384	384
Arginine (Arg)	264	247	795	791
non-essential amino acids (proteinogenic amino acids)				
Aspartic acid (Asp)	25	23	390	378
Serine (Ser)	60	57	395	404
Asparagine (Asn)	49	30	343	292
Glutamic acid (Glu)	308	248	1591	1588
Glycine (Gly)	<10	<10	116	124
Alanine (Ala)	105	93	634	665
Cysteine (Cys)	not determined			
Tyrosine (Tyr)	148	142	814	818
Proline (Pro)	29	40	104	125
Glutamine (Glu)	not determined			
other determined substances				
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	<10	<10	34	41
Cystine (Cys2)	186	189	341	348
Cystathionine (Cystha)	191	173	not analyzable	not analyzable
3-methyl-histidine (3Mehis)	<10	<10	<10	<10

1-methyl-histidine (1Mehis)	<10	<10	<10	<10
Hydroxylysine (Hylys)	<10	<10	<10	<10
Ornithine (Orn)	<10	<10	<10	<10
Hydroxyproline (Hypro)	<10	<10	<10	<10

Results NPN extract (oligopeptides and free amino acids:	**Sample I 5**	**Sample II 5**	**Sample I 9**	**Sample II 9**
	mg/kg test batch			
essential amino acids (proteinogenic amino acids)				
Threonine (Thr)	4813	4641	5223	5266
Valine (Val)	2583	2672	3069	3287
Methionine (Met)	791	154	533	223
Isoleucine (Iso)	2968	2840	3410	3701
Leucine (Leu)	5909	5696	6864	6944
Phenylalanine (Phe)	1590	1564	1890	1846
Lysine (Lys)	5788	5490	6544	6530
Tryptophan (Trp)	not determined			
semi-essential amino acids (proteinogenic amino acids)				
Histidine (His)	1081	1004	1239	1190
Arginine (Arg)	1300	1185	1360	1285
non-essential amino acids (proteinogenic amino acids)				
Aspartic acid (Asp)	5459	5145	not analyzable	2004
Serine (Ser)	3344	2694	3394	3249
Asparagine (Asn)	nn	nn	nn	nn
Glutamic acid (Glu)	11235	9516	9333	8550
Glycine (Gly)	941	873	1038	975
Alanine (Ala)	3279	3123	3225	3176
Cysteine (Cys)	not determined			
Tyrosine (Tyr)	1122	860	1563	1420
Proline (Pro)	4722	4722	4644	4522
Glutamine (Glu)	not determined			
other determined substances				
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	nn	nn	nn	nn
Cystine (Cys2)	747	488	1374	1160
Cystathionine (Cystha)	nn	nn	nn	nn
3-methyl-histidine (3Mehis)	nn	nn	nn	nn
1-methyl-histidine (1Mehis)	nn	nn	nn	nn
Hydroxylysine (Hylys)	nn	nn	nn	nn

Ornithine (Orn)	nn	nn	nn	nn
Hydroxyproline (Hypro)	nn	nn	nn	nn

[0088] After hydrolysis was complete, the batches that were incubated with the endo- and exopeptidases contained about 50 to 60% oligopeptides and about 15% free amino acids, divided into about 10% essential amino acids and 5% non-essential amino acids, based in each case on the total weight of the source protein, in this case 100 g protein.

[0089] The pH, the lactose content, and the taste of the four samples were also tested. The results are summarized in Table 2.

Table 2: Taste analysis of the produced hydrolysates:

	Sample I5	**Sample II5**	**Sample I9**	**Sample II9**	**WPC 80 1:2**
pH value	4.97	5.03	5.04	5.10	
Taste	not bitter	not bitter	not bitter	not bitter	
Lactose [g/100 g]				0.9	0.8

Example 2:

[0090] Whey protein concentrate WPC 80 with a dry matter content of 25% and a protein content of 80% in the dry matter was diluted 1:2 with water. The protein content of the solution prepared in this manner was 9.98% by weight based on the total weight of the prepared solution.

