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(54) Title: PROCESS FOR THE PREPARATION OF PROTEIN COMPOSITIONS FOR USE IN FOOD INDUSTRY

(57) Abstract

Protein composition suitable for use in food industry. Food grade blood is haemolysed, haemolyed blood is denaturated in alkaline medium in the presence of molecular oxygen, the denaturated haemoglobin is decomposed with a proteolytic enzyme capable of functionating in alkaline or a neutral medium, heamin is separated at an acidic pH value, the enzymatic activity of the globin-protein solution is stopped and the product thus obtained is utilized either directly or after freezing of spray-drying. According to the process of the present invention a colourless, odourless product having a high protein content, a full biological value, a neutral creme colour and saline taste is obtained which can be directly used as feed additive.

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Process for the preparation of protein compositions for use in food industry

Technical Field

This invention relates to a process for the preparation of protein compositions for use in food industry from food grade blood.

Background Art

In DOS No. 2,831,338 a process is described for the utilization of slaughter-house blood as human food or animal feed by working-up the blood. Globin-protein and haemin-protein which are the main components of haemoglobin are separated on processing blood. The said separation is to be carried out under mild conditions in order to avoid the damages of globin protein. According to the cited DOS specific proteolytic enzymes are used for this purpose and by this measure attempts are made to obtain protein being free of bitter taste. Specific proteolytic enzymes exhibit their effect in acidic medium and therefore particularly enzymes derived from the Rhozopus rhizbodiformi and Aspergillus niger strains are used. According to our experiments the use of the above enzymes has the drawback that valuable globin protein material is decomposed as well and it is very difficult to control enzymatic decomposition in such a manner that the protein should be devoid of bitter taste.

Disclosure of invention

It is the object of the present invention to provide a process for the preparation of protein compositions suitable for use in food industry from food grade animal blood - particularly from slaughter-house blood -

whereby the protein composition obtained meets the requirements of food industry, has a biological value higher than that of protein compositions prepared by conventional methods since the decomposition of protein can be eliminated. It is a further object of the present invention to provide protein compositions which can be directly worked-up in meat industry and possess a suitable foaming capacity, emulsifying activity and a proper taste and colour.

The protein composition according to the present invention which can be prepared from blood by haemolysis, subsequent alkaline oxidization and proteolysis carried out in alkaline or neutral medium possesses the following characteristic features (related to dry substance):

- minimal protein content: 80 %
- maximal haemin content: 1.2 mg haemin/g
- maximal free amino acid content: 20 %
- maximal ash content: 10 %
- colour: (according to lightness coefficient of the CIE system: minimal Y = 27.13)
- it should be completely soluble in water
- on heating (at a temperature of about 100 °C) it must coagulate at least by 10-15 %
- foaming: 400-500 %
- emulsifying activity: 50-55 %
- protein biological value: at least 68.7 (MORUP-OLESEN index)
- NPU % = 17.1

The preferred characteristic data of the product prepared according to the process of the present invention are as follows:

- protein content: 82-84 %

- haemin content: 0.05-0.8 mg haemin/g
- lysine content of the total amino acid content: at least 7 %
- methionine content: at least 0.8 %
- glutaminic acid content: at least 4.5 %
- ash content: 7-8.5 %.

On using the composition as food additive it causes no change of colour or taste.

According to the present invention there is provided a process for the preparation of protein composition suitable for use in food industry from food grade blood by haemolysis and enzymatic decomposition which comprises subjecting blood haemolyzed by known methods to denaturalization in alkaline medium at a pH value between 10 and 11, oxidizing the denaturalized haemoglobin in the presence of molecular oxygen, treating the product after oxidation with a proteolytic enzyme functionating in alkaline or neutral medium at a rate of 0.006 - 0.05 Au/g protein and an enzyme-substrate ratio of 1:100 - 1:20, adjusting the pH of the medium after enzymatic decomposition to a value between 4.0 and 5.2 and separating the haemin from the solution having a protein content of 4-16 %, terminating the enzymatic activity of the protein solution by thermal treatment and if desired dehydrating the protein solution under mild conditions.

Haemolysis is carried out in a known manner by osmotic methods, in a 1:3-1:2 diluted aqueous solution, or by ultrasonic methods or under an overpressure of 200-300 atm. Oxidation is accomplished in alkaline medium for 10-120 minutes, under stirring, if desired under additional air-blast. The enzymatic decomposition of the

haemolysed product is performed in alkaline or neutral medium, at a temperature of 30-70 °C, for 30-480 minutes. As proteolytic enzyme in the process of the present invention enzymes functionating in alkaline or neutral medium can be used which prefer to cleave the chain at the histidine, leucine, glycine and phenylalanine bonds. The alkalinity of the medium can be adjusted with sodium hydroxide. The protein solution thus obtained being separated from haemin can be directly utilized or if necessary subjected to spray-drying, liophylization or freezing. After enzymatic treatment the proteolitic activity of the treated product is adjusted to 0 Anson unit. Haemin can be separated from the protein solution in a known manner by separation, centrifuging or filtration. The yield of the process amounts to 60-70 % - related to thick blood (haemoglobin).

