(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 03/070017 A2

(51) International Patent Classification[7]: A23J 3/16, A23L 1/305

(21) International Application Number: PCT/IB03/00676

(22) International Filing Date: 24 February 2003 (24.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02004044.0 23 February 2002 (23.02.2002) EP

(71) Applicant *(for all designated States except US)*: **NUTRI PHARMA DANMAKR HOLDING A/S** [DK/DK]; Kongens Nytorv 22, DK-1050 Copenhagen K (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HØIE, Lars, Henrik** [NO/GB]; 8 Blenheim Road, St. Johns Wood, London NW8 0LU (GB). **NÅDLAND, Karl, Johan** [NO/NO]; Åbyv. 9, Kjellstad, N-3400 Lier (NO).

(74) Agent: **BUDDE, SCHOU & OSTENFELD A/S**; Vester Søgade 10, DK-1601 København V (DK).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/070017 A2

(54) Title: NOVEL SOY PROTEIN PRODUCTS

(57) Abstract: The present invention concerns novel soy protein products containing soy protein in a form which result in maximum cholesterol-lowering ingested and also concerns methods for manufacturing said soy protein preparations. Furthermore, the present invention relates to soy protein products suitable for preventing, treating and/or alleviating cardiovascular diseases such as hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and related cardiovascular diseases, for preventing and/or treating type 2 diabetes and/or the metablic syndrome, and for preventing, treating and/or alleviating pulmonary diseases. The present invention also pertains to the use of such soy protein products in the prevention and/or treatment of a cardiovascular disease in a subject suffering from type 2 diabetes.

NOVEL SOY PROTEIN PRODUCTS

FIELD OF THE INVENTION

The present invention concerns novel soy protein products containing soy protein in a form which result in maximum cholesterol-lowering when ingested and also concerns methods for manufacturing said soy protein preparations. Furthermore, the present invention relates to soy protein products suitable for preventing, treating and/or alleviating cardiovascular diseases such as hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and related cardiovascular diseases, for preventing and/or treating type 2 diabetes and/or the metabolic syndrome, and for preventing, treating and/or alleviating pulmonary diseases. The present invention also pertains to the use of such soy protein products in the prevention and/or treatment of a cardiovascular disease in a subject suffering from type 2 diabetes

A soy protein product according to the present invention is particularly useful in preventing and/or reducing the influx of triglycerides and/or cholesterol into the arterial wall and/or reducing the accumulation of cholesterol in the arterial wall of subjects at high risk of developing cardiovascular disease or subjects already suffering from a cardiovascular disease such as atherosclerosis or diabetic subjects. A soy protein product according to the present invention is also useful for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing serum levels of HDL-cholesterol and/or for improving the serum HDL/LDL-ratio in subjects at risk for developing cardiovascular diseases and in subjects already suffering from an arteriosclerotic condition such as e.g. atherosclerosis or a related cardiovascular disease. A soy protein product according to the present invention is also useful in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HLDL-cholesterol in diabetic subjects. A soy protein product according to the present invention is also useful in treating e.g. chronic obstructive pulmonary disease (COPD), inflammation of the airways, asthma, bronchoconstriction, bronchitis, and small airways disease.

In addition the present invention relates to the use of these soy protein products as a medicament and/or in the manufacture of a medicament for treating a subject suffering from cardiovascular diseases, more particularly hypercholesterolemia,

hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and/or related cardiovascular diseases. The present invention also relates to the use of these soy protein products as a medicament and/or in the manufacture of a medicament for treating type 2 diabetes and/or the metabolic syndrome and/or a cardiovascular disease in a subject suffering from type 2 diabetes. Furthermore, the present invention also relates to the use of these soy protein products as a medicament and/or in the manufacture of a medicament for treating a subject suffering from a pulmonary disease, more particularly chronic obstructive pulmonary disease (COPD), inflammation of the airways, asthma, bronchoconstriction, bronchitis, and/or small airways disease.

The present invention also concerns use of a soy protein product according to the present invention in the prevention and/or treatment of said diseases and disorders and for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein in subjects. In addition, the present invention also provides methods for preventing, treating, prophylactically treating and/or alleviating by therapy said diseases and disorders.

