FORM 1

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

620443

APPLICATION FOR A STANDARD PATENT

I\We,

UNILEVER PLC

of UNILEVER HOUSE
BLACKFRIARS
LONDON EC4
ENGLAND

hereby apply for the grant of a standard patent for an invention entitled:

PROCESS FOR PREPARING IMPROVED HYDROLYSED PROTEIN.

which is described in the accompanying complete specification

Details of basic application(s):

Number of basic application	Name of Convention country in which basic application was filed	Date of basic application
88202087.8	EP	26 SEP 88
8824242.5	GB	17 OCT 88

My/our address for service is care of GRIFFITH HACK & CO., Patent Attorneys, 601 St. Kilda Road, Melbourne 3004, Victoria, Australia.

DATED this 25th day of September 1989

UNILEVER PLC

M 012621 250936

GRIFFITH HACK & CO.

TO: The Commissioner of Patents.

CLEMENT HACK & CO

Australia Patent Declaration Form
R 7050 (R)

Forms 7 and 8
AUSTRALIA
Patents Act 1952

DECLARATION IN SUPPORT OF A CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION

No. 41768/89

Names of Applicants	In support of the application made by UNILEVER PLC
Title	PROCESS FOR PREPARING IMPROVED HYDROLYSED PROTEIN
Names and address of person making declaration	I, Dilshad RAJAN Authorized Signatory of Unilever House, Blackfriars, London E.C.4, Great Britain, do solemnly and sincerely declare as follows:-

1. I am authorized by the abovementioned applicant to make this declaration on its behalf.

2. The basic applications as defined by Section 141 of the Act were made in the countries on the following dates by the following applicants namely:

Country, filing date and name of Applicants for the or each basic application	in Europe on 26th September 1988 by UNILEVER N.V. and UNILEVER PLC in Great Britain on 17th October 1988 by UNILEVER PLC

3. The said basic applications were the first applications made in a Convention country in respect of the invention.

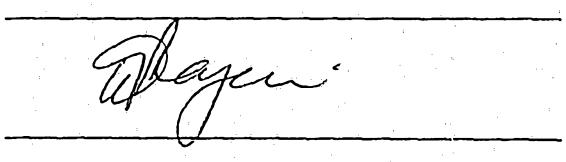
4. The actual inventors of the said invention are

Names and addresses of the or each actual inventor	Johannes Franciscus Maria de ROOIJ, a Dutch subject of G. van Amstelstraat 6, 1213 CK Hilversum, The Netherlands Brian Alan WARD, a British subject of 36 Neville Road, Snodland, Kent ME6 5HX, Great Britain and Maurice WARD, a British subject of 61 Mountbatten Way, Brabourne Lees, Ashford, Kent TN25 6PU, Great Britain

5. The facts upon which the applicants are entitled to make this application are as follows:-

see reverse side of this form for guidance in completing this part	The applicants would be entitled to have assigned to them a patent granted to any of the actual inventors in respect of the said invention and The said applicant UNILEVER PLC is the assignee of the said basic applicant UNILEVER N.V. and the said UNILEVER N.V. was the assignee of the said actual inventor Johannes Franciscus Maria de ROOIJ

DECLARED at London this 20th day of September ,1989



(12) PATENT ABRIDGMENT (11) Document No. AU-B-41768/89
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 620443

(54) Title
PROCESS FOR PREPARING IMPROVED HYDROLYSED PROTEIN

International Patent Classification(s)
(51)[4] **A23J 003/00**

(21) Application No. : **41768/89** (22) Application Date : **25.09.89**

(30) Priority Data

(31) Number	(32) Date	(33) Country
88202087	26.09.88	EP EUROPE
8824242	17.10.88	GB UNITED KINGDOM