According to the process of the present invention it is preferred to use Alcalase 0.6 L (Novo Industri, Denmark), alfa-Kimotripsin (Merck), Corolase PS (Rohm) or Termitase (Rohm) as proteolytic enzyme.

It has been found that if blood cells are denaturalized by haemolysis in alkaline medium, denaturalized haemoglobin is oxidized with molecular oxygen and thereafter haemin is selectively separated from globin-protein by means of a proteolytic enzyme active in alkaline or neutral medium, then the biological value of globin-protein is maintained, its decomposition degree is reduced to a minimal value and a colourless product free from bitter taste is obtained. Oxidation of haemoglobin in alkaline medium promotes the activity of the proteolytic enzyme and the use of enzyme functionating exclusively in neutral or alkaline medium gives optimal results; the latter recognition is just contrary to the teaching of prior art.

Industrial applicability

The invention provides a process for the preparation of protein compositions suitable for use in food industry from food grade blood. The advantages of the invention are that the process is readily feasible and easy to control on industrial scale too, it can be accomplished by using a simple technology, the risks of formation of waste product of inferior quality are low because the process is well reproducible under the given parameters. As slaughter-house blood particularly blood of swine, cattle, horses or sheeps can be used.

Modes of carrying out the invention

Further details of the process of present invention are to be found in the Examples without limiting the scope of protection to the said Examples .

The analysis of the protein obtained is carried out by the following methods:

Determination of protein content: Kjeldahl method (a semi-automatic Kiel-Foss apparatus).

Determination of lysine and glutamic acid content: LABOR MIM and AMINOCHROM type automatic amino--acid analyser.

Determination of methionine content: Barton-Wright: The microbiological assay of the essential amino-acids in compound feeding stuffs, Analyst, 97, 138-141.

Determination of free amino acid content: by using a tin chloride/ninhydrine reagent, related to the L-lysine standard, the

NPU value is

$$\frac{B - B_k}{I} \times 100$$

wherein

B is the nitrogen content of the animal fed with the test-protein;

B_k is the nitrogen content of the control animal

I is the total nitrogen consumption of the feed taken up.

Determination of ash content: in platinum crucible, heated to 650 °C.

Determination of colour: Official Recommendations of Uniform Color Spaces Color-Difference Equations, Metric Color Terms. May 1976 - Supplement No. 2. to CIE Publication No. 15, Colorimetry (E-1.3.1) 1971, page 19.

Determination of haemin content: Gy. Gantner: Acidic--acetonous separation of haem (Husipar 7, 159) dimension: mg of haemin/g.

Determination of emulsion activity: K. Yasumatsu et al: Agric. Biol. Chem. 36, 719 (1972).

Determination of foaming capacity: R.C. Gunter: Chemistry and Characteristics of Enzyme modified Whipping Proteins: J. Am. Oil. Chemist's Soc. March 1979 (V. 56).

MORUP OLESEN index: 10th Int. Cong. Nutr. Abstr. 100, 1235 (Kyoto 1975).

Example 1

To 25 ml of thick blood 75 ml of water are added and

the pH is adjusted to 10.5 by adding a 5 N sodium hydroxide solution. Thick blood is prepared from full complete blood taken with a tubular knife, stabilized with sodium acetate or phosphate and the plasma (blood serum) is separated by centrifuging or separation. Thick blood being diluted with water and adjusted to the suitable pH value is stirred for 120 minutes by using a magnetic stirrer and contacted with air. Under the effect of the alkaline pH and molecular oxygen the protein is denaturalized and the haemoglobin is oxidized.

To 100 ml of haemoglobin solution 0.160 AU Alcalase 0.6 L enzyme are added at an enzyme-substrate ratio of 1:32 and the solution is stirred for 240 minutes. The pH is adjusted to the value of 4.5 by adding concentrated hydrochloric acid. The solution is filled into centrifuge tubes and sedimentated for 30 minutes at 3000 r.p.m. The substance containing haemin is settled. The supernatant is subjected to thermal treatment at 85 °C for 10 minutes and liophylized. The characteristic data of the liophylized product are as follows:
protein content: 80 %
residual haemin content: 0.65 mg/g
CIE lightness factor: Y = 30.4
ash content: 8 %
free amino acid content: 10 %