BACKGROUND OF THE INVENTION

The field of functional foods has increased tremendously in recent years as the health awareness of the population in the industrialised parts of the world has gone up coincident with an increase in lifestyle related syndromes such as obesity, cardiovascular diseases and type II diabetes. Several studies show a significant correlation between the diet of a subject and the risk of the subject of contracting one or more of these diseases and syndromes. This has put the spotlight on the healthiness of everyday diet and as research into the field continues many items are added to the group of ingredients that should be present in a healthy diet.

Intake of soy protein, either alone or in combination with other soybean components, has been shown to have several beneficial effects on humans. These benefits include improvements in plasma lipid and lipoprotein concentrations (i.e. lower LDL cholesterol, lower triglycerides and possibly higher HDL cholesterol). The beneficial effects of soy protein on plasma lipoprotein concentrations have been recognised in 1999, by the U.S. Food and Drug Administration's approval of a health claim that "25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease".

Naturally, the soy protein could be consumed by eating soybeans, but this has little appeal, as soybeans are conceived by some to have an objectionable flavour and furthermore are not part of a traditional diet in the Western world. An alternative way of consuming soy protein is as part of a food product containing soy protein.

Soy protein is used as a food ingredient in the form of products such as soy flour, soy protein concentrate and isolated soy protein. In conventional processes for manufacturing the protein products, the beans are initially cracked, dehulled and flaked. In this process the material is also conditioned, e.g. by boiling them in water for 20-30 minutes to destroy most of the activity of the trypsin inhibitors. The resulting flakes are then extracted with an inert solvent, such as a hydrocarbon solvent, typically hexane, in one of several types of counter-current extraction systems to remove the soybean oil. The defatted flakes, which are the starting material for most commercial protein ingredients, have a protein content of approximately 50%. Moisture content has typically been reduced to 3 to 5% during this process. Any residual solvent may be removed by heat and vacuum, often by employing a deodorising vessel to enable live steam to contact the flakes to remove additional residual solvent. Conditions in the deodorising vessel can be adjusted to produce flakes of varying protein solubility depending on the end-use requirement.

Following milling of the defatted flakes, the soy protein isolates are typically produced by extracting the protein from defatted soybean flakes with water or a solution of water and ethanol at a pH of 6-11, at temperatures up to 80°C to 90°C. The extracted soybean flakes enter a first centrifuge to separate the soluble fraction from the insoluble fibrous residue. The resulting soluble fraction (containing extracted protein and oligosaccharides) is acidified to a pH of about 4,5 corresponding to the isoelectric point of the protein with a food-grade acid. A second centrifuge is used to separate the whey (which includes the soluble oligosaccharides) from the protein curd that typically involve cooling to below room temperature. The curd is then washed with water in a washing tank using any number of multiple washings. The washed curd is commonly neutralised to a pH of about 7 with water and an alkali such as sodium or calcium hydroxide in a neutralisation tank to make the product pH neutral.

Finally the protein curd is normally spray-dried in a dryer at about 150-170°C inlet air temperature and about 80-85°C outlet temperature, to yield the soy protein isolate or

soy proteinate having a moisture content of about four (4) to six (6)% and a soluble protein content greater than about 90%.

Such conventional processes are e.g. described in, "Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design, Rosentahl et al. Trans ChemE. Vol. 76, part C, Dec 1998 and in patents US5674548, US4307014 and US4296026, US4172828 and US3716372.

The resulting products from the conventional manufacturing processes have physical properties and flavour characteristics, which render them useful as food additives. In recent years a number of alternative manufacturing processes aimed at obtaining products with specific characteristics have been described. These are aimed at obtaining a product with certain specified characteristics such as good dispersion properties, low tendency to crystallise, a high content of phytoestrogens etc.

It has for some time been considered a fact that the content of isoflavones where directly involved in the cholesterol lowering effect of soy proteins. Consequently a lot of effort has been devoted to develop processes which reduce the loss of isoflavones. The focus on the isoflavone content has ultimately lead to isoflavones being added to soy protein products after processing.

