

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
14 August 2003 (14.08.2003)

PCT

(10) International Publication Number  
WO 03/066545 A2

- (51) International Patent Classification<sup>7</sup>: **C07B**
- (21) International Application Number: PCT/US03/03450
- (22) International Filing Date: 6 February 2003 (06.02.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/354,270 6 February 2002 (06.02.2002) US  
10/358,135 5 February 2003 (05.02.2003) US
- (71) Applicant: **GREEN EARTH INDUSTRIES, LLC**  
[US/US]; 45600 Terminal Drive, Dulles, VA 20166 (US).

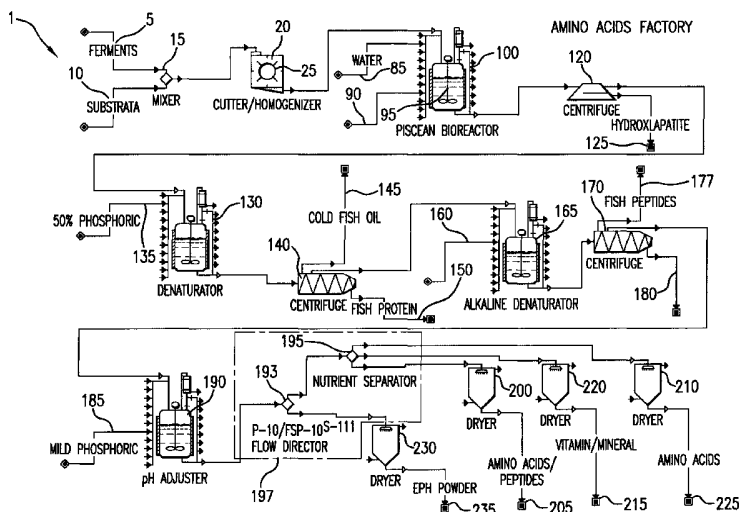
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**  
— without international search report and to be republished upon receipt of that report

- (72) Inventors: **PYNTIKOV, Alexander, V.**; 21184 Sundial Court, Ashburn, VA 20148 (US). **SALERNO, Salvatore, P.**; 12213 Parkstream Terrace, Herndon, VA (US).
- (74) Agent: **RICHARD, Dana, F.**; Green Earth Industries, LLC, 45600 Terminal Drive, Dulles, VA 20166 (US).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: AMINO ACIDS FACTORY



(57) Abstract: A factory for production of an enzymatic protein hydrolysate from cold water marine animals. The factory includes a mixer, a cutter/homogenizer, dual centrifuges, dual denaturators, a pH adjuster, a nutrient separator, and dryers. The enzymatic protein hydrolysate contains 70-90 % free amino acids, 10-20 % peptides, and vitamins and minerals. Production also includes cold fish oil, hydroxylapatite (calcium orthophosphate), and phosphorous salts of calcium. The factory utilizes marine animal entrails containing aggressive enzymes that, and requires raw materials that include little more than marine animals, water, and advanced controls and refinement. The factory provides a greater degree of refining than existing technology through the use of acid and alkaline denaturation in combination with advanced separation and drying technology. The factory may be characterized as resulting in profound enzymatic hydrolysis, and makes possible mass production of amino acids, amino acid mixes, and other nutrients.



WO 03/066545 A2

## AMINO ACIDS FACTORY

### RELATED U.S. APPLICATION DATA

This application claims the benefit of Provisional Application Serial Number  
5 60/354,270 entitled "PROTEOL[YTIC] FERMENTER AND COMPLEX  
TECHNOLOGIES USING THE PROTEOL[YTIC] FERMENTER," filed February 6, 2002.  
This application additionally claims the benefit of Utility Application Serial Number  
(unassigned), entitled "AMINO ACIDS FACTORY" filed February 5, 2003.

### 10 BACKGROUND OF THE INVENTION

#### FIELD OF THE INVENTION

The present invention relates to a factory for industrial production of amino acids and  
other nutrients. More particularly, the invention relates to enzymatic hydrolysis of cold  
15 water marine animal proteins, and resulting products having peptides, specified amino acids,  
minerals, vitamins, and other nutrients.

#### BACKGROUND

The elementary nutrients for cellular growth of life on Earth are amino acids. Every  
20 chemical structure in all living organisms (DNA, RNA, proteins, fats, carbohydrates,  
hormones, vitamins, and the amino acids themselves) are synthesized and broken down by  
means of enzymes that, in turn, are proteins, constructed from amino acids. For  
representatives of all the five kingdoms of living organisms, life on Earth essentially is the  
circulation of amino acids. For example, representatives of the kingdom of plants  
25 continuously make contributions of mass doses of amino acids to global amino acid  
quantities (by way of photosynthesis).

Food is digested in the human body into amino acids, simple sugars and fatty acids.  
Amino acids are not only food for human beings, they are also a base for the rapid  
accumulation of biomass of individual species of organisms in any of the five kingdoms of  
30 the terrestrial life. Humans can help protect their species against vagaries and catastrophes  
within any of the kingdoms of living things on Earth by creating a steadily renewable supply  
of amino acids. Humans can use this resource to benefit a particular kingdom (for the  
production of meat, for example, or gas, or alcohol, or for the purification of water or air, or  
for combating the greenhouse effect on Earth.

Currently, abundant, inexpensive sources of mixtures of amino acids are not readily available. Existing commercial and previously patented processes make use of conventional spray dryer and crystallization technologies to produce amino acid in powder form. These technologies suffer from the following disadvantages. They require steam that adds  
5 substantial costs of production. They often require the use of special additives to enhance nucleation, which adds to the cost of making a final powder product. The temperature ranges over which they operate cause thermal damage to a significant portion of the final products due to hot spots and poor mixing within the drying chamber.

Another disadvantage of existing production methods is that they typically use only  
10 one denaturation step, either acid or alkaline. As a consequence of this approach, much of the amino acid powder that is produced has not been refined or purified to the greatest degree possible.

Methods of hydrolyzing proteins are well known in the art. It is also well known in the art to hydrolyze fish in an alkaline environment so to produce animal feed and other  
15 food products. However there are a number of fundamental problems in the hydrolysis of fish proteins using organic, fish based enzymes. Among those problems have been the inability to obtain a consistent mix of nutrient products on a large-scale basis, shortcomings in the art regarding concentrating, drying, separating, and purifying those products and high relative costs of production.

Russian patent number RU 2103360 by Yermishina *et. al.*, patented January 27,  
20 1998, describes a method for obtaining proteolytic hydrolysates using commercial fish wastes, and the use of the hydrolysates to stimulate the activity of human kidney (eucariot) cell growth. However, Yermishina *et. al.* do not disclose the factory methods or products of the present invention, or their multiple uses.

WIPO application number WO 89/10960 by Mohr V. *et al.*, published November 16,  
25 1989, discloses the use of Antarctic and North Atlantic krill as a source of proteolytic enzymes, including endo- and exopeptidases, e.g. euphauciaceae. The enzymes are used to modify proteins in industrial processes. Mohr *et. al.* do not disclose the use of amino acids for secondary industrial applications, nor do they disclose the methods or products of the  
30 present invention.

WIPO application number WO 01/28353 by Bjarnason, J. *et. al.*, published April 26,  
2001, discloses a method for enzymatically obtaining protein hydrolysates for human consumption, animal feed, and cosmetics. Bjarnason *et. al.* do not disclose the method or products of the present invention.

WIPO application number WO 99/29836 by Walther, B. *et al.*, published November 16, 1989, discloses a procedure for obtaining valuable digestive enzymes--endoproteases (or zozases) from Atlantic salmon eggs using chromatographic procedures. Walther et al do not disclose the method or products of the present invention.

5 U.S. patent number 4,443,540 by Chervan, M. *et al.*, patented April 17, 1984, discloses a method for the preparation of protein hydrolysates. Chervan, *et al.*, do not disclose the method or products of the present invention and utilize a different separation technology.

10 U.S. patent number 5,356,637 by Loosen, P. *et al.*, patented October 18, 1994, discloses a method for the preparation of peptide-rich enzymatic hydrolysate. Loosen, *et al.*, do not disclose the method or products of the present invention.

#### RELATED DEFINITIONS

15 PROTEINS: Proteins are high molecular weight organic compounds with molecular weights ranging from 5000 to many millions of daltons. Proteins are polymers (or copolymers), the monomers of which are amino acids connected to each other by peptide bonds. Peptide chains of smaller sizes (less than 5000 daltons) form peptide and polypeptide molecules. There are various principles for the classification of proteins based on composition, properties, where they are found in nature, and the functions they perform.

20 With respect to composition, proteins are classified as simple or complex (proteins and proteids, or conjugated proteins). The composition of proteins includes, in addition to amino acids, components such as carbohydrates (glycoproteins), nucleic acids (nucleoproteins), fats (lipoproteins), phosphoric acid (phosphoproteins), metals (metalloproteins) or other compounds. One of the criteria used for classification is the solubility of the proteins in

25 various solvents. Albumin and globulin, which dissolve in water and in weak aqueous solutions of electrolytes, are considered soluble proteins, although their stability in solution varies. At the opposite extreme are collagen and keratin – proteins which are insoluble in water and other solvents. In interaction with water, many of the insoluble proteins swell and form a gel (gelatinize). An additional criterion for classification is behavior in an electrical

30 field, depending upon the total electrical charge of their molecules, which are polyions. The electrophoretic mobility of a protein at a specific pH level depends upon its amino acid composition and its isoelectric point (pI – the pH value of the medium at which the protein is electrically neutral). Basic, neutral and acidic proteins are distinguished according to their electrochemical properties. According to biological functions, proteins are classified as

35 biologically active (enzymes, hormones), structural, regulatory, contractile, reserve,

ovalbumin and casein type, transport (blood serum proteins, hemoglobin, myoglobin), protective (antibodies, blood coagulating proteins) and toxins. The whole spectrum of proteins is provided by the particular features of their structures. The protein part of the protein molecule is a polymer chain of amino acids connected by peptide bonds. Amino acids and peptides are formed in the hydrolysis of proteins.