Example 2

25 ml of thick blood prepared according to Example 1 are diluted with 175 ml of water and adjusted with 5 N sodium hydroxide solution to pH 10.5. The solution comprising blood cells haemolysed according to Example 1 is heated to 55 °C and to each 100 ml of the solution 0.024 AU enzyme (Alcalase 0.6 L) are added at an enzyme-

-substrate ratio of 1:100. Enzymatical decomposition is carried out for 480 minutes whereupon the solution is adjusted with concentrated hydrochlorid acid to the pH value of 4 and centrifuged at 3000 r.p.m. for 30 minutes. The globin solution separated from the haemin is allowed to stand at 85 °C for 10 minutes. After inactivation of the enzyme and liophylization 2.5 g of a powder are obtained, the characteristic data there being as follows:

protein content: 88.5 %
ash content: 7 %
residual haemin content: 0.63 mg/g
lightness coefficient Y = 30.4
free amino acid 15 %.

Example 3

25 ml of thick blood prepared according to Example 1 are diluted with 75 ml of water whereupon the pH is adjusted to 10.5 by adding 5 N sodium hydroxide solution. The solution is stirred for 60 minutes with a magnetic stirrer, heated to 85 °C, whereupon a protease enzyme having an activation of 0.40 AU is added and stirred for 60 minutes. After enzymatic decomposition the pH is adjusted to 5 by adding concentrated hydrochloric acid and the mixture is centrifuged for 30 minutes at 3000 r.p.m. From the sedimentated haemine the supernatant is decanted off and the solution is kept at 85 °C for 10 minutes in order to desactivate the enzyme. After liophylization 5.52 g of a product are obtained, having the following characteristic features:

protein content: 84 %
ash content 8 %
residual haemin: 0.32 mg/g
lightness coefficient: Y = 31
free amino acid: 20 %.

Example 4

To 25 ml of thick blood obtained according to Example 1 75 ml of water are added and it is subjected to haemolysis. After haemolysis the solution is heated to 55 °C and an Alcalase 0.6 L enzyme having an activity of 0.28 AU is added at an enzyme-substrate ratio of 1:20. The mixture is stirred for 60 minutes. After enzymatic treatment the pH is adjusted to 5.5 by adding concentrated hydrochloric acid and the solution is centrifuged at 3000 r.p.m. for 30 minutes. The protein solution separated from the haemin is desactivated at 85 °C for 10 minutes and liophylized. Thus 5 g of a product are obtained having the following characteristic data:

protein content: 82 %
ash content: 8.5 %
residual haemin: 1.15 mg/g
lightness coefficient: Y = 27.3
free amino acid: 18 %

Example 5

25 ml of thick blood obtained according to Example 1 are diluted with 75 ml of water and the solution is denaturated for 120 minutes. To 100 ml of the haemoglobin solution thus obtained alfa-Kimoptrisin (Merck) having an activity of 0.4 AU are added at an enzyme--substrate ratio of 1:40 and the system is stirred for 240 minutes. After enzymatic treatment the pH is adjusted to 4.5 by adding concentrated hydrochloric acid and the mixture is centrifuged at 3000 r.p.m. for 30 minutes. The protein solution separated from the haemin is kept at 85 °C for 10 minutes. The powdery product obtained after liophylization weighs 4.8 g and

has the following characteristic data:

protein content: 80 %
residual haemin: 1.2 mg/g
CIE lightness coefficient: Y = 27.0
ash content: 10 %
free amino acid content: 18 %

Example 6

Thick blood obtained according to Example 1 is diluted with water in a ratio of 1 3 and denaturated in alkaline medium for 120 minutes. Denaturalization is carried out under introduction of air. The denaturated haemoglobin is heated to 30 °C, adjusted to the pH value of 5.0 and 0 Corolase PS (Rohm) enzyme having an activity of 0.40 AU is added at an enzyme-substrate ratio of 1:40. The mixture is subjected to thermal treated for 480 minutes and acidified to the pH value of 4.0 with concentrated hydrochloric acid. The mixture is centrifuged in acidic medium at 3000 r.p.m. for 30 minutes. The globin solution is separated from the settled haemin and kept at 65 °C for 10 minutes in order to desactivate the enzyme. The inactivated solution is subjected to liophylization. 5.4 g of a light beige powder are obtained having the following characteristic data:

protein content: 82 %
ash content: 6 %
residual haemin: 0.78 mg/g
lightness coefficient: Y = 29.5
free amino acid content: 15 %

