FORM 1

618554 SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

Express Foods Group Limited, of Victoria Road, South Ruislip, Middlesex, HA4 OHF, UNITED KINGDOM, hereby apply for the grant of a standard patent for an invention entitled:

Process for Obtaining Concentrates Having a High alpha-lactalbumin Content from Whey

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
87 23651	GB	8 October 1987

The address for service is:-

Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia

DATED this TWENTY SEVENTH day of SEPTEMBER 1988

Express Foods Group Limited

By: M J Anderson

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 73482
S&F CODE: 62930

5845/2

618554

SPRUSON & FERGUSON

Commonwealth of Australia

Patents Act 1990

PATENT REQUEST: STANDARD PATENT

We, the Applicant/Nominated Person specified below, request we be granted a patent for the invention disclosed in the accompanying standard complete specification.

[70,71] Applicant/Nominated Person:

Express Foods Group (International) Limited, of Victoria Road, South Ruislip, Middlesex, HA4 OHF, United Kingdom

[54] Invention Title:

Process for Obtaining Concentrates Having a High alpha-lactalbumin Content from Whey

[72] Inventor:

.obin Charles Bottomley

[74] Address for Service in Australia:

Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia **[Code SF]**

Basic Convention Application Details

[31] Application No	[33] Country	[32] Date of Application
87 23651	GB	8 October 1987

DATED this EIGHTEENTH day of OCTOBER 1991
Express Foods Group (International) Limited

John McCann

Registered Patent Attorney

IRN: 73482 INSTR CODE: 62930

AUSTRALIAN PATENT OFFICE

LMM/3533T

(12) PATENT ABRIDGMENT (11) Document No. AU-B-22909/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 618554

(54) Title
PROCESS FOR OBTAINING CONCENTRATES HAVING A HIGH ALPHA-LACTALBUMIN CONTENT FROM WHEY

International Patent Classification(s)
(51)[4] **A23C 009/142** **A23J 001/20** **C07K 003/26**

(21) Application No. : **22909/88** (22) Application Date : **28.09.88**

(30) Priority Data

(31) Number	(32) Date	(33) Country
8723651	**08.10.87**	**GB UNITED KINGDOM**

(43) Publication Date : **13.04.89**

(44) Publication Date of Accepted Application : **02.01.92**

(71) Applicant(s)
EXPRESS FOODS GROUP (INTERNATIONAL) LIMITED

(72) Inventor(s)
ROBIN CHARLES BOTTOMLEY

(74) Attorney or Agent
SPRUSON & FERGUSON , GPO Box 3898, SYDNEY NSW 2001

(56) Prior Art Documents
US 4711753
AU 552141 77719/81 A23J 1/20
AU 538288 59666/80 A23J 1/20

(57) Claim

1. A process for producing an α-lactalbumin-enhanced fraction from a liquid containing whey protein including α-lactalbumin and β-lactoglobulin, wherein said liquid is sweet whey, acid whey, a liquid whey protein concentrate or a reconstituted liquid or solid whey protein concentrate wherein said concentrates are produced from sweet whey or acid whey, which process comprises subjecting said liquid to ultrafiltration using a membrane having a molecular weight cut off of substantially 100,000 to form a permeate having a protein content in which the proportion of α-lactalbumin is enhanced relative to the proportion obtaining in the protein content of said liquid and further ultrafiltering said permeate using a membrane having a molecular weight cut off up to 10,000 to produce a concentrate having an enhanced α-lactalbumin content.

2. The process as claimed in claim 1 characterised in that said concentrate having an enhanced α-lactalbumin content is dried to form a powder.

S & F Ref: 73482

FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION 618554

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

Name and Address of Applicant: Express Foods Group Limited
Victoria Road
South Ruislip Middlesex HA4 OHF
UNITED KINGDOM

Address for Service: Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower, 31 Market Street
Sydney, New South Wales, 2000, Australia

Complete Specification for the invention entitled:

Process for Obtaining Concentrates Having a High alpha-lactalbumin Content from Whey

The following statement is a full description of this invention, including the best method of performing it known to me/us

5845/3

ABSTRACT

"PROCESS FOR OBTAINING CONCENTRATES HAVING A HIGH α-LACTALBUMIN CONTENT FROM WHEY"

The invention is concerned with a process for producing an α-lactalbumin- enhanced fraction from a liquid containing whey protein including α-lactalbumin and β-lactoglobulin characterised by the step of subjecting the said liquid to ultrafiltration using a membrane having a molecular weight cut off of substantially 100,000.