AMINO ACIDS: Amino acids are optically active organic compounds containing an amino group and a carboxyl group at the carbon atom. In the condensation of two amino acids, the amino group of one of them enters into a linkage with the carboxyl group of the other. The linear sequence of amino acids in the polypeptide chain is considered the primary structure of the protein. Proteins are long-chain molecules that are intricately folded into 3-dimensional structures. The chain is composed of strings of small and fairly simple molecules called amino acids. The instructions for making proteins are carried by the DNA. In the 1960s the code for translating DNA sequences into protein structures was discovered, following intensive work since Watson and Crick's discovery of the double helical structure of the DNA molecule in 1953. The compositions of proteins include the 26 amino acids which are most common in nature in the stereo-isomeric form of L-isomers, and more than 10 rare and very rare amino acids. Out of the whole list, there are 8 amino acids that are essential; i.e., they cannot be synthesized in the bodies of animals and have to be obtained exclusively from their diet. Two amino acids are provisionally essential. The other amino acids can be synthesized in animal and plant organisms

PEPTIDES: Proteins are synthesized as a result of the formation of secondary amide bonds between carboxyl groups and amino groups of adjacent amino acids. Such bonds are called peptide bonds, and the structures which emerge as a result of the formation of the peptide bonds between amino acid radicals are called peptides. A peptide that contains two amino acid radicals is called a dipeptide; a peptide that contains three radicals is called a tripeptide, etc. The covalent peptide bonds and disulfide bonds with an energy greater than 35 kcal/mol are the most important bonds in the polypeptide chain. The peptide bonds [(C – N)-group] are partly double bonds and preclude the free rotation of the atoms. Since there is one peptide bond for every 3 bonds in the peptide chain, the free rotation is possible only around the other two bonds. As a result, the peptide chain is spontaneously twisted into a helix. The form of the spiral determines the nature of the secondary structure of the protein. Hydrogen bonds with an energy of 5 kcal/mol play a large role in the formation of the secondary structure. The method for the folding of the structure into its tertiary form is determined by weak interactions within the protein molecule: Coulomb (electrostatic) and Van der Waals. These bonds are very unstable under heating and, like hydrogen bonds, have

a pronounced thermolabile nature. Nevertheless, all the other levels of structural organization and, consequently, the method of self-assembly of the molecules are programmed, as it were, in the amino acid sequences of the proteins. The accessibility of the peptide bonds to enzymes is of importance for the process of enzymatic hydrolysis. However, these bonds are normally masked inside a globule and are attached by hydrogen bonds to the neutral polar groups or form ion pairs. The denaturation of proteins results in unmasking of the susceptible bonds and opens them up to enzyme attack.

There are adequate industrial raw material sources of protein in all the industrially developed countries of the world. Protein sources may include wastes from the meat processing, fish processing and milk processing industries, vegetable proteins, as well as biomass from artificially cultivated microorganisms, fungi or one-celled algae. The criterion for the selection of proteins for hydrolysis is the nutritional value and completeness of the proteins in question. The nutritional value of proteins is determined at present by the calculation (scoring) method on the basis of the concentrations of essential amino acids. Essential amino acids pass into the human body and the bodies of animals with their diet, while bacterial and cell cultures receive them from the nutrient medium. The completeness of the proteins for nutritional purposes is determined by comparison to a protein that has been adopted as a standard. The calculation of the score consists of the quantitative determination of essential amino acids in the protein and the determination of the ratio of the concentration of each to the corresponding concentration in a standard specimen or model mixture. The value of the minimum ratio, i.e., the ratio for the limiting amino acid, is accepted as the score. Proteins from hen's eggs, mother's milk and goose eggs have been adopted as standard proteins. The process for the calculation of corrected amino acid scoring of protein compositions – i.e., the calculation of the score with correction for the susceptibility to breaking down (hydrolysis) of the protein – has been accepted since 1993.

Muscle tissue is a source of proteins which are the most balanced in regard to their amino acid composition, as well as mineral elements, vitamins and growth factors, including substances of a nature which is not yet known. Most biotechnologists have believed and continue to believe that meat cannot be replaced as a source of protein for nutrient media. At the same time, meat is one of the main food products. Therefore, meat is used extremely rarely as a source of raw material in the industrial production of hydrolysates, and it is used as efficiently as possible.

**HYDROLYSIS:** The hydrolysis of proteins is the splitting of the molecule with the destruction of its primary structure (the polypeptide chain). The hydrolysis of the peptide chain is the reverse of the process of formation of the peptide chain; it occurs as a result of

the effects of physical factors (temperature, pressure), chemical reagents (acids, bases) or proteolytic enzymes. As a result of the hydrolysis of a dipeptide, two amino acid molecules are formed, and one molecule of water is absorbed:  $R-CHNH_2-CO-NH-R-COOH + H-OH > R-CHNH_2-COOH + NH_2-R-COOH$ . The products of the full hydrolysis of proteins are amino acids; the products of partial hydrolysis are amino acids and peptides. Typically it is chemical or enzymatic methods that are used for hydrolysis. The chemical methods are used for full (total) hydrolysis of proteins and for partial hydrolysis and are also used in certain cases for the spot (selective) breaking of peptide bonds. The methods of full hydrolysis are used, as a rule, for purposes of analysis. Acid hydrolysis is used often under industrial conditions; alkali hydrolysis is more rarely used.

ACID HYDROLYSIS is conducted primarily with the use of mineral acids at high temperatures (acid-thermal hydrolysis). With the effect of high concentrations of the acids on the protein under heating for a sufficiently long period of time, the protein can be broken down completely into amino acids. Acid hydrolysis is normally performed with sulfuric or hydrochloric acid. An attractive aspect of acid hydrolysis is the possibility of obtaining deep hydrolysates in short periods of time. Another positive factor is the establishment of bactericide conditions in the course of the process, which prevents bacterial growth and makes it possible to store the hydrolysate for a long time without neutralization. However, acid hydrolysis also has its negative aspects. Since acid hydrolysis is not specific to proteins, in the acid-thermal treatment of a complex, mixed raw material of the kind normally used in industry, the breaking down of other biological polymers is also going on at the same time: nucleic acids and polysacharrides. As a result of the hydrolysis of such raw material, the hydrolysis products turn out to be not just amino acids and peptides but carbohydrates (monoses and reducing disacharrides). In acid hydrolysis, melanoids are formed – dark-colored, high molecular weight compounds with a tendency toward aggregation, which have poor solubility in water. The melanoids are cell toxins; therefore, their formation in the hydrolysate sharply reduces its quality. The neutralization of acids in the hydrolysate involves the formation of high concentrations of salts. The higher the concentration of the acid used for hydrolysis, the more salts are formed as a result of neutralization. The increased anion concentration is also a salient factor in the toxicity of the hydrolysate.

ALKALI HYDROLYSIS: In the alkali hydrolysis of proteins, most amino acids are broken down and more importantly, the method for alkali hydrolysis is softer and gentler than acid hydrolysis. As a result, alkali hydrolysis is best used in the industrial production of hydrolysates. Of the known means to hydrolyze proteins, enzymatic hydrolysis is a slightly

alkaline environment at a moderate temperature, is preferred since these conditions do not significantly destroy essential amino acids. However, in the hydrolysis of animal biomass, most animal-produced enzymes, or ferments, are not robust enough to hydrolyze animal proteins under such mild conditions. Hydrolysis occurs in pH ranges that correspond to the peaks in the activity of the enzymes: more often in a neutral, slightly alkaline medium. The optimum temperature is 35 - 50° C. This threshold can be raised to 60° C for certain enzymes, but should not be higher than 80° C. Despite its relative advantages, enzymatic hydrolysis of proteins--using natural ferments--has typically resulted in products whose constituents cannot be accurately predicted on a consistent basis. Further difficulties in industrial scale enzymatic hydrolysis of animal proteins using natural ferments stems from the fact that cost-effective industrial dryer and micro-separation technologies have not been utilized for the production of contaminant-free high-grade amino acids in this environment.

#### SUMMARY OF THE INVENTION

The present invention is a factory for hydrolysis of marine animal biomass and for large-scale production of an enzymatic protein hydrolysate ("EPH," hereinafter). EPH is a mixture of peptides, amino acids, vitamins, minerals, and specified elements. Also produced is hydroxylapatite, fish oil, and phosphoric salts of calcium. The factory utilizes cold-water marine animal entrails containing aggressive enzymes to digest marine animal biomass. The factory contains a mixer, cutter, homogenizer, dual centrifuges and dual denaturators, a pH adjuster, a nutrient separator, and dryers. The EPH may be in liquid form or dried to a powder state. Dry EPH consists of, by weight, 70-90% free amino acids, 10-20% short peptides and 3-5% of other elements, respectively. The proposed production process is fully waste-free and nature-saving. EPH may be applied as an enhancer for the acceleration of known biological and industrial processes, and most particularly, processes depending upon bacterial or cellular action.

The protein hydrolysate is the main product of the proteolytic fermenter. This product can change its form if the proteolytic fermenter is used in combination with a separator that divides the protein hydrolysate into separate components. Together, the two mechanisms compose a device for obtaining free amino acids. Instead of the ferments that arrive with the homogenized biomass, the proteolytic fermenter can use special, previously extracted and immobilized ferments. In this case, the machine can be utilized for the decomposition of distinct, specific types of proteins. The amino acid content produced by



the protein hydrolysate may be the characteristic protein-specific or organ-specific array of amino acids. This may be useful for reconstructive healing of a damaged organ, for example. Such a device based on the proteolytic fermenter is called the device for selective protein-specific fermentative biodeconstruction. If a specific organ is used as input material  
5 for fermentative biodeconstruction, the mechanism will be called the device for selective organ-specific fermentative biodeconstruction. Moreover, the proteolytic fermenter maybe used for the modification of certain substances that contain proteins. For instance, if chitin is input into the proteolytic fermenter with immobilized ferments, ensuring the process of proteolysis, the protein ingredient of chitin will be lost. The product of such a  
10 biotechnological reaction is known as chitosan. It has a number of unique properties, including the ability to effectively absorb metals contained in seawater. The mechanism for obtaining chitosan based on the proteolytic fermenter will be called the biomodifier. With the aid of a biomodifier, other substances may be obtained: high-quality cod-liver oil, minerals, or vitamins.

15 It is an object of this invention to disclose an industrial method and apparatus for the digestion of proteins--using naturally occurring enzymes--into free amino acids and short, biologically active peptides.

It is another object of this invention to solve the problem of harvesting, accumulating and utilizing the entirety of fish to produce amino acids and other nutrients, including  
20 hydroxylapatite, fish oil, and an enzymatic protein hydrolysate.

It is yet an additional object of this invention to solve the problems of drying and separating the components of an enzymatic protein hydrolysate.

It is a further object of this invention to mass produce a nutritious amino acid, vitamin, and mineral mix using digestive enzymes derived from cold-water marine animals.

25 It is another object of this invention to produce an enzymatic protein hydrolysate using *farmed* fish, such as salmon--as both substrate (whole salmon) and as the ferment (salmon viscera)--such that greater consistency is achieved in the product.

It is yet another object of this invention to produce an enzymatic protein hydrolysate employing both farmed fish and computerized control of the hydrolysis process, such that  
30 even greater consistency is present in the hydrolysate and in the final product.

It is a further object of this invention to produce an enzymatic protein hydrolysate from cold water fish that can be used as a nutritious medium for cellular and bacterial growth in other industrial processes.

35

## DESCRIPTION OF THE FIGURES

FIG. 1 is a diagrammatic view of the amino acids factory.

FIG. 2 is a simplified block diagram of the amino acids factory of Fig. 1.

