A PROCESS FOR PRODUCING AN UNDENATURED WHEY PROTEIN CONCENTRATE

Related Applications

This application is a continuation-in-part of U.S. Serial No. 08/315,904 filed September 30, 1994 and a continuation-in-part of U.S. Serial No. 08/175,637, filed December 30, 1993, which are continuations-in-part of U.S. Serial No. 07/989,186 filed December 11, 1992, which is a continuation-in-part of U.S. Serial No. 07/929,347 filed August 13, 1992. The contents of said related applications are hereby incorporated by reference in their entirety.

Background of the Invention

As early as 1982 Bounous et al. (1) showed that dietary whey protein concentrate (W.P.C.) improved the active systemic humoral immune response in a mammal, as measured by sheep red blood cell injections. It was however found that the use of high temperature pasteurization of milk in 1988 and subsequent years following a Salmonellosis epidemic in Europe greatly reduced the effectiveness of commercially available whey protein concentrates in improving the immune response.

In said related applications the discovery that undenatured whey protein concentrate had an enhanced immunological effect was presented. It was furthermore explained that in the conventional high temperature pasteurization of milk the thermosensitive proteins serum albumin and lactoferrin were partially heat denatured and hence precipitated in the curd. Said related applications describe experiments where W.P.C. was prepared using the lowest level of heat treatment of milk compatible with safety standards, so as to obtain a whey protein distribution having a relatively high content of the thermolabile serum albumin.

It was found that the presence in the serum albumin (B.S.A.) of 6 glutamylcyst(e)ine (Glu-Cys) group/molecule (substrate for glutathione (G.S.H) synthesis) and the specific intramolecular disulfide bond related to the undenatured conformation of the molecule, were a key factor in the G.S.H. promoting activity of W.P.C. The term cyst(e)ine implies the presence of a disulfide bond (cystine), and the possible reduction to cysteine. Enhancement of G.S.H. levels in tissues are believed to represent the common denominator underlying the beneficial effect.

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Dietary W.P.C. produced with low level pasteurization improves systemic humoral immune response, increases the resistance of target cells against the carcinogenic effect of chemical carcinogens such as dimethylhydrazine, improves resistance to pneumococcal infection, and provides a moderate but sustained increase of tissue glutathione.

The extent of denaturation produced during whey concentrate production is normally assessed by the loss of solubility at pH 4.6. Clearly the two most important factors of protein denaturation are temperature and pH whereas lower pH values usually enhance the denaturing effect of high temperature. This is why throughout our entire procedure, we have maintained a rather high pH (6) thanks to a reduction of pH lowering bacterial contamination through microfiltration with 1.4 pores. With a denaturation temperature of 64°C. at pH 6, serum albumin appears to be the most easily denatured serum protein since its denaturation is not as reversible as that of alpha-lactalbumin (4). Even a temperature as low as 55°C. causes unfolding of the B.S.A. molecule over a period of time. A pH of at least about 6 is therefore preferably maintained throughout the process.

The object of this invention is to provide an improved process for preparing a whey protein concentrate that has adequate bacterial reduction without protein denaturation. It is a particular object to provide a process that will achieve a whey protein concentrate having a serum albumin of at least 9.5% and adequate bacterial reduction. This approximately 9.5% level of serum albumin was found in our studies to be important for the achievement of sustained increase of tissue glutathione for the properties such as improved systemic humoral immune response described above (References 2 and 3).

We propose to utilize techniques of microfiltration to achieve the objectives of this invention. Microfiltration using ceramic membranes is a membrane based procedure which allows the separation of particles ranging in size between 0.1 and 10 microns.

Microfiltration with membranes having a 1.4 micron porus size has been recently utilized to remove bacteria and other particles from milk in order to obtain a commercially "sterile" milk (5). With this method a 2 to 3 Log reduction of bacterial

count in milk was achieved; hence a bactericidal effect greater than what is obtained in traditional milk pasteurization (72-78°C/15 sec.: 98% bacterial reduction) (6). The industrial methods of separation of the caseins are based on the destabilization of these proteins either by lowering the pH of milk to the isoelectric point (pH 4.6) at 20°C or by enzymatic (rennet) hydrolysis of the Kappa casein which stabilizes the micelles. The first procedure is not suitable for the recovery of native whey proteins because pasteurization at low pH of the whey-concentrate would entail a substantial denaturation of the proteins.