DESCRIPTION

PROCESS FOR OBTAINING CONCENTRATES HAVING A HIGH α-LACTALBUMIN CONTENT FROM WHEY.

The present invention relates to the production of whey protein concentrates having an enhanced α-lactalbumin content.

The isolation of individual proteins from various starting materials is well known. Most individual proteins are recovered from a mixture thereof by using differences in the physical and/or chemical properties of the proteins to advantage. Such isolation processes have usually required a multi-stage process with its attendant expense and difficulty in scaling up to an industrial process.

Whey contains many proteins with excellent properties which are also of a high nutritional value. Their large scale production in the form of whey protein concentrates only became possible with the advent in the early 1970s of ultrafiltration technology and they have become increasingly important in satisfying the needs of the food, dietetic and pharmaceutical industries. Whey protein concentrates in powder form containing all the whey proteins are currently produced by membrane ultrafiltration followed by spray drying and generally have a protein content ranging from 35% to 85%, although lesser or greater amounts of protein can be present.

Such concentrates contain residual non-centrifugeable lipids, principally phospholipids, together with milk lipids and some phospholipo-proteins. These lipoproteins concentrate at the same rate as the other proteins and have amphoteric and amphiphilic properties which lead to their being strongly adsorbed on membrane materials used in ultrafiltration, which was reported to cause irreversible fouling. For this reason LEE AND MERSON (J.Food Sc.41, 402-410) suggested prefiltration of whey to reduce this fouling.

Numerous methods have already been proposed to separate individual whey proteins, but most have not been capable of scaling up to industrially viable processes because of their complexity, their cost in energy, their very low yield and the irremediable degradation of the products due to the use of intensive heat treatments (NIELSEN et al., J.Dairy Science 56 76-83 1973) very alkaline pH (HARRIS and YOELL, 1985) or of high amounts of salts (KUMATA et al., J.Food Science 50 605-609 1985).

PEARCE (Aust.J.Dairy Technol. 38 144-149 1983) has proposed purification of α-lactalbumin based on the low pH solubility of α-lactalbumin under a light heat treatment. However, the α-lactalbumin fraction produced by this process has been reported by J.L. MAUBOIS et al. of the Dairy Research Laboratory

I.N.R.A. at the WPI/IDF International Whey Conference in Chicago, 27th-29th October 1986 to be unsatisfactory due to the high degree of association of the whey lipids and lipoproteins with α-lactalbumin.

J.L. MAUBOIS et al proposed a clarification process which produces a clarified whey which, when subjected to ultrafiltration produces a whey protein concentrate which they suggest is the right product for separation of individual proteins therefrom.

The clarification process has as its object the removal of the lipoproteins using their ability to aggregate through calcium bonding under heat treatment. The clarification process involves six stages which are:

1. cooling the whey to a temperature of 2°C;
2. adjusting the calcium content to 1.2g/kg by addition of $CaCl_2$;
3. raising th pH of the so-treated whey to 7.3 by addition of NaOH;
4. rapidly raising the temperature of the so-treated whey to 55°C;
5. maintaining the whey at this temperature for a period of eight minutes;
6. separating the supernatant from the precipitate by tangential microfiltration (MAUBOIS et al indicated that tangential microfiltration was resorted to because filtration through screen plates

led to loss of lipoproteins to the filtrate, and centrifugation was found to be hopelessly inefficient).

The whey, clarified in the above rather complex manner was then subjected by Maubois et al to ultrafiltration to produce a whey protein concentrate. To obtain -lactalbumin from this concentrate they chose the method described by Pierre & Fauquant in Lait (1986). The details are fully set out in the Maubois et al publication. The method involves the gelling of α-lactalbumin by heating to 55^{o}-65^{o} for 30 minutes at a pH of $\sim$4 for concentrates having an α-lactalbumin content of more than 2g/kg. Maubois et al observed that the supernatant resulting from the gelling contained β-lactoglobulin and that this could be realised in high purity by diafiltration on a UF membrane having a molecular weight cut off of 50,000. For separation of β-lactoglobulin from the gelled α-lactalbumin however, they suggested only that the tangential membrane microfiltration technique used by them for clarification of the heat treated whey might be attempted.

Thus, whilst ultrafiltration has been suggested as one step in a process which involves a number of other physicochemical methods, for the separation of fractions containing enhanced concentrations of

individual whey proteins, the method of purification as a whole has also involved a variety of other procedures which, when scaled up to produce an industrial process, become both complex and expensive to run.