5 FIG. 3 is a block diagram of the amino acids factory showing automated controls of the hydrolysis.

Fig. 4 shows alternate dryers for use in the amino acids factory.

Fig. 5 shows an amino acid factory using a protein-specific array of amino acids.

FIG. 6 shows an amino acids factory producing an organ-specific array of amino acids.

10 FIG. 7 shows an amino acid factory that produces enzymatic protein hydrolysates in addition to Chitozan.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 The basis of the industrial technology of the present invention is the fermentative hydrolysis of protein from a variety of salt-water fish. The hydrolysis is carried out under moderate conditions that imitate the physiological environment within the intestine of mammals and birds during digestion. Like the meat from farm animals and poultry, fish is a valuable food product and a source of biologically active substances. Hence the wastes from  
20 the fish processing industry – substandard fish, heads, tails, fins, entrails, skin and blood – may be used as a protein substrate for hydrolysis in the production of high-quality nutrient media. More than 70 million metric tons of fish annually come from the world's oceans. 35-50% of its total amount is lost as waste products (heads, bony skeletons, viscera) are typically considered substandard raw materials. A large share of the waste is thrown  
25 overboard into the sea. Some is processed to produce fish meal usable as fodder for feeding animals. However, taking into consideration the composition and nutrient value, fish waste products are in no way inferior to fish meat. The amino acids factory shown in FIGURE 1 capitalizes on the concept that fish entrails, particularly cold-water fish entrails, containing hardy enzymes that can actively hydrolyze proteins under cooler temperatures and mild pH,  
30 may be used as an efficient ferment in the proteolytic break down of whole fish.

Meat from fish contains 12.4 – 23.9% raw protein, depending on the species. The proteins from fish are similar to the proteins from the meat of land animals in regard to their fractional and amino acid compositions. The content of water soluble proteins is as high as 30% in individual species of fish; the concentration of salts, which can be extracted with  
35 solutions, can reach 75%; the concentration of insoluble salts is from 3% to 10% in bony and

cartilaginous fish, respectively. The meat from fish (Cod) contains 2.5% free amino acids. Most of this pool is made up of basic amino acids, glycine, alanine, as well as beta-alanine, and taurine. The flesh from fish with white and dark coloring differs sharply in regard to histidine content. The histidine concentration in the former does not exceed 7%, while the  
5 concentration in the latter is from 30% to 80% of the total composition of free amino acids, or 2% of the weight of the meat. Histidine undergoes decarboxylation during storage, with the formation of histamine, which is responsible for the clinical manifestation of allergic reactions and is a cell poison. A specific component of fish meat is the amino acid taurine – a product of the decarboxylation of cysteic acid—and typically not present in the meat of  
10 land mammals. The other low molecular weight nitrogenous compounds in fish include: creatine and creatinine, the dipeptides anserine and carnosine, and the tripeptide glutathione. Scientific data attest to the fact that the amino acid compositions of meat from various species of cold water fish (whole fillets) do not differ a great deal, although certain variations, such as a high histidine content in the meat of the mackerel (twice the average  
15 level), are observed. There is a difference in enzyme content of the entrails between cold water fish and warm water fish, and a slight differences in enzyme content beteen the various types of cold water fish.

The main nutrients in fish hydrolysate, according to the present invention, are amino acids, which are the structural units of proteins and, accordingly, the building blocks for their  
20 construction. The muscle and connective tissues of the body, all the organs of the body, the blood, the brain and the glands for internal secretion are made of proteins; all the extracellular and intracellular enzymes are proteins; most of the hormones are peptides (protein fragments); the transfer of energy, information and oxygen in every living cell is performed by proteins. However, in addition to their plastic function, the amino acids play  
25 an additional, independent role in various processes in the body and have independent biological activity of their own. For hydrolysates to be used for most purposes, they must have a balanced amino acid composition and must contain essential amino acids in the quantities necessary for the body. The essential amino acids for most species of higher and lower animals are arginine, valine, histidine, isoleucine, leucine, lysine, methionine,  
30 threonine, tryptophan and phenylalanine. The nonessential amino acids are the most active participants in many metabolic reactions and act as links in metabolic pathways.

Referring to FIGURE 2, the preferred amino acid factory comprises a raw material preparator **17**, a piscean bioreactor **100**, and a product finisher **122**. These three components further contain subcomponents as shown in FIGURES 1-4 and as described below.

Since an overwhelming majority of marine animals are carnivorous, their fermentative system effectively completes the task of self-digestion. The best candidates for completing such a process are fish and other marine organisms of northern waters, or those inhabiting a colder climate. Their fermentative system is forced to trigger proteolysis at a lower temperature. Consequently, ferments of cold water animals complete proteolysis more effectively than ferments of organisms from warmer climates, even at relatively low temperatures.

Referring to FIGURES 1 and 2, raw material preparator 17 includes a mixer 15 and a cutter/homogenizer 20. The raw materials entering mixer 15 include ferments 5 and substrate 10. In the preferred embodiment, substrate 10 is a single type of cold water fish, preferably whole, uncut farmed salmon. However, the substrate may be any cold-water marine animal. Preferably the protein source is "farmed fish"—again, preferably salmon. However, trout, halibut, and tappai may be used. "Wild catch"—such as cod, mackerel or herring may also be used as a substrate. The substrate may also be "pelagic fish," or it may be "industry fish"—preferably such as blue whiting. shrimp, krill, and blue mussels may also be used as a substrate in the bioreactor. Blue mussels have a high chitin content. The above list of substrates is not exhaustive, however, the listed source materials represent cold water fish protein available in commercially significant quantities.

External objects or materials must never be admitted into the fish by-products. Prior to the loading of raw materials into a grinder, it is necessary to prepare a mixture of tissues containing ferments (internal organs of fish) as well as tissues that provide protein for hydrolysis ("technical" fish: whole fish, heads, tails, skeleton parts, etc.). For complete and profound hydrolysis, an optimal ratio of substrata to ferments has been experimentally selected. It is the ratio of the weight of substrata containing fish tissues to the weight of ferment-containing tissues. For freshly frozen raw materials, this ratio is 1: 1. The resulting homogeneous mass (of ferments and substrate) is transported into the bioreactor, where it is combined with water at a 1: 1 ratio in terms of weight. In this manner, 5 tons of raw materials (2.5 tons of fish internal organs and 2.5 tons of other fish tissues) and 5 tons of water are loaded into a reactor with a 10-ton capacity. That is, in the preferred bioreaction, the total mass of ferments 5 is equal to the total mass of substrate 10.

Ferments 5 preferably come from a specific fish type, and preferably from a specific region or catch. The digestive enzymes vary among all animals, including fish. Some of the more potent proteolytic enzymes in fish include, trypsin, chymotrypsin, elastase, collagenase, amino peptidase, and carboxypeptidase. Even among marine animals, the proteolytic enzymes vary. For example, North Atlantic and Antarctic krill have a potent

proteolytic enzyme, euphanciaceae, and Atlantic salmon have serine proteases. Farmed fish, such as salmon, are optimal for both substrate and for ferment because farmed fish enable greater enzyme consistency in bioreactor 100. In one embodiment, the ferments consist of a specific organ. The resulting EPH mix would include an organ-specific array of amino acids. In another embodiment, the ferments may be synthetic derivatives of cold water fish enzymes, unique or cloned, of any origin. In yet a further embodiment, special, previously extracted and immobilized ferments may be used in place of raw entrails. Pure, deboned fish meat (fillets) may alternatively be used as the substrate. Where pure fish meat and immobilized ferments are utilized, several steps of the factory may be eliminated.

10 In another embodiment as shown in FIGURE 5, amino acid factory 1 may be utilized for the decomposition of distinct, specific types of protein ferments. As shown in FIGURE 5, the amino acid content produced by the protein hydrolysate would reflect the protein-specific array of amino acids from a particular source. Such a device based on the proteolytic fermenter will be called the device for selective protein-specific fermentative biodeconstruction.

15 Referring to FIGURES 1 and 2, before input into bioreactor 100, raw materials 5 and 10 must be broken up with an industrial mixer 15 and reduced to an homogeneous mass. Size reduction equipment in cutter/homogenizer 20 must be designed to produce a homogenous mass of fish remnants such that a typical particle size of 0.1 mm to 0.3 mm results. Preferably, cutter/homogenizer 20 is of stainless steel construction but may be of any other suitable construction known in the art. Blade 25 can be rotary knife type or granulator type as long as size requirements are met.

20 After the material is homogenized, fermentative deconstruction of the proteins begins in bioreactor 100, sometimes referred to herein as a proteolytic fermenter, or reactor. Preferably, bioreactor 100 is a glass-lined, jacketed reactor with integral impeller (20 – 25 rpm). The main body is preferably carbon steel, but may be constructed of any other material suitable for containing upwards of ten tons of liquid material. Connection nozzles are required for addition of fish remnants, water, and pH adjustment chemicals. Connection nozzles are also required for passing steam or cooling water through the reactor jacket.

30 Bioreactor 100 may be of any shape, including rectangular, or tubular, and is restricted by walls of any appropriate smooth surface material--glass, stainless steel, plastics, preferably inert composites--that may be easily cleaned and that are not readily subject to oxidation or enzymatic migration of elasticizers. Preferably bioreactor 100 has a capacity of at least five tons, but may have up to a twenty ton capacity. Specifics of its construction allow for this capacity to contain the necessary material for the large-scale biodeconstruction of proteins.

35

Input materials for bioreactor 100, in addition to including homogenized substrate and ferments, consist of water 85, and chemical reactants 95. Preferably water 85 is deionized and distilled to minimize contaminants. In the preferred embodiment, the mass of water is equivalent to the combined mass of ferments and substrate. The operating  
5 temperature may ranges from 20 to 100 deg. C, but preferably the hydrolysis takes place at between 44.5 and 45 degrees C. Through bioreactor 100, the raw materials begin transforming into a protein hydrolysate. The required alkalinous environment in the bioreactor is created using fractional automatic addition of 20% NaOH solution to the mixture being hydrolyzed. The quantity of NaOH solution may vary greatly and depends on  
10 the level of acidity and the buffering capacity used in the technological process of tap water. Hydrolysis takes place for a maximum of three hours due to the risk of bacterial growth, accompanied by excessive production of foam. Hydrolysis is carried out given a continuous automatic correlation between the pH and temperature levels. Laboratory control of the hydrolysis, every 30 minutes, is accomplished by monitoring the accumulation of amino-  
15 azote in the hydrolysate using formol titration. The completion of hydrolysis is confirmed when the level of amino-azote remains the same between lab tests or grows by a maximum of 5-7%. Hydrolysis is discontinued by the thermal coagulation of contents in the hydrolysate (+ 95°C, 10 min.). This is accomplished by directing steam through the outer cover of bioreactor 100 and simultaneous intensive blending of its contents (20-25 rpm.)  
20 Then, the contents of bioreactor 100 are cooled to + 60°C by allowing cold water to flow between its outer cover and reactor itself.