Example 7

25 ml of thick blood obtained according to Example 1 are diluted with water at a ratio of 1:1 whereupon the pH is adjusted to 10.5 by adding a 5 N sodium hydroxide solution. The solution is stirred for 120 minutes with a magnetic stirrer, thereafter heated to 55 °C and a Thermitase enzyme having an activity of 0.4 AU are added at an enzyme-substrate ratio of 1:40 and at a pH value of 6.8. Enzymatic treatment is carried out for 120 minutes, the pH is adjusted to the value of 4.0 by adding concentrated hydrochloric acid and the system is centrifuged at 3000 r.p.m. for 30 minutes. The supernatant is decanted off the sedimentated haemin and the enzyme is desactivated at 85 °C for 15 minutes. The inactivated solution is subjected to liophylization. Thus 5 g of a product are obtained having the following characteristic data:

protein content: 80 %
ash content: 10 %
residual haemin: 0.88 mg/g
lightness coefficient: Y = 28.8
free amino acid: 16 %.

What we claim is,

1.) Process for the preparation of protein composition for food industry starting from food grade blood by haemolysis and enzymatic decomposition which comprises denaturating blood haemolysed in a known manner in alkaline medium at a pH value between 10 and 11, oxidizing denaturated haemoglobin in the presence of molecular oxygen, decomposing the product obtained after oxidation with a proteolytic enzyme capable of functionating in alkaline or neutral medium at an enzyme-substrate ratio of 1:100 - 1:20, adjusting the pH of the medium after enzymatic decomposition to a pH value between 4.0 and 5.2 and separating the haemin from the solution containing the globin , stopping enzymatic activity of the globin-protein solution by thermal treatment and if desired dehydrating the protein solution under mild conditions.

2.) Process according to Claim 1 which comprises carrying out denaturalization and oxidation in one step, under stirring, within 10-120 minutes, optionally under additional air-blasting.

3.) Process according to Claim 1 or 2 which comprises carrying out enzymatic decomposition of the haemolysed material by using a proteolytic enzyme capable of functionating in alkaline or neutral medium.

4.) Process according to Claim 3 which comprises using enzymes which prefer to cleave the histidine, leucine, glycine, phenylalanine or alanine bonds.

5.) Process according to any of Claims 1-4 which comprises adjusting the proteolytic activity of the medium after enzymatic decomposition to 0 Anson unit by thermal treatment.

6.) Process according to Claim 1 which comprises subjecting the protein solution to freezing, liophylization or spray-drying.

7.) Protein composition for food industry prepared according to any of Claims 1-6 characterized by the following data (related to dry substance):

- minimal protein content: 80 %;
- maximal haemin content: 1.2 mg haemin/g;
- maximal free amino acid content: 20 %;
- from the total amino acid content:
  lysine content: at least 7 %;
  methionine content: at least 0.8 %;
  glutaminic acid content: at least 4.5 %.
- maximal ash content: 10 %;

colour (according to lightness coefficient of the CIE system): Y = at least 27.13;

- according to functional properties the product should be completely soluble in water;
- on heating (at a temperature of about 100 °C) the product coagulates at least 10-15 %;
- foaming capacity: 4-500 %;
- emulsifying activity: 50-55 %;
- protein biological value:
  MORUP-OLESEN index: at least 68.7
  NPU value = at least 17.1 %.

INTERNATIONAL SEARCH REPORT

International Application No PCT/HU 84/00012

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.[3]: A 23 J 1/06, A 23 J 3/00, A 23 K 1/04

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
Int.Cl.[3]:	A 23 J 1/06, A 23 J 3/00, A 23 K 1/04, A 61 K 35/14, A 61 K 37/02, C 12 P 21/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	GB, A, 2 018 121 (SLAGTERIERNES), 17 October 1979 (17.10.79), see abstract; example 1	(1,3-6)
X	DE, A1, 2 637 362 (SOCIETE IDUSTRIELLE DE RECUPERATION DE DECHETS ANIMAUX), 24 February 1977 (24.02.77), see claims 4,5,8,12,14-16	(1,3,5,6)
A	DE, A1, 2 842 918 (RÖHM), 17 April 1980 (17.04.80), see claims 1,3; example 1	(1,3,5)
A	DE, A1, 2 831 338 (NORDFLEISCH AG), 31 January 1980 (31.01.80)	

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* Special categories of cited documents: [15]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
18 May 1984 (18.05.84)	22 May 1984 (22.05.84)
International Searching Authority [1]	**Signature of Authorized Officer [20]**
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Anhang zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

Annex to the International Search Report on International Patent Application No. PCT/HU 84/00012

Annexe au rapport de recherche internationale relatif à la demande de brevet international n°.

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Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
GB-A -2 018 121	17/10/79	DK-A - 465/79	01/03/79
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		FR-B1-2 412 265	17/06/83
		BR-A - 78-08335	07/08/79
		AR-A1- 216 829	31/01/80
		US-A -4 220 723	02/09/80
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