US5994508 relates to a process for providing an isoflavone rich protein isolate, along with the isoflavone rich protein isolate produced thereby. A vegetable material containing protein and at least one isoflavone compound is extracted with an aqueous extractant having a neutral pH. The pH of the extract is adjusted to about the isoelectric point of the protein, in order to precipitate the protein. Following cooling to about 5°C to about 25°C, the protein is separated from the extract. The cool separation temperatures increase the concentration of isoflavones recovered in the protein, while the neutral pH inhibits loss of protein normally observed at cool or cold separation temperatures.

US6013771 relates to a process for providing an isoflavone rich protein isolate, along with the isoflavone rich protein isolate produced thereby. A vegetable material containing protein and at least one isoflavone compound is extracted with an aqueous extractant having a pH above the isoelectric point of the protein, and preferably an alkaline pH. The pH of the extract is adjusted to about the isoelectric point of the

protein in order to precipitate the protein. Following cooling to about 5°C to about 25°C, the protein is separated from the extract. Washing of the separated protein is avoided, or is conducted with minimum amounts of water. The cool separation temperatures and the low wash conditions significantly increase the concentrations of isoflavones recovered in the protein.

US6140469 relates to the production of an isoflavone enriched vegetable protein isolate in which the weight ratio of material to extractant is controlled and washing of the acid precipitated protein curd is avoided or minimised to provide an increased level of isoflavones in the protein isolate.

Similar processes are further described in e.g. US6140469, US6132795, US6083553, US6015785, US6015771, US5994508, US5994508, US5990291 and US5919921. Isolates or extracts having a high fixed level of phytoestrogens/isoflavons are described in e.g. US6140469, US6015785, US6013771, US5994508, US573389, US5637562, US5726034 and US5637561. In most of these methods the resulting soy protein is totally denatured and the globular structure completely unfolded.

In recent years the theory that isoflavones are responsible for the cholesterol lowering effect has been questioned and today evidence that protein components are directly involved has been obtained. For example Sirtori et al., have shown that soy protein or fragments without isoflavones can lower cholesterol levels. This is supported by the demonstration, in major clinical investigations on hypercholesterolemic patients, that marked plasma cholesterol reduction was obtained using isoflavone-poor soybean products. Studies in humans and in animal models have furthermore suggested that the mechanism of soy protein might be linked to the direct activation of LDL receptors in liver cells, or to a modulation of the synthesis and catabolism of LDL by specific dietary amino acids. Such an activation has been demonstrated *in vitro* with both isoflavone-poor soy concentrate, isolated protein subunits α and α' from the 7S soy globulin and synthetic peptides derived from the α/α' sequence in liver cells.

The manufacturing processes for producing soy proteins have thus far not been concerned with what effect the process might have on the proteins with regard to their cholesterol lowering effect. This is probably due to the fact that soy proteins are believed to be relatively heat-stable. Furthermore, the cholesterol-lowering effect

attributed to the proteins themselves or part thereof has been considered as being independent on the conditions by which soy protein products were manufactured.

However, a number of disclosures concern methods aimed at obtaining proteins in a not completely denatured form. These disclosures are mainly concerned with the effect that the degree of denaturation can have on diverse physical-chemical properties. Examples of these methods are:

US6005076 which relates to processes for the preparation and purification of proteinaceous materials from oil seeds and protein meals in a substantially non-denatured form. A process is provided in which a protein isolate is obtained from fat-contaminated oil seed material by carrying out an aqueous salt extraction to solubilise protein and fat and subsequently removing the fat by applying a chilling process. Protein is retrieved as micelles upon decreasing the ionic strength of the solution. The resulting protein is described as having improved functional properties.

US5674548 which relates to soybean milk having a high transparency and a soybean protein material having excellent oil retention and minimised mouthfeel change and also relates to soybean protein providing transparent solutions and a strong and transparent gel. The process involves an aqueous protein extraction step in which the temperature is kept below 40°C. The proteins obtained are characterised in that 85% of the protein is able to permeate a 0,22µ membrane and by optical density at 600 nm.

US4737014 relates to a process for isolation of proteins from soybeans with high yield and in a substantially undenatured form. The process involves an aqueous extraction step in which the pH is adjusted to from about 4.8 to about 5.4 prior to dilution. The protein obtained is substantially undenatured as opposed to proteins obtained by conventional isoelectric precipitation and the yield is better than described in the state of the art.