Upon completion, contents of the reactor are made up of the following components: oil, together with a fraction of light lipo-proteins, bone tissue (hydroxilappatit), and a water-based mixture of hydrolyzed proteins. Since 18% of fish tissue is protein, in excess of one  
25 ton of protein is hydrolyzed from a mass of raw materials that weighs 10 tons. Hydroxilappatit 125 represents significant economic value as a mineral food supplement for people, or in animal feed. For this reason, bone tissue is transported into a separate tank, repeatedly subjected to a decanter in water, separated, dried, minced, and packed. Centrifuge  
30 120 is a high speed decanter for separating bone tissue from hydrolysate. Centrifuge 120 is preferably made from stainless steel and has a nominal rotation speed of 6,000 revolutions per minute. The product of centrifuge 120 is hydroxylapatit 125, which is 25% of the raw materials being hydrolyzed.

After the bone tissue has been separated, the hydrolysate may also be cleaned of cod-liver oil. However, in this case, the oil will be highly contaminated by fragments of low-

density lipo-proteins, which are difficult to eliminate during the cleaning of cod-liver oil. These fragments are denaturated in an acidic environment and then separated from cod-liver oil. Acid denaturation enables separation of highly molecular peptides, glycoproteides, lipoproteides, and nucleoproteides. First denaturing tank 130 (acid denaturation vessel) is preferably a glass-lined, jacketed vessel with an integral impeller. The main body of tank 130 is preferably carbon steel, but may be any other suitable material. Tank 130 has a connection nozzle for the addition of a phosphoric acid solution. There are also connection nozzles for passing steam or cooling water through the jacket of tank 130. There is a discharge nozzle for the denaturated hydrolysate at the down stream end of tank 130. Although other solutions may be used, the combination of NaOH in bioreactor 100 and phosphoric acid in tank 130 is optimal for this enzymatic hydrolysis. Acidic denaturation is a necessary procedure in the process of cleaning the hydrolysate and serves the purpose of freeing it from unhydrolyzed cellular remains and highly molecular products of hydrolysis. The pH level of the bioreactor's contents is brought to 3.5 by fractional automatic addition of a 50% phosphoric acid solution and later heating to +90°C. This temperature is maintained for 15 minutes and then the contents are cooled down to +60°C. The entire procedure of acid denaturation is accompanied by continuous mixing of the bioreactor's contents at 10-12 rpm. The mass of denaturalized fish proteins (highly molecular peptides, glycoproteides, lipoproteides, and nucleoproteides) is separated from the hydrolysate using a high-speed decanter. After that, the hydrolysate is replaced into the bioreactor.

The advantage of using both alkaline and acid denaturation is that their combination helps to maximize the removal of unwanted by-products of the hydrolysis reaction. These steps also help to improve the efficiency of additional separations processes designed to concentrate the various constituents into distinct product streams.

Still referring to FIGURE 1, second centrifuge 140 is a high speed liquid solid separator for separating cold fish oil 145 and fish protein 150 from the hydrolysate. Preferably it has a disc or cell operating at 7,000 revolutions per minute. Fish protein represents significant economic value as a source of food as well as a food supplement. For this reason, the fish protein is transported into a separate tank, repeatedly subjected to decanting in water, separated, dried, minced, and packed. It is possible to pack the protein in batches and to distribute it to the consumer in either fresh or frozen state. The resulting protein weighs 0.3-0.4 tons (net weight) out of the 5 tons of raw materials that undergo hydrolysis. Oil is disconnected from the hydrolysate by way of high-speed separation, at a temperature of 60°C is pumped through a standard separator such as that which is used in the

dairy processing industry. A high speed separator operating at 7,000 rpm is used. Fish oil also represents significant economic value as an independent product. For this reason, the oil is transported into a separate tank and, as it collects, the oil is subjected to additional cleaning, rectification, amalgamation, and packaging. The resulting cold fish oil **145** weighs approximately 1 ton out of the 5 tons of raw materials that undergo hydrolysis.

Second denaturing tank **165** is an alkaline denaturation vessel with an operating pH optimally of 8.5. It is preferably a jacketed reactor with integral impeller operating optimally at approximately 10 – 12 revolutions per minute. The main body may be steel or other suitable material, and it has an operating temperature range from 20 to 100 degrees C. Tank **165** should have connection nozzles for the addition of hydrolysate and preferably a calcium hydroxide  $\text{Ca(OH)}_2$  solution having a concentration of approximately 30 percent. It also has connection nozzles for passing steam or cooling water through the jacket. Separation of Calcium-dependent highly molecular peptides is accomplished through alkaline denaturation in the presence of Ca ions. This ensures a more complete cleansing of the hydrolysate from highly molecular peptides (80,000 D – 30,000 D), as well as from a significant portion of the normally molecular peptides (30,000 D – 5,000 D). Hydrolysate in the bioreactor is neutralized and then alkalinized to a pH of 8.5 using fractional automatic addition of preferably a 30% water-based  $\text{Ca(OH)}_2$  mixture, heated to +90°C, maintained at that temperature for 15 minutes, and then cooled to +60°C.

Referring again to FIGURES 1 and 2, the final component of product purifier **171** is third centrifuge **170**, a high-speed decanter for separating denatured fish peptides and phosphorus salts of calcium. Centrifuge **170** has a preferred rotation speed of 7,000 revolutions per minute. Phosphorous salts of calcium represent high economic value as an independent product utilized in the chemical industry. For this reason, they are centrifuged and harvested. As the contents accumulate, they are dried, freed from organic matter (by way of incineration), batched, and packed. The resulting phosphorous calcium salts (together with calcium hydroxide) weigh approximately 1/3 ton when the hydrolysate is cleaned from 5 tons of raw materials.

Referring still to FIGURE 1, pH adjuster **190** is a carbon steel mixing tank with integral impeller and an operating pH of 5.5. It operates preferably at ambient temperature, and has a connection nozzle for the addition of a mild phosphoric acid solution. The main function of pH adjuster **190** is to aid in product preservation.

As shown in FIGURE 2, the final component of product finisher **122** is product concentrator **196**. Usually the hydrolysate is concentrated 3-4 times, because as the



concentration of a solution increases, their solvency and hydrophobic bonds decrease. At the end of the concentration process, a pH level of 3.5-4.0 is established, which inhibits the growth of microflora. Concentrator 196 includes nutrient separator 195 and alternate driers 200, 210, 220, and 230. Separator 195 is in communication with pH adjuster 190 through  
5 flow redirector 193. Flow director 193 permits production of either an EPH liquid 197, an EPH powder 235, and/or at least three other products. These include an amino acid/peptide mix 205, a vitamin/mineral mix 215, or pure amino acids 225. Nutrient separator 195 can be one of a number of unit operations that can separate one or more amino acids, peptides, and vitamins and minerals from the EPH solution produced by the piscine bioreaction. Nutrient  
10 separator 195 may entail ultra-filtration, piezo-dialysis, or countercurrent extraction of nutrients, as appropriate, in order to produce mixtures that consist mostly of one or two constituents of EPH.

In a piezo-dialysis unit, as described in U.S. patent 4,554,376, by Fujimoto, which is hereby incorporated by reference in its entirety, an amphoteric ion exchange membrane is  
15 used to separate a specific amino acid. Dialysis is carried out at the isoelectric point so that a low molecular weight electrolyte, which is contained as an impurity in the amino acid solution or an amino acid which is not at its isoelectric point, can be selectively allowed to permeate while an amino acid which is at its isoelectric point will not.

In ultra-filtration, a synthetic membrane that allows only certain size spherical  
20 molecules to permeate (typically 2,000 Daltons to 1,000,000 Daltons) is used to remove relatively high molecular weight solutes and particulate components from water and other solvents.

In countercurrent extraction, an organic phosphorous acid extractant, as described in U.S. patent 5,049,279, by Bitar, *et al*, which is hereby incorporated by reference in its  
25 entirety, is used to collect certain amino acids into an extractable liquid phase where transfer into an aqueous phase is possible. The aqueous phase is then charged with hydrochloric acid, producing an acidic concentrated solution of the target amino acid.

Dryer technology is critical to the success of amino acids factory 1. The preferred embodiment is an evaporator followed by an industrial paddle-type dryer 230C. Referring to  
30 FIGURE 4, an evaporator/paddle dryer is a two step process. Evaporator 230B may be either a once-through unit (agitated film, falling film, or climbing film), or a circulation unit (short-tube, forced-circulation, or climbing film). In once through operation, the feed liquor, or hydrolysate passes through a series of tubes with condensing steam providing the heat required for evaporation. The feed hydrolysate leaves the unit as a thick liquor. The  
35 evaporation is accomplished in a single pass. In circulation evaporators a pool of liquid is

held within the equipment. Incoming feed mixes with the liquid from the pool, and the mixture passes through the tubes. Unevaporated liquid discharged from the tubes returns to the pool, so that only part of the total evaporation occurs in one pass. The general purpose of the evaporator is to concentrate the EPH solution prior to final drying.

5           In a paddle dryer **230**, liquid EPH is forced into one end of a chamber. Hot air is injected downward into the liquid material through a horizontal screen, which causes bubbling of the EPH liquid and granulation. Below the screen is a paddle chamber where multiple-paddles mounted on rotating axels continuously scoop up dried granules and move them towards a discharge chute, where the granules are conveyed. An enclosed chamber  
10 above the air nozzles provides freeboard for entrained dry particles to settle back into the mixing area.

          There are several advantages of this type of dryer, patent pending in Norway by Drytec. Paddle dryers operate in a temperature range from 150°C to 200°C using hot air rather than steam. The paddles provide better mixing and fewer hot spots, resulting in less  
15 damage to the EPH powder, and a higher quality product than is the case with conventional drying technology.

          Referring to FIGURE 4, alternate dryers that may be used with factory 1 include a crystallizer **230A**, and an evaporator/spray dryer **230B** to produce EPH in dry powder form. Crystallizer **230A** is equipped with a draft tube, which also acts as a baffle to control the  
20 circulation of the magma, and a downward-directed propeller agitator to provide a controllable circulation within the crystallizer. An additional circulation system, outside the crystallizer body and driven by a circulating pump, contains the heater and feed inlet. Product slurry is removed through an outlet near the bottom of the conical lower section of the crystallizer body. For a given feed rate, both the internal and external circulations are  
25 independently variable and provide control for obtaining the required crystal size distribution.

          Regarding spray dryer **230C**, the concentrated EPH from the evaporator 230B is dispersed into a stream of hot gas in the form of a mist of fine droplets. Water is rapidly  
30 vaporized, leaving residual particles of dry EPH, which are then separated from the gas stream. The flow of EPH and gas (steam or hot air) may be cocurrent, countercurrent, or a combination of both in the same unit. The droplets are formed inside a cylindrical drying chamber, either by spray nozzles or by high-speed spray discs. The drying chamber is typically large (8 to 30 feet in diameter) to prevent the droplets from touching a solid surface before drying has taken place.

