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PROCESS OF ARTIFICIAL DIGESTION OF ALBUMINOID AND FATTY SUBSTANCES

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The invention has for its object to repeat in an industrial manner and "in vitro" the process of the various transformations obtained by the digestion of albuminoid and fatty substances "in vivo". In other words, to divide on the one hand the peptide chains which form the various proteins, on the other hand to dissociate the nitro-lipidic complexes or only the lipidic complexes and finally to bring these disintegrated elements into such a chemical and physical state that they can be readily assimilated by a living organism for subsequent regrouping.

The invention aims at effecting said disintegration by natural means, such as diastases and ferments; said invention protects the material treated and the products obtained from the bacterial actions that are capable either of producing noxious or valueless products, or of degrading the albuminoid materials until ammoniacal products and even ammonia are formed.

The invention is conceived in order:

(*a*) To obtain a biological medium which offers at the opportune instants the optimum characteristics, such as the pH or the pHI (isoelectric point).

(*b*) To subject the albuminoids to the action of diastases or ferments, either contained in the treated materials (autolysis), or brought in from the outside (proteolysis) and thus to produce a disintegration which, at the end of the operation, has not affected the diastases or ferments or the products of poor physical or chemical stability which could not withstand acid or alkaline hydrolyses.

(c) To protect the albuminoids treated from the bacterial actions by acting, without in any way impairing the diastatic or fermentative actions, by conjugated means, on specific groups, this being done without resorting to the use of substances that are difficult to eliminate (sodium chloride), toxic (salts of salycilic acid, of lead, etc.), inflammable (petroleum ether, benzine), dangerous to manipulate or only slightly active on certain bacteria (homologous series of nitromethanes), denaturing (petroleum, carbon tetrachloride), etc. in spite of the more or less limited antiseptic properties of these protecting substances.

(*d*) To eliminate the protecting agents designated above either naturally during the treatment by chemical substitution or hydrolysis, or at the end of the operation by physical means such as heat and vacuum or chemical means such as precipitation in the form of readily separable insoluble substances.

(*e*) To separate the various final elements of the treatment so as to obtain isolated:

The nigrogenous compounds in the simplest form, either free, or in combinations of chemical salts, this being effected without any residue of a protecting agent;

The undigestable substances;

The lipides which accompany the albuminoids, conjointly or in complexes, and which have been able to withstand the saponifications and oxidations of the artificial digestion.

In order to obtain a suitable artificial digestion according to the aims of the invention, it is necessary:

(A) to pulp as carefully as possible the materials to be digested, so as to permit of the deepest action on the proteins both of the temporary protecting agents and of the diastatic and catalytic agents. If the diastases that are relied on for the digestion come from secretion glands added to hte mass, liver, stomach, intestine, said glands must also be very finely ground.

(B) the purpose of the following operation is to introduce the temporary protecting agents, each corresponding to quite specific actions, and also substances capable of effecting, at the desired instant, the transformation of all or part of said agents in inactive and non-toxic products. Said substances may advantageously be the following:

(1) A solution of a polysulphide of magnesium in a mixture of equal parts of acetone, isopropyl alcohol and phenylhydrazine hydrate:

(2) Jointly a mixture of three substances: chloroform, dinitrophenol and an auto-oxidizer such as a terpene pinene, for example betapinene.

Without going into the detail of very complex slow chemical reactions, it may be noted that the invention aims at using the above mentioned substances for the following purposes:

The sulphur-magnesium complex, in the presence of the albuminoids, will become stabilized in the colloidal form, will act its elective properties on the halogens, the hydroxyls and all the electro-negative radicals, and will finally become decomposed during the operation: the magnesium will tend to produce insoluble ammonium magnesium compounds; the sulphur will combine to form halo-organic salts with certain cyclic amino acids when they appear or will effect substitutions of sulfonated form with the phenol nucleus of one of the other agents.