Brief Description of Drawing

Figures 1A and 1B are a schematic representation of the process of this invention for producing a whey protein concentrate with immunoenhancing properties.

Figure 2 illustrates the glutathione promoting activity of said whey protein concentrate to which we have applied the trademark Immunocal, in an *in vitro* assay as described below. In this assay, the activity of said product is compared with a control milk protein (casein i.e. Ca caseinate) and another commercial whey protein concentrate with low serum albumin content (Allicin 855, mentioned in Table 5).

Detailed description of the invention

Material and Methods

Individual whey proteins were measured by polyacrylamide gel electrophoresis. Samples of concentrated whey were applied on 16% polyacrylamide at pH 8 (Laemmli-buffer system) after the samples were reduced with 10% 2-mercaptoethanol. Samples were applied so that each slot received 10-20 microgrammes of protein. Electrophoresis was performed at 200 volts for 70 minutes. The results were also confirmed by chromatography.

Extent of protein denaturation by the process was determined in triplicate by the nitrogen solubility index (NSI) at pH 4.6 and 3000 g. (AOAC 1985 ref. 6). The method utilized in these experiments differs from that described in Reference 2 and represents a more accurate reflection of the undenatured state of protein.

Protein content (N X 6.38) and total lipids of samples were determined in duplicate respectively by the standard method of Kjeldahl and the method of Mojonnier.

Moisture content was determined in duplicate by the AOAC method (7).

Total coliforms count was determined following incubation at 37°C for 18 hours in brilliant green using the most probable number method. Total bacteria count (aerobic mesophiles) was determined following incubation at 32°C for 48 hours in PCA medium. Both methods are approved by the International Dairy Federation and American Public Health Association.

Lactose was measured by the enzymatic method.

Glutathione determination

Intracellular glutathione was measured by enzymatic assay using niacinamide-diphosphate, dithio-nitrobenzene and glutathione reductase. Briefly, MT-4 human lymphocyte T cells were harvested after 72 hours of incubation at 37°C under 5% CO_2 using standard methods of tissue culture in the presence of varying concentration of WPC (0.01 to 1 mg/ml). The cells were then lysed with ice-cold water and 6% sulfosalicylic acid, centrifuged at 600g for 10 min, and supernatants collected and processed. Spectrophotometric analysis was performed for 2 min at 412 nanometres (nm) and results were expressed in nmole of GSH/10^7 cells.

Dr. S. Baruchel is Associate Professor of Pediatric and Oncology at McGill University in Montreal, Canada. He has developed in his laboratory at Montreal Children Hospital Research Institute this in vitro assay (8,9) which is utilized in collaboration with us in evaluating the GSH promoting (potency) activity of each WPC production batch.

The Invention

In accordance with an aspect of this invention, a process is provided for producing an undenatured whey protein concentrate having a serum albumin content of about 9.5% or more as a byproduct of a process for making cheese comprising:

As illustrated in Figure 1A in step 1, cold standardization of the fat content in milk is effected at a temperature not greater than about 4°C. By this approach we reduce the outgrowth of bacteria. Furthermore this method reduces the breakdown of fat globules which would lead to further reactions involving enzymes and bacterial metabolism.

This involves skimming the milk to a desired fat content. The usual practice has been to use a temperature in the range of 50°C to 65°C as this is the most efficient range for skimming to a level of 0.05% of fat. However, we prefer in accordance with this invention to maintain the temperature at a lower level not greater than about 4°C. This low temperature during the fat removal process is necessary to avoid oxidation of lipids which would then tend to continue over time during storage and produce rancidity (liberation of fatty acids) which could in turn damage the conformation without affecting nutritional efficiency of the labile proteins.

In the examples described in this application standardization of milk was carried out with a cold separator (Alfa Laval, CMRPX 714-HGV) coupled with an automatic standardizer (Alfa Laval, Alfast Model 110). All standardization steps were carried out at 4°C. (Figure 1A).

In next step shown in Figure 1, the skim raw milk obtained after step 1, is microfiltered to provide a first permeate having a bacterial count at least as good as the standard applicable to pasteurized milk.

The microfiltration of the skim milk obtained in step 1 was carried out on a microfiltration system with controlled transmembrane pressure as set up by Alfa-Laval filtration system Denmark (MFS-7 system with 1.4 sq m membrane surface). Ceramic membranes were obtained from Membralox (pore size 1.4 micron).