35           As shown in FIGURE 1, enzymatic protein hydrolysate (“EPH”) is the main product

of amino acids factory 1. As previously detailed, other products are derived upstream from nutrient separator 195, including hydroxylapatite 125, cold fish oil 145, fish protein 150, phosphorous salts of calcium, and fish peptides 177. Although part of the novelty of the amino acid factory is the number of different products derived from essentially a singular input source (fish and water), the key product and most commercially valuable nutrient produced by amino acid factory 1 are amino acids and the enzymatic protein hydrolysate (EPH) powder 235, finished through drier 230. EPH consists of 70 to 90 percent free amino acids, 15 to 25 percent highly molecular peptides, and less than 5 percent vitamins and minerals. A less expensive (liquid) form of EPH may be harvested at 197.

Referring now to FIGURE 3, process control system 103 for the amino acids factory makes use of commercially available instrumentation and control equipment with direct electronic links to one or more computers. This system includes one main and several video display screens to indicate system status and key operating parameters, keyboards and switch controls to adjust operating conditions, a central processing unit (CPU) to process system-derived data in real time, and control interlocks for safety and system shutdown in the event of power failure or other emergencies. The CPU will evaluate all monitored process data, such as pH, temperatures, pressures, etc. and convert them to the appropriate mechanical or chemical task required to maintain the process within specified operating ranges through the use of appropriate software. In general, this system will perform the following: a. monitor and display the status of each key piece of equipment in the production process during the manufacturing sequence; b. maintain key operating parameters within their proscribed limits; c. provide alarms during process upset conditions; d. increase the level of process automation during process startups; and e. increase the level of process automation during process shutdown.

According to the preferred embodiment, operating data is fed to process control system 103 from monitoring instruments located throughout the factory. The manufacturing process consists of three principal procedures. These are: a. raw material preparation, b. piscine bioreaction, and c. product finishing. Each of these three principal procedures consists of one or more distinct unit operations that are required to convert one of a number of fish types (e.g., salmon, trout, halibut, tappai, cod, mackerel, herring, shrimp, krill, and blue whiting) into commercial products.

A description of the inputs processed by control system 103, the subsequent outputs, and their relation to providing overall process automation, safety, and ease of operation, is as follows: For raw material preparation, the data inputs for this procedure include: fish type (only one type of fish may be used per batch); the total weight of fish remnants charged to

the cutter/homogenizer; and the operational status of the weigher/mixer and cutter/homogenizer. Ferments and substrata from a single fish type are fed to a mixing vessel mounted on an industrial scale to ensure that the 1:1 weight ratio of substrate to ferments requirement is met, and to determine the total weight of the batch to be processed.

5 The resulting mixture then undergoes size reduction in a cutter/homogenizer to produce a homogeneous mass of fish remnants. The outputs from control system 103 will be: display of ON/OFF status for the weighing/mixer and the cutter/homogenizer, the type of fish being processed and the total weight of the batch (displayed on main and local control panels).

The data inputs for the (second) principal procedure include: the type of fish being  
10 processed, the weight of the batch to be processed, the pH of the bioreaction mixture, the temperature of the bioreaction vessel contents, the bioreaction time, the concentration of amino nitrogen as hydrolysis takes place, and the bioreaction vessel pressure. The weight of the batch of fish remnants (substrata and ferments in a 1:1 weight ratio, respectively) determines the amount of water to be charged to the bioreaction vessel for hydrolysis. The  
15 ratio of fish remnants to water is 1:1. The pH of the bioreaction mixture is maintained between 7.8 and 8.0 by the addition of a caustic solution fed by a metering pump controlled by a signal from a pH probe located within the bioreactor. The amount of caustic solution used is dependent on the pH of the local water supply and its buffering capacity. Once the bioreactor is fully charged and the pH is within the desired range, hydrolysis begins. The  
20 bioreaction mixture is continuously agitated by an integral mixer. The progress of the bioreaction is monitored by measuring the concentration of amino nitrogen in the bioreactor at regular time intervals. Once the concentration of amino nitrogen begins to level off, hydrolysis is discontinued by raising the temperature of the bioreaction mixture to 95 C over a time interval of approximately ten minutes. This deactivates the enzymes catalyzing the  
25 bioreaction and causes thermal coagulation of the bioreactor contents. The bioreactor contents are then cooled to 60 C. In the unlikely event that a batch becomes contaminated, there will be unwanted bacterial growth accompanied by the excessive production of foam. For this reason, the pressure within the bioreactor is monitored to provide pressure relief and system shutdown to prevent it from exploding.

30 The data outputs from the computerized control system will be: the quantity of caustic solution metered into the bioreactor, agitator speed, the quantity of water to be charged to the bioreactor, the quantity of non-contact steam required to raise the temperature of the bioreactor to 95 C, the quantity of non-contact cooling water required to lower the temperature of the bioreactor to 60 C, signals for the activation of pressure relief valves,

safety alarms, and system shutdown should the bioreactor be contaminated and/or generate excessive pressure.

The inputs for the last principal procedure include: the operational status of the hydroxylapatite/EPH centrifuge, the pH of the acid/neutral denaturation vessel contents, the  
5 temperature of the acid/neutral denaturation vessel contents, the operational status of the fish oil/fish protein/EPH centrifuge, the pH of the alkaline denaturation vessel contents, the temperature of the alkaline denaturation vessel contents, the operational status of the fish peptides/Ca-P/EPH centrifuge, the pH of the adjusting vessel contents, the temperature of the pH adjusting vessel contents, the temperature of the nutrient separation unit, the pressure  
10 (or pressure drop) of the nutrient separation unit, the pH of the nutrient separation unit, the temperature of the dryer, and the pressure drop across the dryer.

Upon completion of hydrolysis, the contents of the bioreactor are fed to a centrifuge for separation and removal of bone tissue (hydroxylapatite). The EPH solution is then fed to the acid/neutral denaturation vessel. Acid denaturation removes a significant portion of high  
15 molecular weight peptides, glycoproteids, lipoproteids, and nucleoproteids from the EPH solution. The pH of the denaturation vessel contents is adjusted to 3.5 by the addition of a phosphoric acid solution fed by a metering pump controlled by a signal from a pH probe located within the denaturation vessel. The amount of acid solution used is dependent on the pH of the hydrolysate and its buffering capacity. The denaturation vessel' contents are  
20 continuously agitated by an integral mixer. Once a pH of 3.5 is maintained, the temperature of the denaturation vessel contents are raised to 90 C and held there for 15 minutes. Then the denaturation vessel contents are cooled to 60 C.

Upon completion of acid denaturation, the vessel contents are fed to a centrifuge for separation of cold fish oil, fish protein, and EPH. The EPH solution is then fed to the  
25 alkaline denaturation vessel. Alkaline denaturation in the presence of calcium ions provides additional cleansing of the EPH from high molecular weight peptides (30,000 D to 80,000 D), with significant removal of moderate molecular weight peptides (5,000 D to 30,000 D). The pH of the denaturation vessel contents is adjusted to 8.5 by the addition of a 30 percent calcium hydroxide solution fed by a metering pump controlled by a signal from a pH probe  
30 located within the denaturation vessel. The amount of calcium hydroxide solution used is dependent on the pH of the EPH and its buffering capacity. The denaturation vessel' contents are continuously agitated by an integral mixer. Once a pH of 8.5 is maintained, the temperature of the denaturation vessel contents are raised to 90 C and held there for 15 minutes. Then the denaturation vessel contents are cooled to 60 C.

Upon completion of alkaline denaturation, the vessel contents are fed to a centrifuge for separation of fish peptides, phosphorus salts of calcium, and hydrolysate. The EPH solution is then fed to pH adjuster 190. Adjuster 190 brings the pH of the hydrolysate to 5.5 using a phosphoric acid solution fed by a metering pump controlled by a signal from a pH probe located within the pH adjusting vessel. The pH adjuster's contents are continuously agitated by an integral mixer. Upon completion of pH adjustment, the EPH is fed to a dryer for the production of dry, powdered product. The drying unit can be any one of a number of configurations that use steam to evaporate the water content of the EPH and produce a dry powder.

10 An alternate process unit can also be used to provide further separation of EPH into product streams comprised of major EPH constituents. For example, certain specific amino acids may be isolated by the use of an ion exchange resin. Other constituents of EPH, such as bioactive peptides, may be separated by the use of ultra-filtration. Key monitored operating parameters are typically temperature, pressure (or pressure drop), and pH.

15 The data outputs from the computerized control system will be: display of ON/OFF status for the hydroxylapatite/EPH centrifuge, the quantity of phosphoric acid solution metered into the acid/neutral denaturation vessel, the acid/neutral denaturation vessel agitator speed, the quantity of non-contact steam required to raise the temperature of the acid/neutral denaturation vessel to 90 C, the quantity of non-contact cooling water required to lower the temperature of the acid/neutral denaturation vessel to 60 C, display of ON/OFF status for the cold fish oil/fish protein/EPH centrifuge, the quantity of 30 percent calcium hydroxide solution metered into the alkaline denaturation vessel, the alkaline denaturation vessel agitator speed, the quantity of non-contact steam required to raise the temperature of the alkaline denaturation vessel to 90 C, the quantity of non-contact cooling water required to lower the temperature of the alkaline denaturation vessel to 60 C, the ON/OFF status for the fish peptide/Ca-P salts/EPH centrifuge, the quantity of phosphoric acid solution metered into the pH adjusting vessel, the pH adjuster agitator speed, the quantity of steam required by the dryer to make EPH powder.

20 The enzymatic protein hydrolysate (EPH), envisioned by the current invention, is produced by profound enzymatic protein hydrolysis of cold water animals. Approximately 30 70% – 90% of that EPH is free amino acids, as itemized below. An alternate mixture of EPH may also contain Tryptophan, OH-proline, OH-lysine. The EPH also contains approximately 10% – 20% highly molecular peptides. In addition, the EPH contains approximately 3% – 5% vitamins, minerals and oils. The EPH minerals include cadmium, lead, mercury, arsenic, natrium, potassium, calcium, phosphorous, magnesium, iron, zinc, 35

copper, manganese, selenium, cobalt, molybdenum, chromium. The vitamins in the EPH include thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid, biotin, folic acid, cyanocobalamine, meso-inositol, vitamin E. For each amino acid below, their effect on the functioning of various organs, tissues and systems of the human body, as well as the possible uses of the amino acids in the treatment of specific diseases are briefly described, as follows:

Lysine-- $C_6H_{14}N_2O_2$ —is an essential amino acid.

Promotes the adequate absorption of calcium by bone tissue; participates actively in the synthesis of collagen, which makes up the basis for cartilage and connective and bone tissue; effectively stimulates the synthesis of humoral antibodies and a number of hormones and enzymes. Research has demonstrated that lysine can be effective against the herpes virus, since it stimulates the production of substances that suppress the growth of the virus. A deficiency of lysine in the diet is accompanied by rapid fatigue, an inability to concentrate, irritability, bloodshot eyes, retardation of growth, hair loss, anemia and reproductive problems.