In the presence of the colloidal sulphur, of the chloroform (or of a substance of the harmonic series of same) and of the pinene, there will be

formed, by substitution, oxidation or displacement, monosulfonic orthophenol, nascent chloromethane (or chloroethane or again parachlorodinitrephenol) and finally, by bonded oxidation of the pinene, paraphthalic acid (or a monochloro derivative of same). It would moreover be advantageous to associate the dinitrophenol with a sulfonic acid chloride so as subsequently to sulfonate the phenol nucleus more surely.

These substances, which are powerfully antiseptic in small doses (less than 4 per 1000 of the mass to be treated), possess the property of forming slowly and then of dissociating slowly by hydrolysis in an acid medium, precisely when at a given instant the pH of the treated mass spontaneously reaches 6.4, ionic acidity due to the nature of the amico-acids then formed.

They can be replaced by vicinal substances of the harmonic series, without the principle of the invention being modified thereby: for example, nitro-methane may be substituted for the chloroform, camphrene for the betapinene, diphenylaminosulfone or metaphenylenediamine for the dinitrophenol, etc. In any case, the substitution substances obtained by reaction are comparable in nature and in effect, and the chlorinated residues as well as the NO_2 radicals that may persist are fixed on the lipides whence they can be readily extracted by heating to 80° C. None of the above referred to agents offers any opposing action to the reaction of metallic catalytic agents that it might be considered advisable to employ in order to accelerate the artificial digestion.

It must furthermore be noted that betapinene or any other strongly auto-oxidizing agent is employed in order to act, by bonded oxidation, on the arginine (one of the first amino-acids released from the peptide chain) so as to prevent the formation of free guanine. It is moreover advantageous to use it in a methylated or methylic form, so as to assist the formation of insoluble methylguanidine. This substance also acts tine the subsequent formation of the peroxiacids produced by the desintegration of glycerines of unsaturated fatty acids.

(C) A very thorough stirring by means of appropriate apparatus is effected in order to mix first of all the previously described solution of polysulphide of magnesium, then the chloroform-pinene-dinitrophenol mixture. The mass thus treated can be kept and stored indefinitely: there is therefore in this part of the invention a means for protecting from bacterial actions all perishable materials which can previously withstand a state of fine division.

(D) But a mass thus treated may not under certain conditions, be protected from aerobic bacteria, mildew, spores, etc. which are capable of developing and of producing secondary fermentations. Protection therefrom is obtained by covering the mass stored in a tank with a fine layer of oil in which p-hydroxybenzoic acid has been dissolved (in the hot state) to form a $\frac{1}{2000}$ solution. A larger quantity of this 1 per 2 mil oily solution carefully mixed with the mass to be treated will prevent the oxidization of the unsaturated fatty acid lipides, at any rate in the cold state and for a limited time.

(E) Before placing the mass to be digested in the maturation tanks, it is necessary to bring it to the suitable pH for starting the diastatic reactions. Said pH varies of course according to the material: fish, meat of herbivorous animals, ovalbumin, flesh or organs of carnivorous animals and even leguminous materials. But the concentration is always less than pH7 and the optimum point is obtained by slight acification of the mass.

This operation is effected at the same time as the mixing with the protecting substances hereinbefore defined, in the case of an immediate digestion. On the contrary, in the case of storing, it is only effected at the time when the mass is placed in the maturation tank, by means of a second mixing since it would start a slow lysis in the cold state of the mass during storage.

(F) The mass of proteins and diastases thus prepared is then placed to digest in a maturation tank of any content. The temperature is such that the nitrogenous disintegration takes place as quickly as possible, without however there being a coagulation of the free albumins and a destruction of the diastases or ferments. The bringing to the required temperature should be effected quickly: it is therefore necessary to act on a divided mass which is stirred continuously or not, avoiding local overheating and the formation of crust.