[0091] The pH of this solution was originally 6.32 and was then adjusted to 5.45 with citric acid.

[0092] The batch volume was 2 liters in each case. An endopeptidase with an activity of about 1000 SAPU/g and an exopeptidase with about 900 CPGU were added. Enzymatic hydrolysis was performed at 50 °C.

[0093] Table 3 shows the hydrolysis scheme:

Table 3: Hydrolysis scheme

Day	**Time**	**Batch EZ 40**	**Batch EZ 41**
Day 1	7:05	2 g exopeptidase	2 g exopeptidase
	9:05	2 g exopeptidase	2 g exopeptidase
		2g exopeptidase	2g exopeptidase
	11:10	2 g exopeptidase	2 g exopeptidase
		2g exopeptidase	2g exopeptidase
	14:10	End	4 g exopeptidase
			4g exopeptidase
Day 2	9:00		End
Total quantity of endopeptidase [g/100 g source protein]		3 g	5 g
Total quantity of exopeptidase [g/100 g source protein]		2 g	4 g

[0094] Samples were then taken and analyzed in the manner described in Example 1.

[0095] Table 4 shows the composition of the analyzed samples.

Table 4: Sample composition

	Results free amino acids:		**Results NPN extract (oligopeptides and free amino acids):**	
	EZ40	**EZ41**	**EZ40**	**EZ41**
	mg/kg test batch			
essential amino acids (proteinogenic amino acids)				
Threonine (Thr)	143	434	3782	4482
Valine (Val)	166	513	2222	2694
Methionine (Met)	114	257	871	978
Isoleucine (Iso)	101	283	2458	2956
Leucine (Leu)	808	<2000*	4643	>5500*
Phenylalanine (Phe)	143	354	1217	1486
Lysine (Lys)	1147	<2000*	<4000*	>4000*
Tryptophan (Trp)	not determined			
semi-essential amino acids (proteinogenic amino acids)				
Histidine (His)	124	253	727	949
Arginine (Arg)	256	536	956	1214
non-essential amino acids (proteinogenic amino acids)				
Aspartic acid (Asp)	46	163	4136	5076
Serine (Ser)	125	289	2557	2957

Asparagine (Asn)	61	138	<50	<50
Glutamic acid (Glu)	261	666	>8000*	>8000*
Glycine (Gly)	19	48	685	878
Alanine (Ala)	124	381	2637	>3000*
Cysteine (Cys)	not determined			
Tyrosine (Tyr)	193	515	1064	1470
Proline (Pro)	21	79	>4000*	>4000*
Glutamine (Glu)	not determined			
other determined substances				
Taurine (Tau)	68	83	87	90
Citrulline (Cit)	<10	<10	<50	<50
Cystine (Cys2)	103	261	399	516
Cystathionine (Cystha)	<10	<10	<50	<50
3-methyl-histidine (3Mehis)	<10	<10	<50	<50
1-methyl-histidine (1Mehis)	<10	<10	<50	<50
Hydroxylysine (Hylys)	<10	<10	<50	<50
Ornithine (Orn)	<10	<10	<50	<50
Hydroxyproline (Hypro)	<10	<10	<50	<50

[0096] The samples were also analyzed for phase separation, pH, and taste. The results are summarized in Table 5.

Table 5: Taste analysis of the produced hydrolysates:

	Sample EZ 40	**Sample EZ 41**
Phase separation	no	yes
pH	4.50	4.47
Taste	bitter	bitter

Example 3:

[0097] Whey protein concentrate WPC 80 with a dry matter content of 25% and a protein content of 80% in the dry matter was used in water-diluted or undiluted form.

[0098] Table 6 shows the composition of the test batches with their pH values. A pH adjustment was not necessary.