Methionine-- $C_5H_{11}NO_2S$ --is an essential amino acid.

Is the main source of sulfur in the cells of the body, necessary for the normal growth of hair, nails and skin; promotes a lowering of the level of cholesterol in the blood; increases the production of lecithin in the liver; reduces the level of fat in the liver and protects the kidneys; a natural gelatinizing (binding) agent for heavy metals; regulates the formation of ammonia in the urine and promotes the formation of urine without ammonia, which reduces bladder irritation; strengthens the structure of the hair and promotes its growth.

Phenylalanine-- $C_9H_9NO_2$ --an essential amino acid that is used intensively in the brain for the synthesis of norepinephrine – a chemical agent which serves for the transmission of signals between both nerve cells and brain cells; promotes physical activity, reduces hunger, acts as an antidepressant and improves memory.

Threonine-- $C_4H_9NO_3$ --an essential amino acid.

Is an extremely important structural element of collagen, elastin and the proteins that make up tooth enamel; prevents the fatty degeneration of the liver; promotes the normal functioning of the gastrointestinal tract.

Leucine-- $C_6H_{13}NO_2$ -- essential fatty acid that is a decisive component in the synthesis of extremely important biologically active compounds responsible for the generation and transfer of energy in all the cells of the body; stimulates the working of the brain.

Isoleucine-- $C_6H_{13}NO_2$ --essential fatty acid that is decisive in the synthesis of extremely important biologically active compounds responsible for the generation and transfer of energy in all the cells of the body; stimulates the working of the brain.

Arginine-- $C_6H_{14}N_4O_2$ --a nonessential amino acid which strengthens the immune response to viral, bacterial and parasitic infections; promotes the healing of wounds and the regeneration of tissues of the parenchymatous organs; inhibits the growth of tumor cells; stimulates the synthesis of growth hormone.

5 Tyrosine-- $C_9H_{11}NO_3$ --a nonessential amino acid.

Transmits impulses from nerve cells to brain cells; helps to overcome depression; improves memory; promotes concentration; supports the healthy functioning of the thyroid gland, the adrenal glands and the pituitary gland.

Glycine-- $C_2H_5NO_2$ --a nonessential amino acid.

10 Promotes the delivery of oxygen and energy in cellular synthesis; is responsible for the strength of the immune response; stimulates the synthesis of a number of hormones.

Serine-- $HOCH_2$ --a nonessential amino acid.

Responsible for the full assimilation of glucose by the liver and the muscles; strengthens the synthesis of humoral antibodies; participates in the synthesis of the fatty acid sheath around  
15 the nerve fibers.

Glutamic Acid— $C_5H_9NO_4$ --a nonessential amino acid which is considered “brain food”; improves mental abilities; helps with fatigue; speeds up the healing of wounds; is used in the treatment of schizophrenia, alcoholism and diabetes mellitus.

20 Aspartic Acid— $C_4H_7NO_4$ --a nonessential amino acid. Promotes the excretion of ammonia from the body, since ammonia is a highly poisonous substance for brain cells. Recent research has demonstrated that aspartic acid increases resistance to fatigue and improves endurance.

Taurine-- $C_2H_7NO_3S$ --a nonessential amino acid which is formed from cysteine in the liver or from methionine in other organs. It is present in milk and in some sea animals.  
25 Taurine participates in the regulation of the process of stimulation in the central nervous system; it has an antiarrhythmic effect on the cardiac muscle; it is a factor in the control of many of the biochemical reactions that take place in the process of aging; it promotes the neutralization of free radicals that appear in the cells of the body from environmental factors; it inhibits aging processes; it binds free radicals; it neutralizes toxins; it is an active  
30 participant in the restoration of epidermal tissue damaged by burns or by transplants or other surgical effects. The hair and skin contain up to 14% cystine.

Histidine-- $C_6H_9N_3O_2$ --a nonessential amino acid.

Found in abundance in hemoglobin; widely used in the treatment of rheumatoid arthritis, allergic diseases, ulcers and anemia. A histidine deficiency is a cause of poor hearing.

35 Alanine-- $C_3H_7NO_2$ --a nonessential amino acid which is an important source of energy



for muscle tissue, the brain and the central nervous system; strengthens the activity of the immune system and improves the humoral immune response; promotes the metabolism of sugar and organic acids.

5 Proline-- $C_5H_9NO_2$ --a nonessential amino acid. It is the main component of all connective tissue and is necessary for the normal functioning of the joints, tendons and ligaments; supports the working fitness of the cardiac muscle.

Ornithine-- $C_5H_{12}N_2O_2$ --a nonessential amino acid. It has not been isolated from proteins except after alkali hydrolysis.

## 10 CLAIMS

We claim:

1. an amino acid factory, comprising:  
a raw material preparator;  
15 a piscean bioreactor connected to said material preparator; and  
a product finisher connected to said bioreactor.
2. The factory according to claim 1, wherein said product finisher contains a nutrient separator selected from among the group consisting of a piezo-dialysis unit, an ultrafiltration unit, and a countercurrent extraction unit.
- 20 3. The factory according to claim 1, wherein said raw materials consist essentially of marine animal parts and water, and wherein said product finisher produces a nutrient mix consisting essentially of amino acids.
4. The factory according to claim 3, wherein said product finisher produces a nutrient mix essentially consisting of taurine, aspartine, threonine, serine, glutamic acid, praline,  
25 glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine.
5. The factory according to claim 3, wherein said product finisher produces a nutrient mix comprising cadmium, lead, mercury, arsenic, natrium, potassium, calcium, phosphorus, magnesium, iron, zinc, copper, manganese, selenium, cobalt, molybdenum, and chromium.
- 30 6. The factory according to claim 3, wherein said mix further comprises the following vitamins: thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid, biotin, folic acid, cyanocobalamine, meso-inosite, and vitamin E.
7. An amino acid factory, comprising:  
a mixer having a multi-ton capacity into which raw materials are fed;  
35 a cutter conjoining said mixer;

a reactor connected to said mixer and said cutter, into which feeds a first alkaline PH adjuster;

a first centrifuge connected to said reactor, said first centrifuge producing an hydroxylapatite;

5 a first denaturing tank, connected to said first centrifuge, into which feeds a first acidic PH adjuster;

a second centrifuge connected to said first denaturing tank, said second centrifuge producing cold fish oil;

10 a second denaturing tank, connected to said second centrifuge, into which feeds a second alkaline PH adjuster;

a third centrifuge, connected to said second denaturing tank, said third centrifuge producing a calcium-based product;

a PH adjuster, connected to said third centrifuge; and

a concentrator connected to said PH adjuster.

15 8. The factory according to claim 7, wherein said raw materials further comprise:

a substrate of essentially whole marine animals;

ferments comprising marine animal entrails having a mass content that is essentially equivalent to the mass content of said substrate; and

20 purified water having a mass content that is essentially equivalent to the mass content of said animal protein.

9. The factory according to claim 8, wherein said marine animals consist of a single species of farmed cold-water fish.

10. The factory according to claim 8, wherein said ferments are derived from a single organ, such as the stomach of a fish.

25 11. The factory according to claim 8, wherein said marine animals consist of a single species of cold-water wild catch, such as cod, mackerel, and herring.

12. The factory according to claim 8, wherein said marine animals consist of a single species of pelagic fish.

30 13. The factory according to claim 8, wherein said marine animals consist of shrimp or krill.

14. The factory according to claim 8, wherein said marine animals consist of industry fish, such as blue whiting.

15. The factory according to claim 8, wherein said raw materials are selected from among the group consisting of trout, halibut, tappai, and cod.

35 16. The factory according to claim 9, wherein said cold-water fish is salmon.

17. The factory according to claim 9, wherein said cold-water fish is krill.

18. The factory according to claim 9, wherein said concentrator produces a powder-  
5 based enzymatic protein hydrolysate (EPH) consisting essentially of free amino acids.

19. An enzymatic protein hydrolysate consisting of 70-90 % of the free amino acids:  
taurine, aspartine, threonine, serine, glutamic acid, praline, glycine, alanine, valine,  
methionine, isoleucine, leucine, tyrosine, phenylalaine, oxyhedral proline, ornithine, lysine,  
10 histidine, arginine; and a 5-25% mix of cadmium, lead, mercury, arsenic, natrium,  
potassium, calcium, phosphorous, magnesium, iron, zinc, copper, manganese, selenium,  
cobalt, molybdenum, and chromium.

20. An amino acid factory, comprising:  
a mixer having a multi-ton capacity;  
a raw material solution feeding into said mixer, said solution essentially  
15 comprising, by weight, 25% fish entrails, 25% whole fish, 50% purified water, and a basic  
solution;  
a cutter conjoining said mixer;  
a fermentation reactor connected to said mixer and said cutter, into which feeds  
a first alkaline PH adjuster;  
20 a first centrifuge connected to said reactor, said first centrifuge producing an  
hydroxylapatite;  
a first denaturing tank, connected to said first centrifuge, into which feeds a  
first acidic PH adjuster;  
a second centrifuge connected to said first denaturing tank, said second  
25 centrifuge producing cold fish oil;  
a second denaturing tank, connected to said second centrifuge, into which feeds  
a second alkaline PH adjuster;  
a third centrifuge, connected to said second denaturing tank, said third  
centrifuge producing phosphorous salts of calcium;  
30 a PH adjuster, connected to said third centrifuge, into which feeds a second  
acidic PH adjuster;  
a protein hydrolysate concentrator, connected to said PH adjuster,  
comprising a nutrient separator and a dryer;  
wherein, said concentrator produces an enzymatic protein hydrolysate

consisting essentially of taurine, aspartine, threonine, serine, glutamic acid, praline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, OH-proline, ornithine, lysine, histidine, and arginine, bioactive peptides, and select vitamins and minerals.