Table 1 below shows the operating conditions during microfiltration of skim raw bovine milk using a ceramic membrane, as previously described having a pore size of 1.4 microns.

TABLE 1

Temperature	≤ 50°
Transmembrane Pressure (TMP)	0,5 bar
Tangential velocity	>6.6 m/s
Concentration Factor (CF)	20
Flux	950 L/h/m^2

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Raw milk should be used that has been skimmed at a temperature not in excess of about 4°C.

The temperature used during microfiltration of the raw milk should be in the range of about 40°C to 45°C. As previously noted significant denaturation of serum albumin does not begin to occur until about 55°C.

Table 2 shows the microbial counts of the milk following microfiltration. We have noted a 2-3 log reduction in bacterial counts in accordance with the literature (5). This confirms the efficacy of the method. As expected, the lipids are in the retentate. The protein content of the permeate is only marginally different from that of the retentate and there is no difference between permeate and retentate in the type of proteins as measured by electrophoresis. Hence no selected retention of proteins is noted over a 1.4 micron membrane.

TABLE 2

Microbial counts (per/g) expressed in log

Experiment number	Raw Milk		Retentate		Permeate		Reduction of total bacterial counts[1]
	Total (T)	Coliforms (C)	T	C	T	C	
1	4.40	--	6.78	--	2.37	--	2.03
2	6.05	4.25	6.64	4.23	3.07	0.48	2.98
3	3.93	1.80	4.66	1.29	1.76	0	2.17
4	5.72	3.08	6.33	3.18	3.35	0.3	2.37
5	5.49	3.67	6.32	--	2.53	--	2.96
6	5.03	3.08	7.20	--	2.89	--	2.14
7	6.03	4.60	7.51	--	4.03	2.41	2.00
8	6.90	5.32	8.45	7.51	4.27	2.48	2.63
9	6.52	4.00	7.80	5.78	3.76	1.80	2.76

[1]Reduction in log from raw milk to permeate.

The microbial counts of the permeate compare favourably with standards applicable to conventional pasteurization.

The next step, step 3 according to Figure 1A, is pasteurization of the microfiltered milk. The temperature used in this invention should be below 75°C and preferably about 70°-72°C for up to about 20 seconds.

The following step 4 involves cooling of the microfiltered milk. In accordance with the examples of this invention the temperature is accordingly immediately reduced, such as by flash cooling to a temperature of about 30°C prior to cheese making.

At this preliminary stage of cheese making, calcium chloride is usually added to milk to increase the firmness of the curd during the manufacture of cheese. The net result is an increase in cheese yield due to better precipitation and less losses of cheese curd particles in the whey.

Based on the report of Shimada & Matsushita (1981)(10) denaturation of BSA is enhanced by calcium ions. Therefore we avoid the addition of calcium ions contrary to normal practice in cheese manufacture.

Additives of this sort are avoided during production of cheese if the whey generated is being used for this invention. Yield can also be increased in conventional practice by the addition of milk derivatives (casein, whey protein) to milk being transformed into cheese. This procedure is avoided because it influences the quality of the protein content of the whey. The various fraction found in normal whey will be modified resulting in a whey protein concentrate having less undenatured bovine serum albumin per unit of total protein. The pH is to be maintained at not less than 6 at this chilling stage and until concentration of the whey.

Repining follows in step 5 and this involves the addition of cultures to milk about 24 hours before starting cheese making. In the examples the only additives are the usual lactic cultures.

The next step in manufacturing cheese is curd making, illustrated in step 6, Figure 1, which includes the following stages namely:

(a) Rennetting

(b) Coagulum cutting

(c) Stirring

(d) Heating (cooking)

(e) Post stirring

Following post stirring the curd is prepressed and separated from the whey.

Conventional additives are used for rennetting. The making and subsequent handling of the cheese are generally represented in Figure 1B at step 7. This step will depend on the type of cheese. They therefore will not further be described.

What is important however for the purposes of this invention is to avoid any additives or treatment that will deleteriously affect the whey. Thus the production of Emmental cheese requires a temperature of more than 50°C. Raising the curd to this temperature before separation of the whey and maintaining such temperatures would affect the serum albumin content. It is highly desirable to avoid any cheese making steps that involve a temperature in excess of 40°C.

No additional whey is collected for the purposes of this invention during shaping and final pressing of the curd as the pH by then of any whey associated with the curd would be below pH 6.