Table 6: Composition of the test batches

	EZ 42	EZ 43	EZ 44	EZ45
Protein content [%]	11.27	20.43	11.27	10.00
pH	5.33	5.34	5.33	5.60
Batch volume [l]	2	2	2	1

[0099] An endopeptidase with an activity of about 1000 SAPU/g and an exopeptidase with about 900 CPGU were added. Enzymatic hydrolysis was performed at 50 °C.

[0100] Table 7 shows the hydrolysis scheme.

Table 7: Hydrolysis scheme:

Day	Time	EZ 42	EZ 43	EZ 44
1	10:00	2 g endop.	2 g endop.	10 g endop.
	11:00	2 g endop.	2 g endop.	
	12:00	2 g endop.	2 g endop.	
	13:00	2 g endop.	2 g endop.	
	14:00	2 g endop.	2 g endop.	
	16:00	End of batch no. 1		
2	7:00	End of batch no. 2		
	7:15	2 g exop.	2 g exop.	8 g exop.
	8:15	2 g exop.	2 g exop.	
	9:15	2 g exop.	2 g exop.	
	10:15	2 g exop.	2 g exop.	
	12:15	End of batch no. 3		

Day	Time	EZ 45
1	7:15	2.5 g endop.
	13:15	End of batch no. 1
2	8:45	End of batch no. 2
	7:30	2 g exop.
	12:30	End of batch no. 3

[0101] Samples were then taken and analyzed in the manner described in example 1.

[0102] Table 8 shows the composition of the analyzed samples.

Table 8: Sample composition

Results	**EZ 42**			**EZ 43**		
Free amino acids:	**6h**	**21h**	**26h**	**6h**	**21h**	**26h**
	mg/kg test batch					
essential amino acids (proteinogenic amino acids)						
Threonine (Thr)	85	267	671	71	263	992
Valine (Val)	60	205	631	96	229	818
Methionine (Met)	150	341	453	203	415	632
Isoleucine (Iso)	39	156	398	70	186	465
Leucine (Leu)	522	1351	2536	582	1407	3049
Phenylalanine (Phe)	126	335	709	165	354	886
Lysine (Lys)	1102	1731	2898	1598	2126	3723
Tryptophan (Trp)	not determined			not determined		
semi-essential amino acids (proteinogenic amino acids)						
Histidine (His)	68	164	303	74	150	400
Arginine (Arg)	230	408	421	220	448	359
non-essential amino acids (proteinogenic amino acids)						
Aspartic acid (Asp)	33	134	464	40	121	574
Serine (Ser)	69	169	368	70	170	613
Asparagine (Asn)	nn	164	386	39	99	609
Glutamic acid (Glu)	141	614	1909	194	553	2305
Glycine (Gly)	26	63	133	30	67	195
Alanine (Ala)	85	239	554	112	248	751
Cysteine (Cys)	not determined			not determined		
Tyrosine (Tyr)	64	260	626	58	251	681
Proline (Pro)	nn	76	150	nn	72	236
Glutamine (Glu)	not determined			not determined		
other determined substances						
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	nn	nn	156	nn	nn	431
Cystine (Cys2)	nn	62	150	18	49	57
Cystathionine (Cystha)	89	151	68	115	145	63
3-methyl-histidine (3Mehis)	nn	nn	nn	nn	nn	nn
1-methyl-histidine (1Mehis)	nn	nn	nn	nn	nn	nn
Hydroxylysine (Hylys)	nn	nn	nn	nn	nn	nn
Ornithine (Orn)	nn	19	55	nn	nn	96
Hydroxyproline (Hypro)	nn	nn	nn	nn	nn	nn
Total	2889	6909	14039	3755	7353	17935