Thus the provision of a simple and commercially viable process for fractionating whey proteins and in particular α-lactalbumin, has eluded the researchers.

The ultrafiltration method uses membranes which will allow only molecules up to a given size to pass through into the permeate. Although theoretically the membranes could be constructed to provide any given molecular weight cut-off in practice they are manufactured to give molecular weight cut-off at 3,000, 10,000, 30,000, 50,000, 100,000 and 500,000. The cut-off is also not precise. For example, although the molecular weight of α-lactalbumin is substantially 14,000 some α-lactalbumin will pass into the permeate when ultrafiltration using a membrane having a molecular weight cut-off of 10,000 (e.g. a Romicon PM10 membrane) is practiced on whey. The molecular weight cut-off designated for a given membrane is therefore an average figure.

β-lactoglobulin has a molecular weight of about 18,000, but as it normally exists as a dimer it behaves as if it had a molecular weight more in the region of 36,000 or even somewhat larger. It would be

expected therefore that if a liquid containing both α-lactalbumin and β-lactoglobulin were subjected to ultrafiltration using a 30,000 molecular weight cut off membrane that separation of the two proteins would result and the ratio of α-lactalbumin to β-lactoglobulin in the permeate would be considerably higher than in the original liquid. It would also be expected that even using a 50,000 molecualr weight cut off membrane that some increase in the ratio would occur due to the apparent molecular weight of the β-lactoglobulin, but that with a 100,000 molecular weight cut off membrane (e.g. a Romicon PM100) substantially no increase would be expected.

It has now been surprisingly found that when such an ultrafiltration is performed using a 100,000 molecular weight cut off membrane on a whey protein concentrate obtained using a 10,000 molecular weight cut off membrane, or whey itself, that the ratio of α-lactalbumin to β-lactoglobulin in the permeate is substantially greater in the permeate than in the whey protein concentrate starting material. It has also been surprisingly found that the ratio of α-lactalbumin/β-lactoglobulin in the permeate using a 100,000 molecular weight cut off membrane is not significantly lower than that in the permeate resulting from the use of a 50,000 molecular weight cut off membrane but that the rate of permeation

of α-lactalbumin through the 100,000 molecular weight cut off membrane is significantly higher than its rate of permeation through a 50,000 molecular weight cut off membrane. The rate of permeation of the α-lactalbumin through the membrane is important in achieving commercial viability and the higher rate of permeation through the 100,000 molecular weight cut off membrane over that through a 30,000 or 50,000 molecular weight cut off membrane coupled with the surprisingly high ratio of α-lactalbumin/ β-lactoglobulin in the permeate resulting from use of a 100,000 molecular weight cut off membrane unexpectedly provides a viable industrial process for producing a commercially viable α-lactalbumin enhanced whey protein concentrate.

According to the present invention therefore, there is provided a process for producing an α-lactalbumin-enhanced fraction from a liquid containing whey protein including α-lactalbumin and β-lactoglobulin, wherein said liquid is sweet whey, acid whey, a liquid whey protein concentrate or a reconstituted liquid or solid whey protein concentrate wherein said concentrates are produced from sweet whey or acid whey, which process comprises subjecting said liquid to ultrafiltration using a membrane having a molecular weight cut off of substantially 100,000 to form a permeate having a protein content in which the proportion of α-lactalbumin is enhanced relative to the proportion obtaining in the protein content of said liquid and further ultrafiltering said permeate using a membrane having a molecular weight cut off up to 10,000 to produce a concentrate having an enhanced α-lactalbumin content.

The liquid containing the whey proteins may be whey itself but is preferably a whey protein concentrate or such a concentrate diluted with deionized water (usually to a protein content in the range of about 4-6% by weight) to give optimum results from the particular type of ultrafiltration equipment and UF

membrane used. The α-lactalbumin-containing permeate resulting from ultrafiltration of the said liquid through the 100,000 molecular weight cut off membrane is preferably concentrated by further ultrafiltration using an appropriate membrane such as a 10,000 molecular weight cut off membrane. The resulting liquid concentrate may then be dried to form a powder by the usual spray drying method.

The invention will be further illustrated by the following Examples, which are purely illustrative.

The wheys used in the Examples are from Cheddar cheese production, the whey pasteurisatiron conditions being 72 to 73°C/18 sec.