US5936069 provides a method for producing an improved soy protein concentrate having low raffinose, stachyose; and fiber content, as well as minimal lipoxygenase activity and/or good flavour. The improved soy protein concentrate is high in naturally occurring vitamins, minerals and isoflavones, and may also be high in phytoestrogens. The resulting soy protein concentrate has a protein content of about 60% to about 80% of available protein. Further, the improved soy protein concentrate contains less

than 0.3% fibre. Also, since it is not necessary to use any alcohol or acid to produce the improved soy protein concentrate, the final product has not been denatured and is highly functional. The final product is high in water-soluble minerals, proteins and isoflavones, and may generally be higher in phytoestrogens.

In none of the above references is any other means of determining the degree of denaturation than differential scanning calorimetry (DSC) described. Furthermore, none of the above references describe the degree of denaturation of the obtained products, nor contemplates that this would have any effect on the potential cholesterol lowering effect or other health benefits obtainable by ingestion of the produced soy protein. It has furthermore not been questioned whether subsequent processing steps which would normally be considered to have an effect on protein structure, such as heat treatment, would affect e.g. the ability of the soy protein to lower cholesterol. This may be due to the fact that soy proteins have been considered to be heat-stable and due to early studies which showed that soy protein incorporated into baked muffins had a cholesterol lowering effect. One should however take into consideration that the conditions (i.e. for example temperature/humidity) in the internal part of a baked product may not cause complete denaturation of the proteins.

It has now surprisingly been found that soy proteins can lose their ability to lower blood cholesterol and to promote other health benefits when ingested, if excessive heat or other denaturing conditions are applied in the manufacturing process of the soy protein product and/or in subsequent processes where the soy protein is for example incorporated into food products.

Thus, without wishing to be bound by specific theory it is currently believed that certain peptides, which are breakdown products of soy protein formed in the digestive tract, could thus be responsible for the reduction of plasma cholesterol. If the formation of these peptides is dependent on the three-dimensional structure of the protein, this structure will determine the cholesterol-lowering effect of ingesting the soy protein. Processes, which result in structural changes of the soy protein, may therefore compromise the health benefit of ingesting the protein.

It is thus the aim of the present invention to provide soy protein products containing soy protein in a form which retain the cholesterol-lowering effect and also to provide a process for the manufacture of said protein products.

SUMMARY OF THE INVENTION

The present invention provides soy protein products which retain the ability to lower blood cholesterol levels upon ingestion, characterised by having more than a further specified minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility.

The present invention further provides a method for manufacturing soy protein products which retain the ability to lower blood cholesterol upon ingestion, characterised by having more than a further specified minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility.. Said method is further characterised by process conditions which render the 7S and 11S subunits intact. Without wishing to be bound by theory it is believed that ingestion of soy proteins with characteristics such as minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B),), a minimum 7S enthalpy peak or a minimum solubility facilitate the formation of breakdown products in the digestive tract, in the form of peptides which elicit an effect in the form of cholesterol lowering.

The present invention also provides the use of soy protein products which retain the ability to lower blood cholesterol levels upon ingestion, characterised by minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility as a food ingredient, and also provides for the use of the soy protein products which retain the ability to lower blood cholesterol levels upon ingestion to obtain a health benefit.

The globulins are the major part of the soy proteins. These can be divided in 7 S and 11 S globulins. The ratio between 7S and 11 S can vary with the cultivars from 0.5-2.0.

7 S is a glycoprotein. The main component is β-Conglycinin, which is a trimer, molecular mass of 150-200 kDa. Three main sub-units: α' (72 kDa), α (68 kDa) and β (52 kDa). Seen from a nutritional point of view, the α' is the best due to the higher content of cysteine, methionine and tryptophan.

11S is a glycosylated protein, glycinin. It is a hexamer with ba globular mass of 300-380 kDa. Each sub-unit (out of six)is composed of a acidic polypeptide (A), 35 kDa

and a basic polypeptide (B), 20 kDa. The two polypeptides are linked together by a disulfide bond.