The whey obtained in step 6 should be chilled to about 4°C. as soon as it is separated from the curd, and this is illustrated on Figure 1B as step 8. By this procedure, the metabolism of the lactic acid bacteria is reduced and acidity does not increase. No additives (such as H_2O_2) should be used as antibacterial agents. Instead we use low temperature inhibition of bacterial metabolism. The pH of the whey should never be below pH 6 before it is concentrated.

The whey that has been collected is first centrifuged to take out excess fat (Alfa-Laval M\HMRPX-214TGV) that was present during cheese production, chilled to 4°C. and stored till ultrafiltration (U.F.) is carried out.

During next step 9, the temperature is then raised at 40°C for the ultrafiltration using, for example, Romicon cartridge (2.3 sq.m.) with a cut off of 50,000 dalton. During U.F. the retentate is submitted to diafiltration by adding distilled water so as to reduce the lactose level in the dry matter to less than 10% of total solids. 1 volume of retentate to 1 volume of water is used and this procedure is

performed two or three times. The retentate following completion of U.F. has a total solids of 19-20%. The conditions of ultrafiltration are set forth below in Table 3.

TABLE 3

TEMPERATURE	approx. 40°
TRANSMEMBRANE PRESSURE (TMP)	1,0 bar
TANGENTIAL VELOCITY	TANGENTIAL VELOCITY >7m/s
CONCENTRATION FACTOR (CF)	29
FLUX	24.5 $l/m^2/h$

The membrane flux increases 2 to 2.5% per degree centigrade, giving a similar increase in capacity of a manufacturing plant. This means that without special reasons for operations at low temperature, it is an advantage in conventional practice to operate at as high a temperature as possible.

The temperature utilized in most other commercial methods during this procedure is 50°C. This level of temperature facilitates a higher flux through the membranes hence more retentate production per unit of time and per unit of membrane. In our method, the draw-back of less production per unit of membrane is compensated by increasing the membrane surface.

The objective of not exceeding 40°C. is obtained throughout the system by fine tuning the points of input and output in the system so as to avoid a heat producing unbalance between the two.

Next, the temperature is lowered to 4°C. in step 10 and kept at that temperature till freeze drying or spray drying is started. The microbial counts of the retentate compare favourably with standards applicable to conventional pasteurization.

These standards differ in each jurisdiction. As an example, the Province of Quebec, Canada, requires that total bacteria count (aerobic mesophiles (32°C) be maintained below 50,000 (log 4.69), both in the factory and in the final product in the case of powdered milk products. Coliforms are to be below 10. The Province of

Quebec has a standard of a bacteria count of 25,000 (log 4.39) and a coliform count of 5 in the factory for milk products that have not been pasteurized or fermented.

Concentration to produce a dry product by lyophilization (freeze drying) is performed in step 11 at temperatures under 0°C for 15 to 18 hours. This does not denature a significant proportion of the thermolabile proteins.

Table 4 illustrates the composition of whey protein concentrate powder obtained using the principles described above.

Certain factors cannot be controlled during normal production of whey concentrate powders. Seasonal variation of milk composition and bacterial metabolism that occurs in milk at each step of the cheese making process will be mainly responsible for the differences observed in the composition of the concentrate. However, the practice of the principles of this invention may be expected to produce a consistently high level of thermolabile proteins such as serum albumin and to avoid substantial loss of the glutamylcysteine group containing protein, together with bacterial counts substantially lower than that obtained with the method disclosed in the PCT/CA93/00518 (Bounous et al.) and in the United States Application SN.08/175,637 (Lange et al.), both assigned to Immunotec Research Corporation Ltd., which is the assignee of the present application. Clearly the combination of microfiltration as in the first part of the method disclosed in PCT/CA93/00518 (Bounous et al.) and pasteurization low temperature lenient procedures as disclosed in United States Application SN.08/175,637 (Lange et al.) greatly reduced the bacterial content of the end product. With this method of production, it is possible to obtain desired bacterial results with less stringent conditions than are disclosed in these prior applications. This is important for large scale productions on industrial scale.