Results NPN-extract (oligopeptides and free amino acids):	**EZ 42**			**EZ 43**		
	6h	**21h**	**26h**	**6h**	**21h**	**26h**
	mg/kg test batch					
essential amino acids (proteinogenic amino acids)						
Threonine (Thr)	4151	5074	4963	5574	5355	1927
Valine (Val)	4016	3634	3687	5923	5821	5951
Methionine (Met)	423	768	755	1098	1073	307
Isoleucine (Iso)	3953	3864	4059	6293	6110	6356
Leucine (Leu)	8149	7809	7971	12565	12778	13637
Phenylalanine (Phe)	2316	2175	2261	3186	3285	3533
Lysine (Lys)	7261	6975	7228	10794	10943	12236
Tryptophan (Trp)	not determined			not determined		
semi-essential amino acids (proteinogenic amino acids)						
Histidine (His)	1536	1327	1544	2057	2103	2547
Arginine (Arg)	2308	1487	1321	2253	2599	1666
non-essential amino acids (proteinogenic amino acids)						
Aspartic acid (Asp)	7188	6941	7287	10038	10948	10159
Serine (Ser)	2344	3104	3039	3161	2787	798
Asparagine (Asn)	nn	nn	nn	nn	nn	nn
Glutamic acid (Glu)	12268	12614	12217	15289	16814	7777
Glycine (Gly)	1763	1274	1386	1800	2119	2186
Alanine (Ala)	4958	4383	4159	7320	7204	7185
Cysteine (Cys)	not determined			not determined		
Tyrosine (Tyr)	938	1316	1611	1522	1463	269
Proline (Pro)	5506	5271	4993	8859	8653	9694
Glutamine (Glu)	not determined			not determined		
other determined substances						
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	nn	nn	130	nn	nn	nn
Cystine (Cys2)	1898	1175	1617	2515	3922	3235
Cystathionine (Cystha)	nn	nn	nn	nn	nn	nn
3-methyl-histidine (3Mehis)	nn	nn	nn	nn	nn	nn
1-methyl-histidine (1Mehis)	nn	nn	nn	nn	nn	nn
Hydroxylysine (Hylys)	nn	nn	nn	nn	nn	nn
Ornithine (Orn)	nn	nn	59	nn	nn	260
Hydroxyproline (Hypro)	nn	nn	nn	nn	nn	nn
Total	70976	69191	70287	100247	103977	89723

Results	**EZ 44**			**EZ 45**		
Free amino acids:	**6h**	**21h**	**26h**	**6h**	**25.5 h**	**30.5 h**
	mg/kg test batch					
essential amino acids (proteinogenic amino acids)						
Threonine (Thr)	76	463	695	35	385	517
Valine (Val)	49	417	612	11	364	471
Methionine (Met)	170	398	532	100	286	317
Isoleucine (Iso)	33	246	481	34	220	301
Leucine (Leu)	576	1798	2731	237	1142	1592
Phenylalanine (Phe)	146	498	757	93	347	479
Lysine (Lys)	1141	2192	2961	691	1350	1831
Tryptophan (Trp)	not determined			not determined		
semi-essential amino acids (proteinogenic amino acids)						
Histidine (His)	76	208	330	36	128	209
Arginine (Arg)	219	462	406	126	340	394
non-essential amino acids (proteinogenic amino acids)						
Aspartic acid (Asp)	48	251	232	28	93	156
Serine (Ser)	61	257	384	39	219	278
Asparagine (Asn)	nn	220	356	nn	146	154
Glutamic acid (Glu)	156	1070	2099	118	924	1382
Glycine (Gly)	10	94	152	nn	63	101
Alanine (Ala)	93	369	606	66	291	431
Cysteine (Cys)	not determined			not determined		
Tyrosine (Tyr)	80	382	684	27	250	412
Proline (Pro)	nn	94	173	nn	nn	194
Glutamine (Glu)	not determined			not determined		
other determined substances						
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	nn	58	105	nn	nn	58
Cystine (Cys2)	27	59	228	nn	23	55
Cystathionine (Cystha)	48	89	136	31	48	nn
3-methyl-histidine (3Mehis)	nn	nn	nn	nn	nn	nn
1-methyl-histidine (1Mehis)	nn	nn	nn	nn	nn	nn
Hydroxylysine (Hylys)	nn	nn	nn	nn	nn	nn
Ornithine (Orn)	nn	13	146	nn	29	54
Hydroxyproline (Hypro)	nn	nn	nn	nn	nn	51
Total	3009	9638	14806	1672	6648	9437