Sirtori, Lovati & al have performed a number of experiments in a hepatoma cell line (HepG2), an in vitro model of human liver cells that are highly sensitive to factors regulating LDFL receptor expression, cholesterol biosynthesis and breakdown. Their studies have identified that the peptide corresponding to positions 127-150 (or a part of it) in the α' of the 7S globulin of the soy protein is the really active part responsible for the upgrading of the LDL receptors in the liver. Which part of this peptide is really the active peptide is not defined yet (Lovati, Sirtori & al, J Nutrition 2000, 130:2543-2549). No upgrading effects were found on the α and the β-unit at all. This finding support the hypothesis that if one or more peptides from the α' units can reach the liver after been exposed to the enzymes in the stomach and intestine, they should elicit a cholesterol-lowering effect. In addition also the peptide comprising amino acids 10-32 from 7Sα' may be active in the upgrading of the LDL receptors in the liver.

Clinical trials on soy proteins have been performed over many years. But the reported reductions in LDL give broad variety in the results, in fact from around 6% for patients > 5.7 mmol/l cholesterol (Bakhit & al, 1994, American Institute of Nutrition) and % up to 22-23 % for patients high in cholesterol, > 7 mmol /l (Sirtori & al, Lancet, 1977 i:275-277). The Bakhit study used ISP, baked in muffins as the application from for the study. The Sirtori study used a textured soy protein.

A summary is found in the meta-analysis by James Anderson & al,(The New England Journal of Medicine, 1995, August 3, 276-282). This analysis was performed on 38 clinical studies. These studies (performed from 1975 to 1995) used either ISP or textured soy protein. The average reduction in LDL was in average 12.9 % (6.8- 24 % (for patients with high cholesterol levels)).

It is relevant also to mention the hypocholesterolemic effect is directly correlated to the patient's cholesterolemia. Minimal effect or little reductions occur at cholesterol levels of 6 mmol/l or less and with most benefits with cholesterol of greater than 7 mmol/l (Sirtori, Current Atherosclerotis Reports 2001, 3: 47-53 and J. Anderson, meta-analysis 1995. A new meta-analysis done by J. Anderson, was reported in poster form at the International Symposium on the Role of Soy, November 4-7, 2001 in San Diego, California, USA. The new analysis conclude that regular intake of ISP's can decrease

the LDL by 6.1 %. The figure is based on only ISP based clinical studies from the later years. One other conclusion is that isoflavon poor soy protein is less effective than isoflavon-rich ISP in reducing serum LDL. This is probably very much related to the study shown in Arch of Internal Medicine, volume 159, September 27: 2070-2076, where Crouse III & al describe a clinical, double blind study on 156 patients. In addition to place the patients were given one of 5 daily diets, placebo 25 g of casein, the rest 25 g of soy protein with 3, 27, 37 and 62 mg isoflavons (specified as aglycons). As glycosides these figures corresponds to (specified as mg isoflavons, glycoside forms per g soy protein) 0.2, 1.8, 2.5 and 4.2. The results were as average: 2.5 %, 2.5 %, 4.2 % and 6 % reduction in LDL to placebo. For the patients > 6.5 mmol/l in cholesterol level, the figures were: 3.8 %, 4.5 %, 8.3 % and 9.8 %. For patients <6 mmol/l the reduction was not significant.

On the other hand have Sirtori and his group did an evaluation of the LDL-receptor stimulatory activity (hepatoma cell line (HepG2) of the major soy isoflavon, genistein, up to a concentration of 30 mg/l and failed to demonstrate any evident changes. Isoflavons are known to be inhibitors of tyrosine kinase. This means that they regulate negatively the cellular LDL receptor activity, shown in different experimental systems (Grove & al, J Biochem 1991, 266: 18194-18199, (Sirtori, Current Atherosclerotis Reports 2001)

Studies of the effect of ingesting isoflavons in man (separate from soy proteins) have failed to show any cholesterol lowering effect (Hodgson JM and al, 1998, J Nutr 128: 728-732; Hsu CS and al, 2001, J Reprod Med 46 : 221-226; Nestel and al, 1999, J Clin Endocrinol Metab 84: 895-898).