(43) Publication Date : **29.03.90**

(44) Publication Date of Accepted Application : **20.02.92**

(71) Applicant(s)
UNILEVER PLC

(72) Inventor(s)
JOHANNES FRANCISCUS MARIA DE ROOIJ; BRIAN ALAN WARD; MAURICE WARD

(74) Attorney or Agent
GRIFFITH HACK & CO , GPO Box 1285K, MELBOURNE VIC 3001

(57) Claim

1. A process for improving HCl-hydrolysed protein characterized by subjecting an aqueous solution thereof to gel permeation chromatography using a porous material having an equivalent average pore diameter between 0.5 and 2.5 nanometers characterized by eluting a fraction which is at least free from detectable amounts of monochloropropanediols whilst at least 40% of the sodium chloride is retained.

8. A method according to any of the preceding claims so that after amino acids and salt have been eluted, elution is continued with at least twice the first amount of eluant before another amount of protein hydrolysate is introduced.

AUSTRALIA

620443

PATENTS ACT 1952

Form 10

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE

Short Title:

Int. Cl:

Application Number:
Lodged:

Complete Specification-Lodged:
Accepted:
Lapsed:
Published:

Priority:

Related Art:

TO BE COMPLETED BY APPLICANT

Name of Applicant:
UNILEVER PLC

Address of Applicant: UNILEVER HOUSE
BLACKFRIARS
LONDON EC4
ENGLAND

Actual Inventor:

Address for Service: GRIFFITH HACK & CO.,
601 St. Kilda Road,
Melbourne, Victoria 3004,
Australia.

Complete Specification for the invention entitled:
PROCESS FOR PREPARING IMPROVED
HYDROLYSED PROTEIN.

The following statement is a full description of this invention including the best method of performing it known to me:-

Process for preparing improved hydrolysed protein

The invention relates to a chemical process for improving hydrolysed protein, in particular to HCl-hydrolysed protein.
The hydrolysis of proteins by treatment with hydrochloric acid was developed by Liebig in the middle of the last century. Since then the method has been extensively employed for the commercial production of food supplements and flavours.

In commercial operations it is customary to hydrolyse the mainly vegetable proteins by boiling with strong hydrochloric acid followed by cooling and neutralization of the hydrolysate with sodium carbonate or sodium hydroxide and removal of the solid non-hydrolysed material. The hydrolysis temperature is normally in the range from 100 to 120°C and the reaction time from 2 to 24 hours.

The degree of hydrolysis normally is between 60 and 85% of the amide groups. In the case of pure protein starting materials higher degrees of hydrolysis can be obtained.

Suitable protein hydrolysate starting material materials can be for example casein, soya bean protein, gluten and oil seed cake materials. The protein hydrolysis is carried out in the conventional way whilst stirring the mixture in a reactor which is inert to HCl at high temperatures.

Studies have shown that protein hydrolysates prepared with hydrochloric acid contain a quantity of dichloropropanols (DCP's), especially 1,3-dichloropropane-2-ol and monochloropropanediols (MCP's) and the problem of their elimination has arisen.

According to GB-A-2 183 659 (Société Des Produits Nestlé

SA) hydrochloric acid (HCl) hydrolysed protein is first freed from the insolubles and then subjected to steam distillation under reduced pressure while keeping the density of the hydrolysate at a substantially constant level in order to eliminate any 1,3-dichloropropane-2-ol present.

Also it is known from EP-A- 209 921 (Unilever) to desalinate protein hydrolysate dissolved in a polar solvent by fractionating the solution by means of gel filtration over a porous material having a pore diameter between 0.5 and 2.5 nanometers.

Whilst the elimination of 1,3-dichloropropane-2-ol from HCl-hydrolysed protein according to GB-A-2 183 659 is desirable in these food supplements and flavours there has been a need for methods which remove the full range of MCP's and DCP's more efficiently from these products.