If further bacterial reduction is desired a second pasteurization or microfiltration procedure can be applied to the whey between steps 8 and 9, and namely prior to ultrafiltration. Further evidence of the leniency of this method of production is the relatively high level of lactoferrin (Table 4) in comparison to commercial WPC. This important protein is similar to serum albumin in that it contains 4 Glu-Cys peptides (Reference 11) and it is similarly thermolabile. The analysis of lactoferrin as reported in Table 4 were performed by Drs. G. Regester and

G. Smithers at the CSIRO Dairy research Laboratory in Victoria, Australia, and will likely be published soon in the Journal of Nutrition.

TABLE 4

COMPOSITION OF WHEY PROTEIN CONCENTRATE POWDER

	EXAMPLE 1	EXAMPLE 2	EXAMPLE 3
PROTEIN (%)	78.04	77.00	77.08
α-Lactalbumin (%)	22.00	22.80	22.20
β-Lactoglobulin (%)	57.80	56.30	54.45
Serum Albumin (%)	11.7	11.06	10.85
Lactoferrin (%)	0.67	0.70	0.69
Immunoglobulin (%)	8.39	9.20	11.10
FAT (%)	3.	4.	3.5
LACTOSE (%)	9.40	9.20	9.4
MOISTURE (%) maximum	4.	4.	4.
Ash	3	3	3
MICROBIOLOGY			
Salmonella (/100g)	No growth	No growth	No growth
Coli (/100g)	<5	<5	<5
Staphylococcus (/g)	<5	<5	<5
Total count (/g)	<1000	<1000	<500
NITROGEN SOLUBILITY INDEX	99%	98.7%	99%

The protein composition and solubility of the final product in powder form after concentration by ultrafiltration and lyophilization (Table 4) meets the requirements previously identified herein as essential for the development of immunoenhancing activity and tissue GSH promotion: serum albumin concentration around 9.5% or more and minimal degree of denaturation. In Table 5 and Table 6 are presented for comparison the concentration of serum albumin in currently commercially available W.P.C.'s and the nitrogen solubility index determined by De Wit in some W.P.C. products, as shown in Table 6. Table 5 is derived from Bounous et al. (2)

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TABLE 5

WHEY PROTEIN CONCENTRATE	BOVINE SERUM ALBUMIN in % of Total Whey Protein
Promod (TM)	4±1
Alacen 855	4±1
Lacprodan-80	4.8±2
Sapro	4±0.1
Savropro-75	4±1
Bioisolate	5±1
Promix	4.3±1

TABLE 6

WHEY PROTEIN CONCENTRATE	NSI AT pH 4.6
Normal UF WPC	83%
Neutral UF-DF-WPC	78%
Acid UF-DF-WPC	42%
De-fatted UF WPC	91%
Sphèrosil 'OMA' WPC (TM)	79%
Sphèrosil 'S' WPC (TM)	35%
Vistec WPC (TM)	35%
Demin. delact. WPC	72%

From De Wit J.N. et al. Neth. Milk Dairy J. 37 (1983) pp. 37-49

Table 7 represents analysis results of an undenatured whey protein concentrate prepared generally in accordance with the present invention made by the Japan Governmental Food analysis centre.

The Japanese Government Food analysis centre also conducted a comparison of the serum albumin content of the undenatured whey protein concentrate generally made in accordance with this invention with three best commercial whey protein concentrates available on the market. The serum albumin content for the product of this invention was found to be 11.7 as compared with 7.7, 7.7 and 5.5 respectively for the conventional products.

The process of this invention therefore provides a practical procedure for making undenatured whey protein concentrate. Furthermore, it has the advantage of using a by product of cheese production which is a high pollutant. It is a promising prophylactic and therapeutic approach which utilizes what was until now a continuing financial problem for the dairy industry which is responsible for the disposal of this major water pollutant.

In conclusion, it is the objective of this invention to preserve intact the conformation of the labile whey proteins in the W.P.C. This objective of leniency is obtained through several inter-dependent steps involving microfiltration, temperature, ions content, ultrafiltration flux and drying technique.