As clearly outlined by many research groups, heat treatments of soy proteins weaken the bonds that maintain their secondary and tertiary molecular structure. As thermal denaturation occurs, the protein molecules expose to the solvent (water) the hydrophobic areas that are buried in their native confirmation, This molecular change generates an aggregation process of the partially unfolded protein molecules as a consequence of the imbalance between the attractive and repulsive forces of particles. At concentrations above a certain level, the aggregation process sends in the formation of a network. Therefore, any thermal history that changes the suspension from a stabilized colloidal state to the network formation give rise to the following sequences of events:

Denaturation -------→ Aggregation ----------→ Gelation

(R.Schmidt, ACS Symposium Series 147, American Chem. Soc., Washington DC, 1981; A. Clark and Ross Murphy, Adv. Polym. Sci. 1987, 83: 57-192; J.M. Anguilera, Food Technology 1995, 40 (10): 83-89).

The unfolding of the proteins is accompanied by enthalpic changes and can easily be followed by differential scanning calorimetry (DSC), which is the most used technique. The protein aggregation can be followed by gel electrophoresis (SDS-PAGE). In addition the denaturation will adversely affect the solubility of the protiens and this may be measured by standard techniques. By use of such techniques the effect of heat treatment on structure, through denaturation, aggregation and effect on the properties of the ISP, are studied (Petroccelli and Añón, J. Agric. Food Chem. 1991, 39: 1386-1391; 1991, 39: 1029-1032; 1994, 42: 2161-2169; 1995, 43: 3035-3041).

The denaturation temperature for both 7S and 11S is influenced by thermal energy, pH, water concentration and to some degree also ionic strength. The denaturation temperatures increase a lot with reduced water content in the suspension of the soy proteins. High and low pH will also reduce the denaturation temperature. The 7S protein is the most vulnerable and will normally start denaturation at around 70 °C for 10 % dispersion in water, but approx. 130 °C at a moisture content of around 30 %. At 80 °C it will take only a few seconds before all 7S will be completely denatured and the structure unfolded (J. Renkema & al, J. of Biotechnology 2000, 79:223-230; S.Petruccelli and M. Añón, J. Agric. Food Chem., 1996, 44: 3005-3009; A. Morales and J.L. Kokini, J. Rheol. 1999, 43: 315-325).

All results from the clinical tests compared to the technical/physical data and knowledge, indicate clearly that the more thermal energy that is used in the production process, the more denatured the proteins will be (unfolding of structure, aggregation of the strands to bigger clusters of insoluble particles), the less probable it is that the molecules will still have the "active" part of the molecule.

A test on commercial available ISPs before 1990, showed a broad varieties of qualities regarding the denaturation status (Arrese, Wagner and Añón, J. Agric. Food Chem. 1991, 39: 1029-1032). A similar test on commercial available ISPs to day show that all

of them are completely denatured on the 7S protein part. Further heating as in preparation of muffins will worsen the possibility to have any α' of the 7S intact. Our data shows that the ISPs are much more denatured than normal qualities of soy flour which is normally the starting point for manufacturing of the ISP. The normal raw material for texturing of soy protein, is soy flour. The less denatured your starting point is before further addition of thermal and mechanical energy, the less you will change the protein structure and less α' will be ruined.

In order to keep the ISP (or what ever type of soy product) at a high nutritional quality it is important to keep the level of the Trypsin inhibitors (Bowman-Birk inhibitor and Kunitz Trypsin inhibitor) at a level of 5-20 % of the natural occurring in the soy bean (below 50 UTI/mg). There might be a compromise in reaching this level and at the same time denature the soy proteins as little as possible. It will definitely be important to use lowest possible moisture in the heating process step of the manufacturing of the new and improved ISP. It is also important to avoid high pH at any steps in the production (not higher than pH 9.5).