Results NPN-extract (oligopeptides and free amino acids):	**EZ 44**			**EZ 45**		
	6h	**21h**	**26h**	**6h**	**25.5 h**	**30.5 h**
	mg/kg test batch					
essential amino acids (proteinogenic amino acids)						
Threonine (Thr)	5627	2394	6272	2782	4669	3677
Valine (Val)	3457	3624	3881	3236	3310	3361
Methionine (Met)	950	455	1300	444	714	510
Isoleucine (Iso)	3503	3849	4129	3443	3566	3598
Leucine (Leu)	7474	7984	8521	6684	6916	7196
Phenylalanine (Phe)	2091	1891	2503	1721	1955	1994
Lysine (Lys)	6759	7179	7806	5881	5975	6121
Tryptophan (Trp)	not determined			not determined		
semi-essential amino acids (proteinogenic amino acids)						
Histidine (His)	1247	1419	1695	1115	1229	1327
Arginine (Arg)	1567	1349	1336	1264	1444	1332
non-essential amino acids (proteinogenic amino acids)						
Aspartic acid (Asp)	6711	6333	8226	5793	6098	6405
Serine (Ser)	3763	1070	4374	1523	2960	2174
Asparagine (Asn)	nn	nn	nn	nn	nn	nn
Glutamic acid (Glu)	13549	10107	14520	10546	11651	10480
Glycine (Gly)	1163	1324	1571	1010	1114	1208
Alanine (Ala)	4496	4273	4609	4061	3880	3918
Cysteine (Cys)	not determined			not determined		
Tyrosine (Tyr)	1379	545	1995	546	1322	1086
Proline (Pro)	5490	5579	5819	5146	5035	4910
Glutamine (Glu)	not determined			not determined		
other determined substances						
Taurine (Tau)	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable	not analyzable
Citrulline (Cit)	nn	nn	nn	nn	nn	nn
Cystine (Cys2)	742	1765	1276	1207	1059	1583
Cystathionine (Cystha)	nn	nn	nn	nn	nn	nn
3-methyl-histidine (3Mehis)	nn	nn	nn	nn	nn	nn
1-methyl-histidine (1Mehis)	nn	nn	nn	nn	nn	nn
Hydroxylysine (Hylys)	nn	nn	nn	nn	nn	nn
Ornithine (Orn)	nn	nn	139	nn	nn	64
Hydroxyproline (Hypro)	nn	nn	nn	nn	nn	nn
Total	69968	61140	79972	56402	62897	60944

[0103] The samples were examined specifically for their taste. The samples were not bitter.

Patentkrav

1. Fremgangsmåde til fremstilling af en sammensætning, der omfatter frie aminosyrer, oligopeptid og polypeptid
kendetegnet ved, at
fremgangsmåden mindst omfatter følgende trin:

tilsætning af mindst to forskellige peptidaser, der forefindes i renset form, til en sammensætning, der omfatter mindst ét protein, hvor mindst en exopeptidase og mindst en endopeptidase tilsættes sammensætningen, der omfatter mindst ét protein, og hvor

(a) de mindst to forskellige peptidaser er af mikrobial oprindelse;
(b) sammensætningen, der omfatter mindst ét protein, har en proteinkoncentration på 5 til 30 vægtprocent i forhold til den samlede vægt af sammensætningen, der indeholder mindst ét protein;
(c) sammensætningen, der omfatter mindst ét protein, har en pH-værdi på 4 til 6 efter tilsætning af de mindst to forskellige peptidaser og inkuberes ved en temperatur på 40 °C til 60 °C med mindst to forskellige peptidaser; og
(d) den samlede mængde af tilsat peptidase er fra 2 til 10 gram pr. 100 gram af det mindst ene protein i den sammensætning, der indeholder mindst ét protein.