The pH falls gradually to 6.4 then rises towards 6.8. When it reaches this approximate value and stays there, bussering is effected with soda until the pH1 is obtained: the basic function of the amino-acids (which are amphoteric) will thus act freely and salts of said amino-acids will thus form, for example histidine sulphate.

(G) The digestion is stopped when the pH has returned of its own accord to pH6.8 and stays so for twenty four hours. The total duration of digestion varies according to the nature of the substances to be treated, to the temperature, to the nature and the importance of the diastases.

(H) The pasty mass which has been placed to digest appears at the end of the operation in the form of a liquid in which an undigested layer has sedimented. The liquid part is formed by an aqueous solution of polypeptides and of amino-acids (or of salts), of free lipides and of peroxidized lipides in suspension or of saponified materials. These various substances are separated by decantation, defecation with tanin and with lime, centrifugation, filtration and ultracentrifugation, its being possible for these mechanical processes to be combined, used in the hot or the cold state, according to whether it is desired to isolate in such and such a part soluble substances above a given temperature.

Finally there is obtained:

(*a*) Undigestible substances: cartilage, conjunctive substances, bones, fish-bones, scales, etc, according to the substances treated;

(*b*) Nitrogenous products in aqueous solution; these products no longer exhibit any trace of the temporary protecting agents; it is therefore necessary to concentrate them since otherwise various bacterial actions may occur. As the liquid keeps indefinitely as soon as it is deprived of 50% of its constitutive water, the excess of water may advantageously be removed either in vacuo, or by streaming, or by exposing in a thin layer, or by cryogeny or adsorption or by any method which neither causes a decarboxllation nor a deamination but which on the other hand removes certain ammoniacal products. This concentration may be continued until the pasty or even the dry state is reached; the amino-acids or their salts then appear in a crystalline form;

(c) Fatty degrases remaining on the filter or separated by decantation or centrigugation. These degrases represent in reality a unidigested mixture, chromoproteins, peroxides of fatty acids

and of various lipides, salts of various phosphoric acids, amino-alcohols, lecitins, higher alcohols, often with fixation of chlorinated residues or of nitrile radicals.

(I) After replacing said degrases to digest in order to finally eliminate the undigested materials which has been stopped at the digestion ceiling during the first maturation, the various products are exposed as a thin layer either to a vacuum or to a suitable heat respecting the organoleptic diastases so as to eliminate the volatile chlorinated residues.

From these fresh exhausted degrases, is separated the excess of free lipides by decantation, pressure or centrifugation. Then the residual mass is treated with suitable solvents in such a manner as only to carry away the fatty substances. The products which might be dissolved, other than the lipides, will in this case be precipitated in the solvents. Finally, a heterogeneous mass of phosphoaminated substances will be obtained: that can, if necessary be readily separated by the usual washing, fractionated solubility and chemical precipitation means.

Finally, by operating according to the described phases of this invention, a slow but absolutely complete artificial digestion has been obtained, which is certainly carried much farther than in the natural operations of living organisms: The complex protein molecules have been extremely divided (chief object of the operation); a part of the lipides has also been converted into elementary substances. The whole of the products obtained, save the undigestible substances, represent elements that are particularly assimilable by a living organism, that have a high nutritive power and a high therapeutical value.

Food products are thus obtained which, save for the wilful separation or elimination of at least one half of the radicals hereinbefore defined, contain all the amino-acids produced by the lysis of the treaded materials. These products offer the feature of being able, save for wilful separation, to contain the whole gammut of the radicals which initially existed in the proteins of animal or vegetable origin subjected to disintegration, but said radicals, instead of forming part of complex organic chains, are in the free state in the form of amino-acids or combined in the form of salts of these latter.

It should be noted, on the other hand, that these finished products are stable although they contain neither sodium chloride nor any other preserving agent.

Preferably, the products are so treated as to be in the form of a medium that is unsuitable for sowing, so as not to be capable of uncontrolled subsequent transformations.

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