According to a new surprising correlation we have found between the denaturing status of the soy protein and the cholesterol lowering effect, it is questionable what effect - if any - the isoflavons could have. We believe that the correlation between effect and the level of isoflavons is mostly a consequence of the process conditions which are applied in the manufacture of the ISP. Process conditions which keep the soy protein in a relatively undenatured form also favour a high level of isoflavons. An example is alcohol extraction which will increase unfolding and also dissolve not only the fat soluble ingredients, but also some of the most hydrophobic amino acids. It is well known that in the folded protein structure, which is a disc in shape, the hydrophobic part is towards the inside of the disc, where the isoflavons are linked to this hydrophobic part of the amino acids by hydrogen bonds. Even in denatured 7S part of the soy protein, the hydrophobic part is still protected inside the protein molecules, still linked to the isoflavons. Used in this form and status, it is possible that the enzymes in the gastric and intestinal juices will not brake up the polypeptides which are linked to the some part of the 7S part of the proteins.

Early preparations of soy protein, which were not subjected to the same processing conditions as are used today, had a much more pronounced soy flavour and odour. The product quality of the ISP's, which were developed in last 5-7 years, is good, almost neutral in flavour and odour and tailor made for specific applications (for liquid

products, bars and powders). The producers deliberately change the structure to get the specific properties they want depending on the application. Some of the qualities are also enzymatic treated as part of the process to obtain the preferred properties.

The present invention for the first time offers a solution as to how high amounts of soy protein which has not been processed in a way which deanture the soy proteins, can be incorporated in food products without resulting in objectionable flavour or odour.

A substantial knowledge has been obtained in industry (too a large extent as secret know how) and in scientific research programs in the universities. Studies have in particular been aimed at the effect of the different process parameters (heat, pH, temperature/time) on both structure and on how to achieve specific properties (Food Proteins and their Applications, F. Damodaran and A. Paraf, 1997, chapter 9: Structure-Function Relationships of Soy Proteins; Shigeru Utsumi, Yasuki Matsumura and Tomochiko Mori: 259-291, Marcel Dekker Inc, New York, ISBN: o-8247-9820-1). (Hermansson & al, J. Texture Stud. 1978, 9: 33-58; Hermansson & al, J. Am. Oil Chem. Soc., 1986, 63: 658-666).

The term "processed soy protein product" as used throughout the present specification and the appended claims shall be taken to mean any product containing soy protein obtained by processing of soy beans.

The term "intact subunit" as used throughout the present specification and the appended claims shall be taken to mean a protein subunit which has the molecular weight of the full-length protein subunit as well as a protein subunit which is associated with other subunits by e.g. disulfide bridges or non-covalent bonding. The content of such "intact subunits" can for example be performed by analysis by SDS-PAGE under reducing conditions.

The terms "7S subunits" and "11S subunits" as used throughout the present specification and the appended claims shall be taken to mean the subunits which constitute the globulins in legume plants. These are in soy beans also referred to as β-conglycinin which is a trimer of α, α' and β and glycinin which is a hexamer comprising A and B subunits.

The term "ability to lower blood cholesterol" as used throughout the present

specification and the appended claims shall be taken to mean being effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or in increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of high-density lipoproteins (HDL) and/or in generating a decrease in serum levels of low-density lipoproteins (LDL). It is desirable to achieve an elevated serum HDL/LDL-cholesterol ratio since this may result in an increased reverse cholesterol transport and a subsequent excretion.

The term "health benefit" as used throughout the present specification and the appended claims shall be taken to mean any beneficial effect in the prevention or treatment of disease.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect the present invention provides soy protein products which retain the ability to lower blood cholesterol levels upon ingestion, characterised by a minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility. The soy protein products more specifically are believed to facilitate the formation of peptides which has a cholesterol lowering effect.

In another aspect, the present invention provides a method for manufacturing a soy protein product which retain the ability to lower blood cholesterol levels upon ingestion and characterised by a minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility, which method comprises process conditions which render the 7S and 11S subunits intact/undenatured.

In another aspect, the present invention provides a soy protein product prepared by a method according to the present invention.

In yet another aspect, the present invention provides for the use of a soy protein product according to the invention as a food ingredient.

In yet another aspect, the present invention provides for the use of a soy protein product according to the invention to obtain a health benefit, such as cholesterol lowering.

The preferred starting material for the process of the invention is soy beans. Normally the soybeans are heated in almost boiling water after de-hulling to reduce the activity of Trypsin inhibitors (TI), Bowman-Birk inhibitor and Kunitz Trypsin inhibitor. This heating is typically carried out for only a very short time, preferably less than 15 minutes.