The analysis figures in percent by weight for the liquid whey are:-

	Liquid Whey
Total solids	6.1
Protein (Nx6.38)	0.8
Fat	0.04/0.25*
Ash	0.52
Sodium	0.053
Potassium	0.15
Calcium	0.062
Magnesium	0.008
Phosphorus	0.046
pH	6.3

* separated/unseparated

Example 1

3750 litres of white Cheddar whey at a pH of 6.18 were subjected to ultrafiltration in an Alfa-Laval UFS-4 plant fitted with Romicon PM10 membranes with a surface area of $10m^2$. The whey was maintained at a temperature of 50^oC and the ultrafiltration resulted in the production of 240 litres of whey protein concentrate with a content of 7.9% protein equivalent to 57% of total solids.

The concentrate so produced was diluted with demineralised water to a 5% protein content (350 litres total volume) and was subjected to ultrafiltration at 50^oC on an Alfa-Laval UFS 4 fitted with Romicon PM100 membranes. This resulted in the production of 78 litres of α-lactalbumin depleted concentrate and 302 litres of an α-lactalbumin enriched permeate containing 1.8% protein equivalent to 4.55% of total solids.

The permeate obtained above was run again through the Alfa-Laval UFS 4 plant fitted with Romicon PM10 membranes and 25 litres of αlactalbumin rich concentrate were obtained having a 14.1% protein content, equivalent to 16.7% of total solids.

The final liquid concentrate was then spray-dried to obtain 3.5 Kg of powder containing 78.3% protein.

The ratios of α-lactalbumin/βlactoglobulin

(based on HPLC (TSK G3000SW Column) profiles, absorbance at 280 nm) were:-

Starting Whey	1.1
Step 3 Liquid conc.	2.0

Example 2 Using the same conditions and equipment as in Example 1:

i. 1390 litres of whey were concentrated by ultrafiltration (PM10 membranes) to 91 litres having 18.7% by weight solids and 10.6% by weight total protein.

ii. 147 litres of deionised water were added to the concentrate from (i) to reduce the protein concentration to 4.06% by weight. The resulting diluted material was subjected to ultrafiltration using a PM 100 membrane to yield 180 litres of α-lactalbumin rich permeate.

iii. The permeate resulting from (ii) was concentrated by ultrafiltration using a PM10 membrane to 16 litres; the concentrate was spray dried to yield 2.6 kg of powder having the composition:-

Total protein	80.3% by weight
True protein	59.3% by weight
Moisture	6.4% by weight

The ratio of α-lactalbumin to β-lactoglobulin (based on the HPLC (TSK G3000SW Column) profiles absorbance at 280nm) was 7:4.

Example 3 350 litres of white Cheddar whey were subjected to ultrafiltration (Alfa Laval UFS-4, 4xPM10 at 50°C) and concentrated to 14 litres having a solids content of 12.5% by weight. 16 litres of deionised water were added to the concentrate and the resultant 30 litres of diluted concentrate subjected to ultrafiltration at 20°C on an Amicon DC10 unit equipped with an Amicon H5P100 membrane with a surface area of 0.45m^2 to generate 25.5 litres of α-lactalbumin rich permeate. The permeate was further ultrafiltered at 20°C using the same Amicon equipment but with a H5P10 membrane having a surface area of 0.45m^2 to produce 3 litres of concentrate. The concentrate was spray dried to yield a powder having the composition:-

Total Protein	81.6% by weight
True protein	73.6% by weight
Total solids	94.1% by weight

HPLC (TSK G3000SW Column) profiles both for the starting whey and the final product were prepared and are reproduced in the accompanying drawing, Fig. 1 being the profile for the starting whey and Fig. 2 the profile for the final product. The ratio of α-lactalbumin/β-lactoglobulin was 3.0.

.....

The claims defining the invention are as follows:

1. A process for producing an α-lactalbumin-enhanced fraction from a liquid containing whey protein including α-lactalbumin and β-lactoglobulin, wherein said liquid is sweet whey, acid whey, a liquid whey protein concentrate or a reconstituted liquid or solid whey protein concentrate wherein said concentrates are produced from sweet whey or acid whey, which process comprises subjecting said liquid to ultrafiltration using a membrane having a molecular weight cut off of substantially 100,000 to form a permeate having a protein content in which the proportion of α-lactalbumin is enhanced relative to the proportion obtaining in the protein content of said liquid and further ultrafiltering said permeate using a membrane having a molecular weight cut off up to 10,000 to produce a concentrate having an enhanced α-lactalbumin content.

2. The process as claimed in claim 1 characterised in that said concentrate having an enhanced α-lactalbumin content is dried to form a powder.