The present invention provides a method for improving HCl-hydrolysed protein by subjecting an aqueous solution thereof to gel permeation chromatography (using a porous material having an equivalent pore diameter between 0.5 and 2.5 nanometers in which a fraction is eluted which is substantially free from monochloropropanols, and optionally free from dichloropropanols whilst at a substantial quantity of say at least 40, preferably at least 50% (w.w.) of the sodium chloride is retained.

It should be noted that the desalination process known from EP-A- 209 921 in view of the fact that the molecular weight and size of monochloropropanediols and dichloropropanols resembles that of some amino acids, would not seem suitable for the separation of amino acids from chloropropanols.
According to this known process the amino acids are first eluted and the salt remains on the porous material. It was subsequently found that the MCP's and DCP's also remained on the porous material and would be

eluted when a subsequent batch of protein hydrolysate was being processed on the same column.

In a preferred embodiment of the invention the gel permeation technique is combined with a steam distillation step. It is this combination of steps which leads to a more efficient purification of the protein hydrolysate, because gel permeation can be conducted at a higher throughput when this is followed by distillation. Where gel permeation and (steam) distillation are combined it is preferred that the gel permeation step precedes the distillation step. It is preferred to allow the amount of water in the hydrolysate to drop during the distillation step.

For good results it is recommended that the porous material has an equivalent pore diameter between 0.5 and 2.5 , preferably between 1.0 and 2.0 nanometers and such materials as cross-linked dextrans are conveniently and readily available. Very suitable materials are e.g. Sephadex G 10 and G 15 (Sephadex is a tradename of Pharmacia AB, Uppsala, Sweden.)
It is normally recommended to carry out the gel permeation step in such a way that after the sodium chloride has been eluted, elution is continued with at least twice the amount of eluant required for removing amino acids and salt before introducing another amount of protein hydrolysate.
In a preferred embodiment of the invention gel permeation chromatography (GPC) and distillation are combined in such a way that the gel permeation technique is carried out in a cyclic pattern of operation. Injections of HCl-hydrolysed protein are made so that a fraction is eluted which contains dichloropropanols together with amino acids and salt (which fraction is substantially free from monochloropropanediols), from which fraction the dichloropropanols are subsequently removed by (steam) distillation. This distillation step can be conducted so that the amount of water in the

hydrolysate is decreased or so that the amount of water remains substantially constant (steam stripping).
When the process according to the present invention is carried on a larger scale it is recommended to use a plurality of columns packed with porous material which are alternately rinsed and used for separation.
By applying the process according to the present invention one generally obtains a product which contains still at least 50% of the original content of sodium chloride, but which is free from detectable amounts of monochloropropanediols and dichloropropanols.

The protein hydrolysate prepared according to the present invention can be used with advantage as a savoury flavour, in foodstuffs, such as soups, beefburgers,sausages, sauces, goulash etc.

The improved protein hydrolysates according to the present invention are also excellent starting materials in the preparation of reaction flavours in which the hydrolysate is reacted with mono- and di-saccharides, cysteine/cystine, thiamine etc. in which reaction flavour a major part of the starting amino acids remain unchanged.

The detection method for the various chloropropanols used according to the present specification is a modification of the method described on page 5 of GB-A-2 183 659 (Soc. Prod. Nestlé), which method has been extended by improved extraction techniques as to permit also MCP determination.

The invention is illustrated by the following examples:

General

For all examples use was made of a thermostatic gel-permeation (GPC) column having a length of 100 cm and an internal diameter of 113 mm which was filled to a height of 70 cm with pre-swollen Sephadex G-10 resin (a cross-linked dextran ex Pharmacia AB, Uppsala, Sweden) with an equivalent pore diameter between 0.5 and 2.5 nanometers. The column temperature was maintained at ambient.