2. Fremgangsmåden ifølge krav 1, **kendetegnet ved, at** de mindst to forskellige peptidaser er af bakteriel eller fungal oprindelse.

3. Fremgangsmåden ifølge mindst ét af kravene 1 eller 2, **kendetegnet ved, at** sammensætningen, der omfatter mindst ét protein,

(a) har en proteinkoncentration på 10 til 20 vægtprocent i forhold til den samlede vægt af sammensætningen, der omfatter mindst ét protein;
(b) inkuberes i mindst 1 time, fortrinsvis i et tidsrum på 1 time til 24 timer, særligt foretrukket i et tidsrum på 4 timer til 12 timer, mest foretrukket i et tidsrum på 6 timer til 10 timer med mindst én af de to mindste forskellige peptidaser;

(c) inkuberes ved en temperatur på 45 °C til 60 °C, særligt foretrukket ved en temperatur på 45 °C til 55 °C med de to mindste forskellige peptidaser;

(d) ved tilsætning af mindst to forskellige peptidaser har en pH-værdi på 4,5 til 6, hvor pH-værdien eventuelt tilpasses gennem tilsætning af mindst én organisk syre, specielt valgt fra mælkesyre, citronsyre og æblesyre; og/eller

(e) omfatter mindst en proteinkilde, der er valgt fra valle, valleproteinkoncentrat, mælkeproteinkoncentrat, slam fra levnedsmiddelproduktion såsom destillerislam eller bryggerislam, ærter, linser, bønner, soja og en kombination af to eller flere heraf.

4. Fremgangsmåden ifølge mindst ét af de foregående krav, **kendetegnet ved, at** fremgangsmåden desuden omfatter følgende trin: tilsætning af kalciumkarbonat og/eller magnesiumkarbonat, hvor tilsætningen sker efter afslutning af inkubationen af sammensætningen, der omfatter mindst ét protein, med de mindst to peptidaser.

5. Fremgangsmåden ifølge mindst ét af de foregående krav, **kendetegnet ved, at**

(a) den samlede mængde af tilsat peptidase er hver fra 500 til 20.000, fortrinsvis fra 1000 til 15.000, særligt foretrukket fra 2000 til 10.000 pr. 100 gram af det mindst ene protein i sammensætningen, der omfatter mindst ét protein;

(b) mindst én af de mindst to peptidaser gentagne gange føjes til sammensætningen, der omfatter mindst ét protein,

(c) mindst én af de mindst to peptidaser gentagne gange tilsættes sammensætningen, der omfatter mindst ét protein, i hovedsageligt lige store mængder og i hovedsageligt lige store tidsintervaller; og/eller

(d) tilsætningen af mindst én endopeptidase sker før tilsætning af den mindst ene exopeptidase.

6. Fremgangsmåden ifølge mindst ét af de foregående krav, **kendetegnet ved, at** sammensætningen, der indeholder mindst ét protein, inkuberes først i 1 til 10 timer, fortrinsvis i 2 til 6 timer, særligt foretrukket i ca. 5 timer med den mindst ene endopeptidase og derefter i 3 til 40 timer, fortrinsvis i 20 til 40 timer, særligt foretrukket i ca. 30 timer med den mindst ene exopeptidase, hvor inkubationen sker med exopeptidasen fortrinsvis ved tilstedeværelse af mindst én endopeptidase.

7. Fremgangsmåden ifølge krav 6, **kendetegnet ved, at** den mindst ene endopeptidase og/eller den mindst ene exopeptidase tilsættes flere gange, fortrinsvis to gange, tre gange, fire gange, fem gange eller seks gange i hovedsageligt lige store mængder og med hovedsageligt lige store tidsmæssige afstande.