5

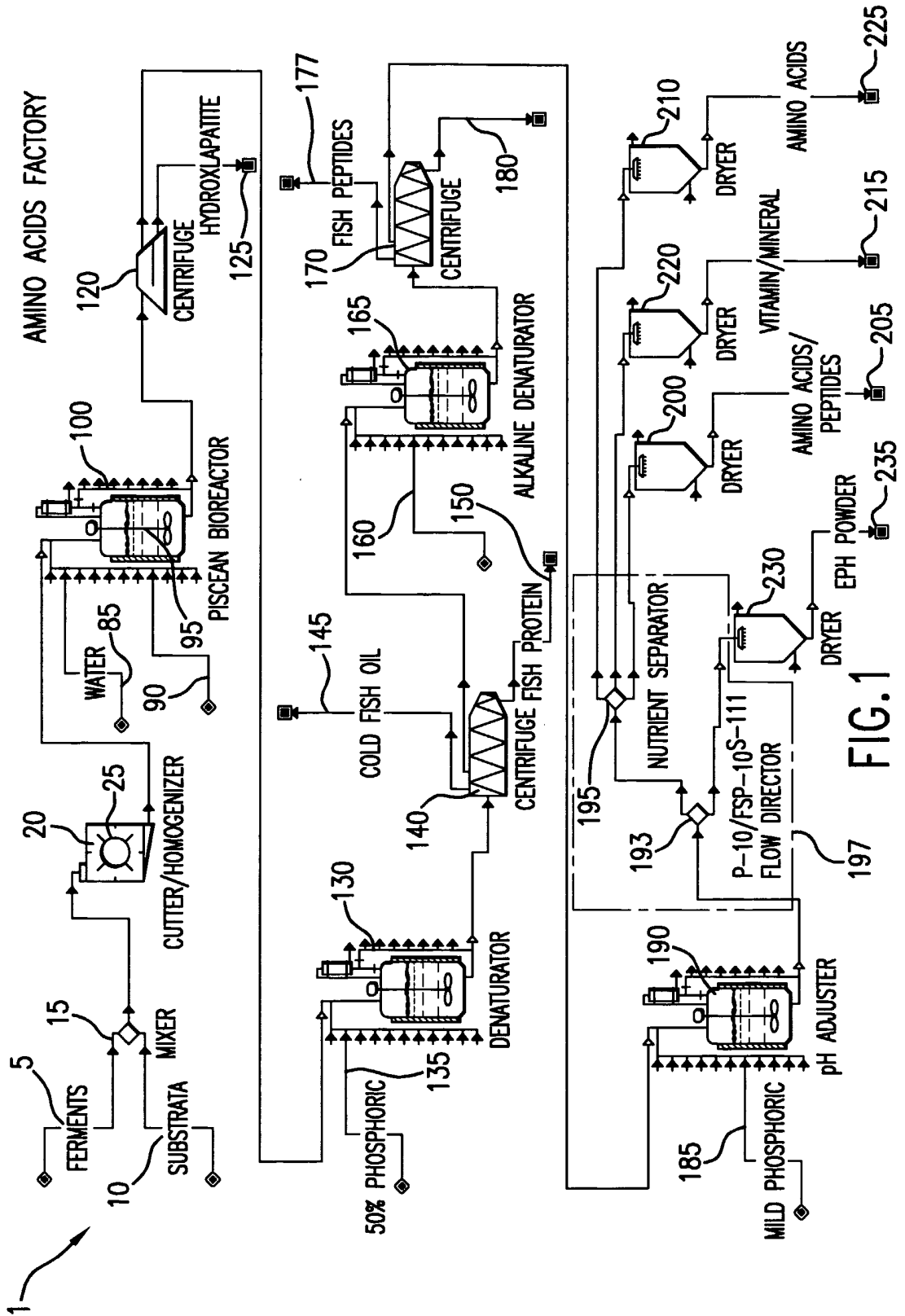


FIG. 1

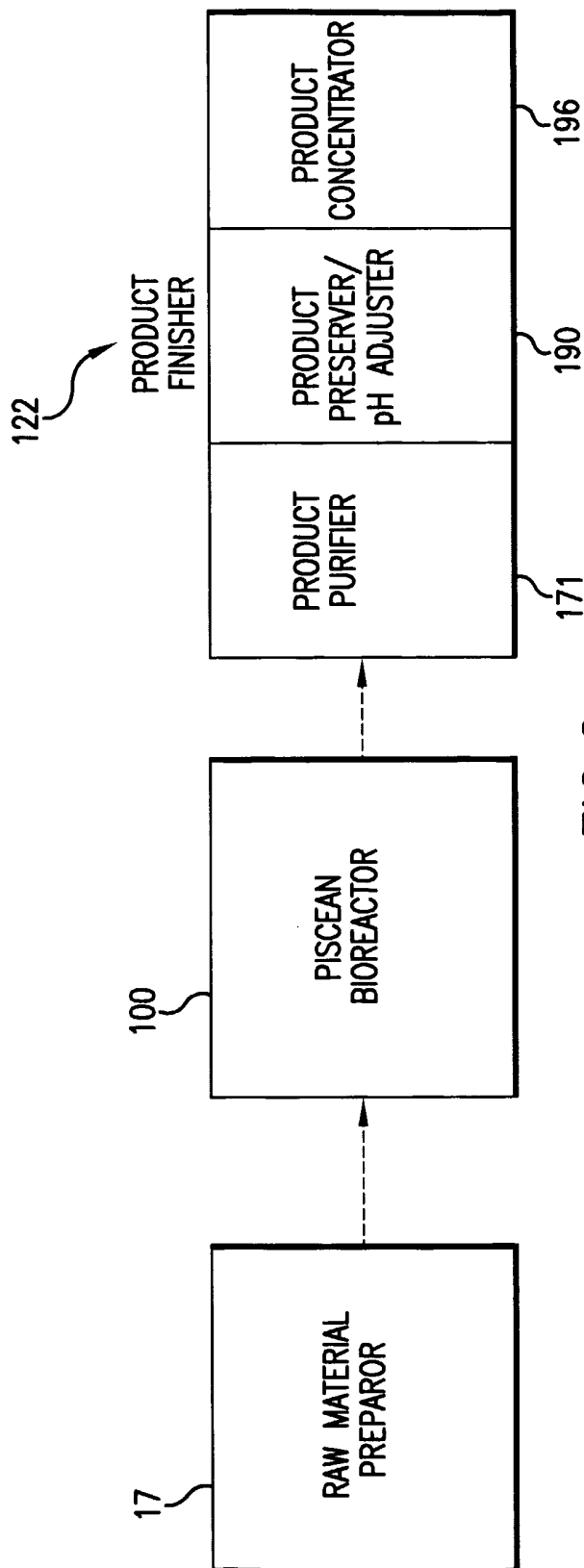


FIG.2

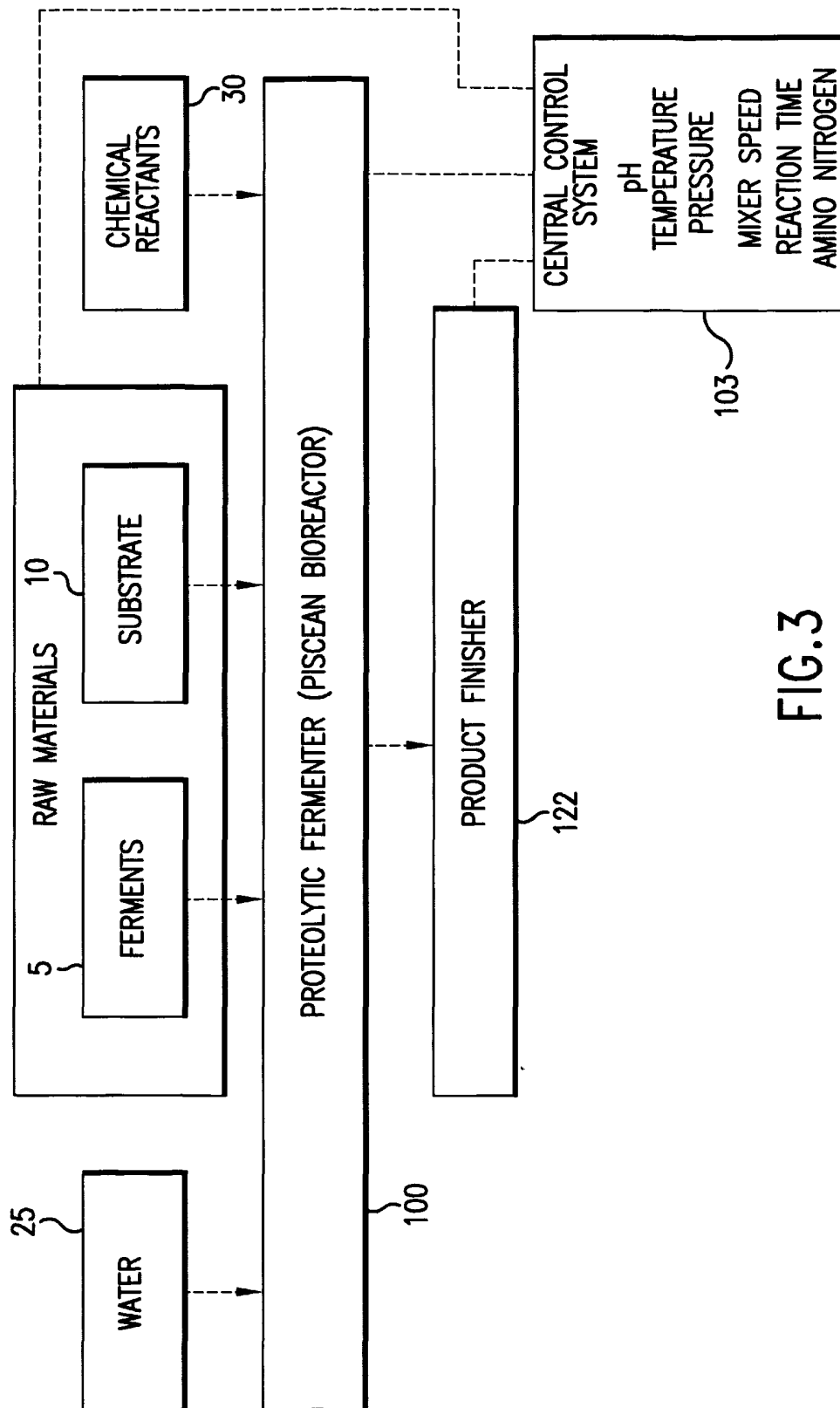


FIG.3

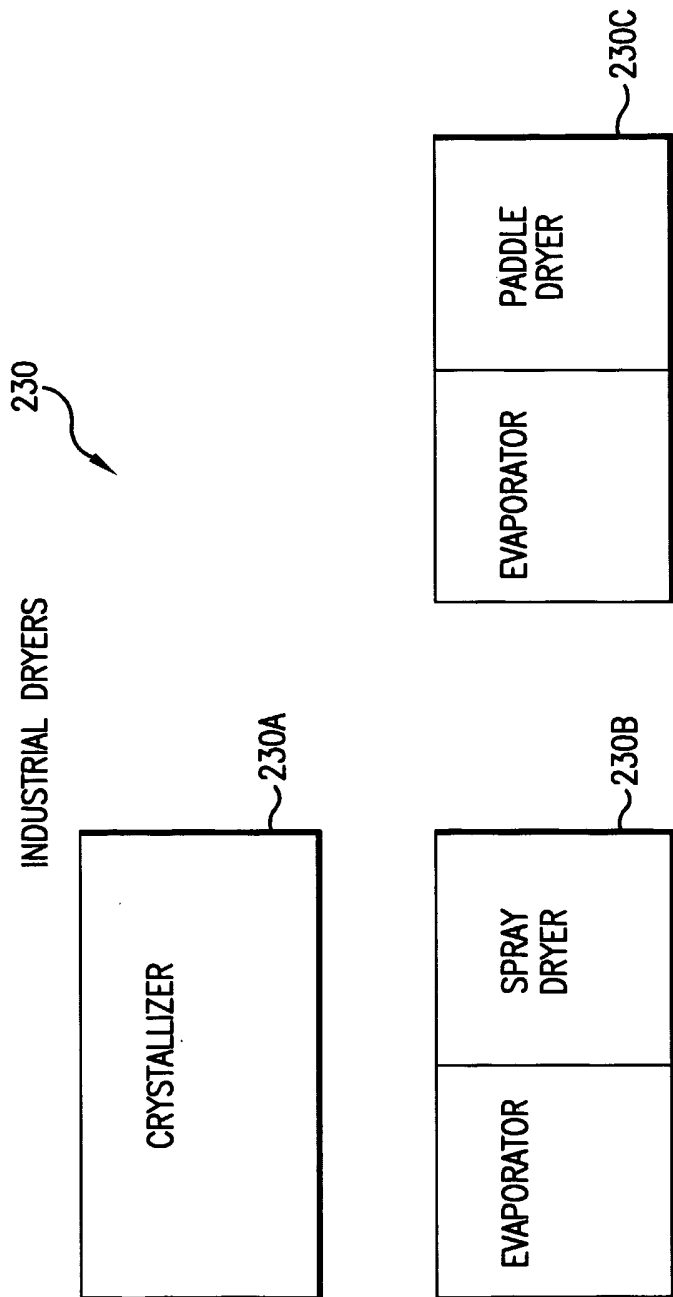