Example I

Protein hydrolysate with an NaCl and total solids content of 19.9% and 37.5% respectively and a content of 21.6 ppm for the MCP's and 8 ppm for the DCP's was diluted 50% v/v with deionised water to give a homogeneous mixture. This mixture was then pumped to the GPC column and eluted from the column with deionised water. The eluate from the column was collected as fractions and analyzed for salt, solids, MCP and DCP content. The contents of MCP's and DCP's were below their levels of detection of 1 and 0.05 ppm respectively. Initial and subsequent fractions, which when bulked together contained 80% of original salt, were combined and concentrated using a rotary evaporator to 40% total solids. The concentrate and distillate were then again analyzed for MCP and DCP content and also found to be below the levels of detection.

Example II

Using an equivalent homogeneous mixture to that described in Example 1 a small quantity of 1,3-dichloropropane-2-ol was added to increase the concentration by a factor of 10. This mixture was then pumped to the GPC column and eluted from the column with deionised water and collected as fractions. The elution of material from the column was extended to ensure that all of the 1,3-dichloropropane-2-ol had been flushed from the column. All fractions were then analyzed for salt, solids and MCP and DCP content. The

presence of 1,3-dichloropropane-2-ol in fractions was detected long after the elution of salt had ceased. Initial and subsequent fractions which, when bulked together contained 80% of the original salt, were combined with fractions containing 1,3-dichloropropane-2-ol and concentrated to 40% solids using a rotary evaporator. The concentrate and distillate were then again analyzed for the presence of DCP's and found to be below the level of detection of 0.05 ppm in the concentrate but detectable in the distillate.

Example III

Using an equivalent homogeneous mixture to that described in Example I, a small quantity of 3-chloropropane-1,2 diol was added to increase the concentration of this substance by a factor of 10. This mixture was then pumped to a GPC column and eluted from the column with deionised water and collected as fractions. The elution of material from the column was extended to ensure that all of the 3-chloropropane-1,2-diol had been flushed from the column. All fractions were then analyzed for salt, solids , DCP's and MCP's. The presence of 3-chloropropane-1,2-diol was detected in the latter fractions containing salt. Initial and subsequent fraction which, when bulked together contained 80% of the original salt, were combined with fractions containing 3-chloropropane-1,2-diol and concentrated to 40% solids using a rotary evaporator. The concentrate and distillate, were then analyzed for the presence of MCP's which was found to be below the level of detection (0.05 ppm) in the distillate but detectable in the concentrate.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A process for improving HCl-hydrolysed protein characterized by subjecting an aqueous solution thereof to gel permeation chromatography using a porous material having an equivalent average pore diameter between 0.5 and 2.5 nanometers characterized by eluting a fraction which is at least free from detectable amounts of monochloropropanediols whilst at least 40% of the sodium chloride is retained.

2. A process according to claim 1 characterized in that the gel permeation technique is combined with a distillation step.

3. A process according to claim 2 characterized in that the gel permeation step is followed by a steam distillation step.

4. A process according to claim 2 characterized in that the gel permeation step is preceded by a distillation step.

5. A process according to claim 2,3 or 4 characterized in that during the distillation step the water content of the hydrolysate is reduced.

6. A process according to any of the preceding claims characterized in that the gel permeation technique is carried out in a cyclic pattern with injections of HCl hydrolysed protein and that a fraction is eluted which is free from monochloropropandiols and optionally dichloropropanols but contains amino acids and salt.

7. A process according to claim 2 or 3 characterized in that any dichloropropanols are substantially removed from the eluate by steam stripping.

8. A method according to any of the preceding claims so that after amino acids and salt have been eluted, elution is continued with at least twice the first amount of eluant before another amount of protein hydrolysate is introduced.

9. A process according to any of the preceding claims characterized in that a plurality of columns packed with porous material are involved which are alternately rinsed and used for separation.

10. A process according to any of the preceding claims in which at least 50% (w.w.) of the sodium chloride is retained.

DATED this 25th day of SEPTEMBER 1989.

UNILEVER PLC
By its Patent Attorneys:

GRIFFITH HACK & CO.

Fellows Institute of Patent
Attorneys of Australia.