8. Sammensætningen, der omfatter frie aminosyrer, oligopeptid og polypeptid, som opnås gennem fremgangsmåden ifølge mindst ét af de forrige krav, **kendetegnet ved, at** sammensætningen (a) omfatter fra 0,5 vægtprocent til 25 vægtprocent frie aminosyrer i forhold til den samlede vægt af frie aminosyrer, oligopeptider, polypeptider og protein uden de tilsatte peptidaser og (b) omfatter fra 25 til 65 vægtprocent oligopeptider på grundlag af den samlede vægt af frie aminosyrer, oligopeptider, polypeptider og protein uden de tilsatte peptidaser.

9. Sammensætningen ifølge krav 8, **kendetegnet ved, at**

(a) sammensætningen omfatter fra 2 vægtprocent til 20 vægtprocent, særligt foretrukket fra 5 vægtprocent til 15 vægtprocent frie aminosyrer i forhold til den samlede vægt af frie aminosyrer, oligopeptider, polypeptider og proteiner uden de tilsatte peptidaser;
(b) sammensætningen omfatter fra 0,3 vægtprocent til 13 vægtprocent, fortrinsvis fra 1,2 vægtprocent til 12 vægtprocent, særligt foretrukket fra 3 vægtprocent til 9 vægtprocent af følgende frie aminosyrer i alt i forhold til den samlede vægt af frie aminosyrer, oligopeptider, polypeptider og protein uden de tilsatte peptidaser: isoleucin, leucin, lysin, methionin, fenylalanin, threonin, tryptofan og valin;
(c) sammensætningen omfatter en pH-værdi på 4 til 6, særligt foretrukket på 4,5 til 6; og/eller
(d) vægtforholdet mellem magnesium og calcium i sammensætningen udgør fra 1,25:1 til 3:1, fortrinsvis fra 1,5:1 til 2,5:1, særligt foretrukket ca. 2:1.

10. Sammensætningen ifølge krav 8 eller 9, **kendetegnet ved, at** 0 vægtprocent til 40 vægtprocent, fortrinsvis 0 vægtprocent til 20 vægtprocent, særligt foretrukket 0 vægtprocent til 10 vægtprocent af mindst ét protein som uspaltet protein.

11. Anvendelsen af sammensætningen ifølge mindst ét af kravene 8 til 10 til fremstilling af et næringsmiddel, specielt til fremstilling af en frugtsaftdrik, en mælkedrik, en valledrik, en yoghurt, en ayran eller en kefir.

12. Anvendelsen ifølge krav 11, **kendetegnet ved, at** en del af vandet i et næringsmiddel erstattes med sammensætningen ifølge mindst ét af kravene 8 til 10.

13. Anvendelsen af sammensætningen ifølge mindst ét af kravene 8 til 10 til fremstilling af et kropsplejeprodukt, specielt til fremstilling af en sæbe, en shampoo, en hårkur, en hårmaske, en hårpakning, en hårvand, en balsam, en badetilsætning, en body lotion, en kropsgel, en lotion til behandling af rosacea, en creme til behandling af akne, en ansigtsmaske eller en ansigtscreme.

14. Anvendelsen ifølge krav 13, **kendetegnet ved, at** en del af vandet, fortrinsvis mindst 80 % af vandet, af et kropsplejeprodukt, fortrinsvis en sæbe, erstattes med sammensætningen ifølge mindst ét af kravene 8 til 10.

15. Næringsmiddel, der omfatter sammensætningen ifølge mindst ét af kravene 8 til 10.

16. Næringsmidlet ifølge krav 15, **kendetegnet ved, at** den samlede andel af frie aminosyrer, oligopeptider og polypeptider i næringsmidlet udgør (a) fra 2 vægtprocent til 25 vægtprocent, fortrinsvis fra 5 til 10 vægtprocent; og/eller udgør (b) fra 5 til 50 gram, fortrinsvis ca. 10 gram.

17. Kropsplejeprodukt, der omfatter sammensætningen ifølge mindst ét af kravene 8 til 10.