FIG. 4



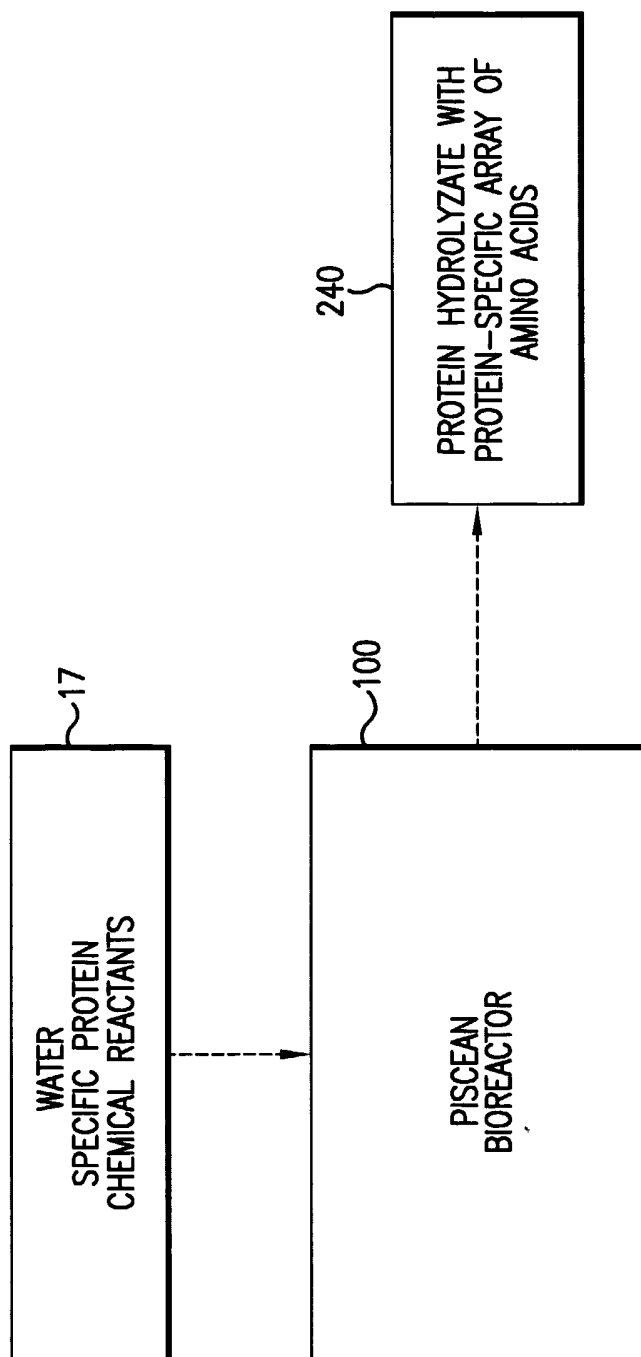


FIG.5

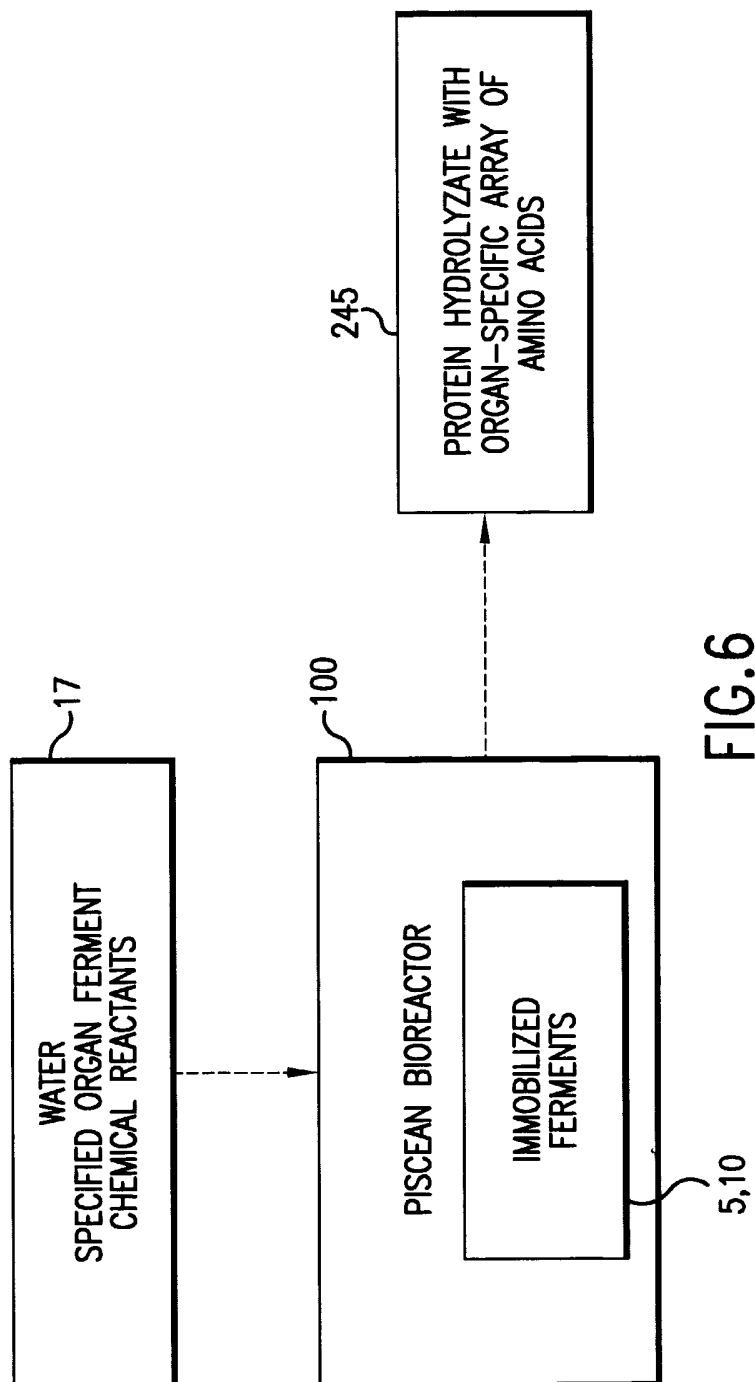


FIG. 6

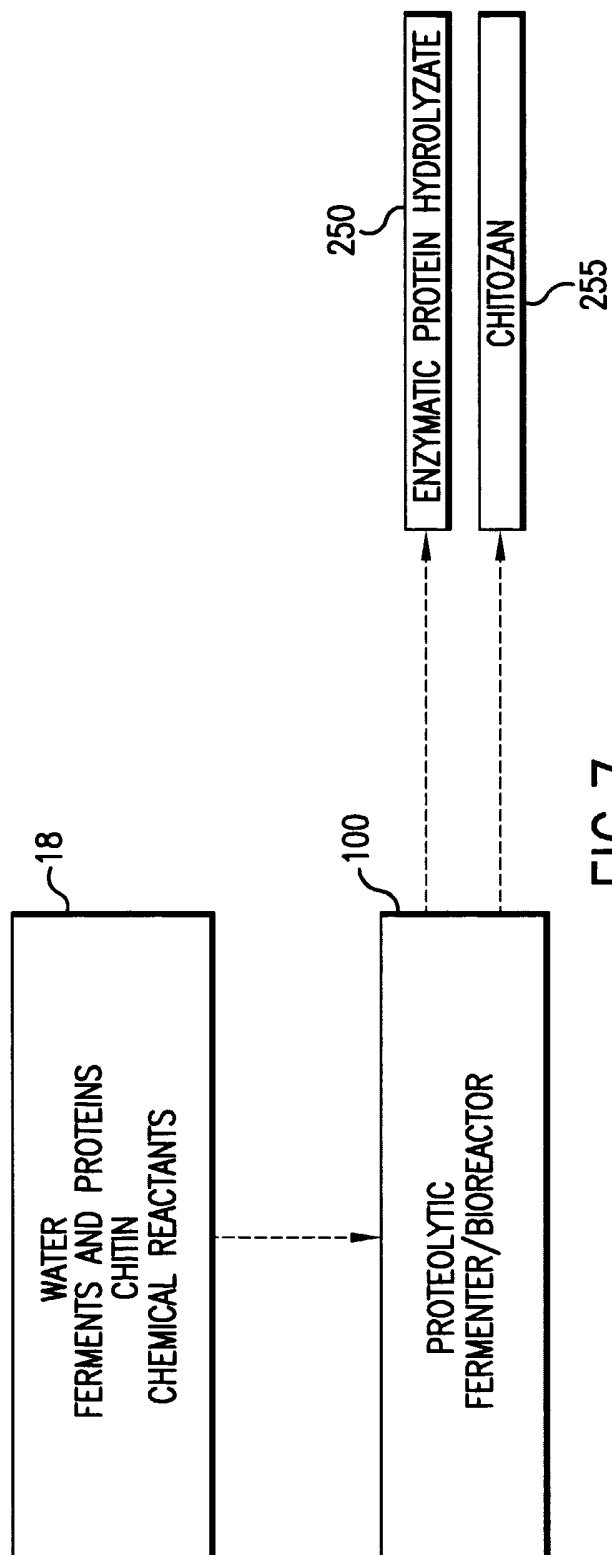


FIG.7