TABLE 7

ITEMS	RESULTS	METHOD
Water	4.4%	Air Oven Method
Protein	83.1%	Kieldahl Method (Nx6.38)
Lipid	6.2%	Roese-Gottlieb Method
Moisture	2.2%	Ignition at 550°
Carbohydrate	4.1%	100-(water + protein + lipid + moisture)
Energy	423kacl/100g	protein x 4.22 + lipid + 9.16 + carbohydrate x 3.87
As	Absent (<0.1ppm)	Silver Diethyldithiocarbamate Spectrophotometric Method
Pb	Absent (<0.05ppm)	Atomic Absorption Spectrophotometric Method
Cd	Absent (<0.01ppm)	Atomic Absorption Spectrophotometric Method
Total Hg	Absent (<0.01ppm)	Cold Vapor Atomic Absorbtion Spectrophotometric Method
Sn	Absent (<1ppm)	Polarography Method
Cu	1.52ppm	Atomic Absorbtion Spectrophotometric Method
Total Aerobic Bacteria	$8.7x10^3/g$	Standard Agar Plating Method
Coliforms	Absent/2.22g	BGLB Broth Inoculating Method
S. Aureus	Absent/0.01g	Surface Spread Plating Method
Salmonella	Absent/25g	Enrichment Culture Method
Pseudomonas	Absent/0.1g	Enrichment Culture Method

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A process for producing whey protein concentrate having a serum albumin of about 9.5% or more as a by-product of a process for making cheese, comprising the steps:

(a) cold standardization of the fat content in milk, for producing skim milk, at a temperature not greater than about 4°C;

(b) spore and bacteria reduction by microfiltration of the skim milk through a 1.2 to 1.7 micron pore membrane;

(c) optional pasteurization prior to cheese making at a high temperature for a time short enough to avoid denaturing of the protein in the skim milk;

(d) chilling the skim milk preliminary to cheese production while maintaining the pH at 6 to 6.5 and without the addition of calcium ions;

(e) making curd and whey at a temperature in the range of about 30 to 45°C;

(f) separating the curd from the whey;

(g) removal of excess fat from the whey;

(h) microfiltration with a membrane having a pore size in the range 1 to 1.3 microns, or pasteurization, if needed, at a high temperature for a short time enough to avoid denaturing of the protein in the milk, to remove further bacteria from the milk and produce a commercially sterile permeate;

(i) removal of further water and small water-soluble molecules by ultrafiltration of the resulting whey at a temperature in the range of 35 to 42°C to provide a retentate;

(j) concentrating and drying said retentate under conditions of temperature and time that will not denature at least 95% of the protein.

2. A process as in claim 1 in which pasteurization is at a temperature in the range of 70 to 75°C for a predetermined time.

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3. A process as in claim 2 wherein said predetermined time is between 10 and 20 seconds.

4. A process as in claim 1, in which the step of chilling the skim milk preliminary to cheese production comprises flash cooling to a temperature of approximately 30°C.

5. A process as in claim 1, in which ultrafiltration is accompanied by diafiltration to reduce the lactose level in the final dry product to less than 10% of total solids.

6. A process as in claim 1, in which the retentate in step (j) is concentrated by lyophilization (freeze-drying).

7. A process as in claim 1, in which the whey for further processing is separated in a prepressing operation, prior to shaping and final pressing of the curd.

8. A process as in claim 1, in which the retentate in step (j) is concentrated by spray drying.

9. A process as in claim 1, wherein the conditions for drying step include freeze-drying at a temperature below 0°C.

10. A process as in claim 9, wherein the time of freeze-drying is about 15 to 18 hours.

11. A process as in claim 9, wherein the resulting product has a moisture content of about 3% to 5%.

12. A process as in claim 9, wherein the resulting product has a moisture content of about 4%.

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13. A process as in claim 1, wherein the removal of excess fat from the whey in step (g) is carried out by centrifugation.

14. A process as in claim 1, wherein the removal of excess fat from whey in step (g) comprises cooling the whey at less than but close to 4°C, maintaining the pH of about 6 and centrifugation of whey, while no additives are supplied during this step.

15. A process as claimed in claim 1, wherein said membrane is a ceramic membrane.

16. A process for producing a substantially undenatured whey protein concentrate having a biologically effective amount of proteins containing glutamylcyst(e)ine peptides comprising cold standardization of milk, microfiltration, pasteurization, the formation of curd and whey, separation of the whey and filtering and drying the whey under conditions of time and temperature that will avoid substantial loss of said glutamylcysteine peptide containing protein.

17. A process as in claim 1 or 16 in which the whey protein concentrate comprises serum albumin, alfa-lactalbumin and lactoferrin proteins which are cystine rich, in an amount effective to replenish depleted cellular glutathione (GSH), enhance the immune response and exert anti-cancer effects.

18. A process as in claim 17, in which the serum albumin is bovine serum albumin.

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