3. The process as claimed in claim 1 or claim 2, characterised in that said liquid is acid whey or sweet whey.

4. The process as claimed in claim 1 or claim 2, characterised in that said liquid is a whey protein concentrate diluted with deionised water.

5. The process as claimed in claim 4 characterised in that said whey protein concentrate is produced by subjecting whey to ultrafiltration using a membrane having a molecular weight cut off of substantially 10,000 or less.

6. A process for producing an α-lactalbumin-enhanced fraction from a liquid containing whey protein including α-lactalbumin and β-lactoglobulin, wherein said liquid is as defined in claim 1, which process is substantially as hereinbefore described with reference to any one of the Examples.

7. An α-lactalbumin-enhanced fraction when produced by the process of any one of claims 1 to 6.

DATED this EIGHTEENTH day of OCTOBER 1990
Express Foods Group Limited
Patent Attorneys for the Applicant
SPRUSON & FERGUSON

LMM/375Z

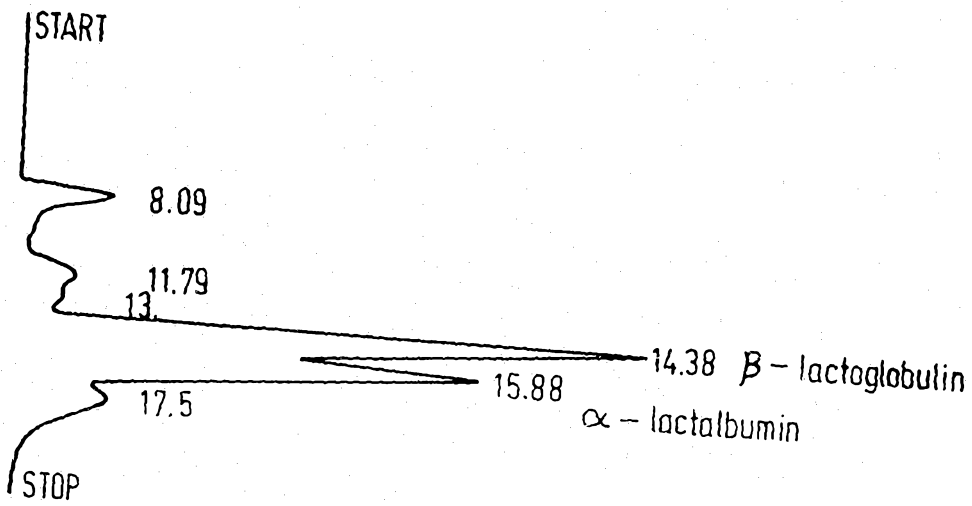
START
8.09
11.79
13.
14.38 β – lactoglobulin
15.88
17.5
α – lactalbumin
STOP

Fig.1

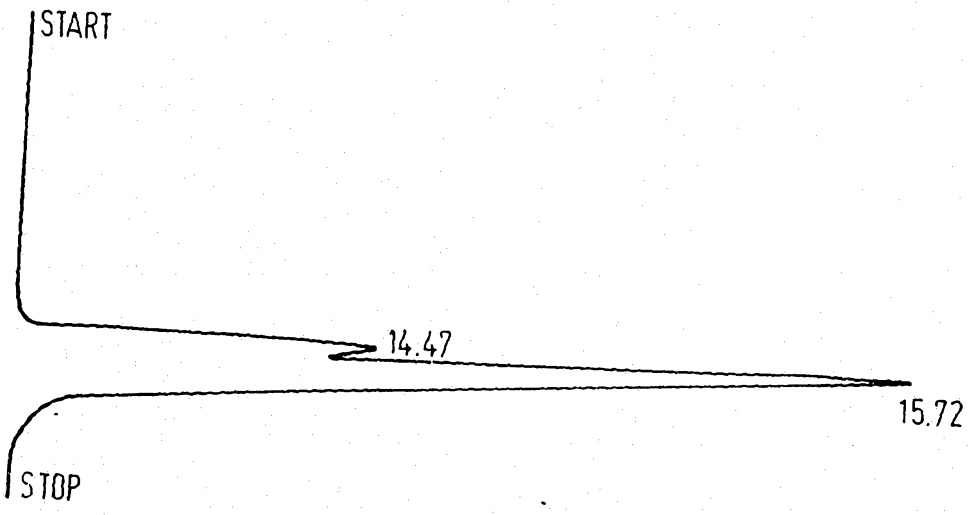
START
14.47
15.72
STOP

Fig.2