According to a currently especially preferred embodiment of the present invention the level of active Trypsin inhibitors in the final soy protein product is less than 20 % of the original activity in the beans, such as e.g. less than 19%, eg. less than 18%, such as less than 17%, eg. less than 16%, such as less than 15%, eg. less than 14%, such as less than 13%, eg. less than 12%, such as less than 11%, eg. less than 10%, such as

less than 9%, eg. less than 8%, such as less than 7%, eg. less than 6%, such as less than 5%.

According to a process according to the present invention, this preferred level of trypsin inhibitors is in a particularly preferrd embodiment, reached by selecting processing conditions which allow for the separation of TI from the globular proteins. It is thus possible to obtain soy protein products with low levels of TI without applying heating, chemical means or separation using e.g. membranes. If such measures are applied it may be in the form of e.g. the NADP/thioredoxin system (from Escherichia coli or wheat germ) (e.g. as described by Jin-an Jiao et al, J. Agric.Food Chem, 40:2333-2336) or by combining the initial heat treatment with small quantities of Sodium Sulphite (Na_2SO_3). This treatment will break the S-S bonds in the globular protein molecules and thus open the protein structure.

Even though as described above the preferred starting material for the process of the invention is soy beans, also soy flakes or flour may be used as a starting material for the process according to the invention. In this case a presently preferred starting material for the process of the invention is soy flakes or flour from which the oil has been removed by solvent or mechanical extraction, and which may be produced from soybeans according to conventional processes.

Thus, below part of the general process of the invention will be described with respect to a soy flour starting material, although other soy protein and vegetable protein containing starting materials e.g. flakes may also be used. The general process combines several precipitation steps under conditions which do not denature the globular 11S and 7S proteins. The process allows for two important objectives to be met. Firstly, it allows for removal of TI from the globular soy proteins without applying traditional methods that might denature the globular proteins and complicate the process. Secondly, the process allows for a partial fractionation of the globular proteins. The first precipitate obtained at modestly acidic pH is thus enriched in 11S globular proteins, whereas the precipitate obtained at more acidic pH is enriched in the 7S globular proteins and thus also in 7Sα'. It is thus possible to obtain soy protein products with very high amounts of specific globular proteins. Further processing of a fraction from the acidic precipitation which is enriched in 7Sα', by for example size fractionation to remove molecular species of small size, would therefore provide a convenient method to obtain soy protein comprising very high amounts of the 7Sα'

subunit. Given the current focus on the effects of the 7Sα' subunit soy protein products with increased amounts of this subunit may prove to be of particular interest.

To obtain an ISP with a much higher content of 7S - and thus 7Sα' - a cultivar with a natural relation between 7 and 11s of up to 2:1 can be selected and/or further separation steps may be applied. Membrane filtration technology could for example be used to concentrate the 7 S fraction without denaturing the globular structure further. An example of such a technology is "crossflow" membrane filtration as the liquid is flowing tangential to the membrane surface and thus inhibits formation of deposits. In this case we have globular proteins in the form of ISP obtained by the method according to the invention. This could for example be a product produced as described in example 4 or a fraction precipitated out at pH 3.5 and partly or fully neutralized afterwards (pH 3.5-8). The proteins in these two ISPs can be separated according to molecular size in three fractions:

1) below 100 kDa
2) between 100 and 250 kDa
3) above 250 kDa

The separation can be carried out in two steps:

Step 1: Removal of molecules with a molecular weight below 100 kDa and collection of a product fraction with the higher molecular weights (concentrate).

Step 2: Removal of the 11S fraction by applying a membrane with a cut-off at approximately 250 kDa. The product fraction, which is the permeate is enriched in 7S proteins.

The equipment is typically tubular with a housing of stainless steel. The tubes are equipped with the suitable membrane for the separation. The first step can be an ultrafiltration step with an operating pressure below 10 bars. The second step can be a microfiltration with an operating pressure below 3 bars. (Two different membranes is used in the two membrane filtration steps).

As the separation has to be performed on a molecular level, the ISP must be completely dissolved in the water phase. The separation is normally carried out at at

pH 6.5-7 and the ISP is dissolved in water in less than 4 % w/v at from 5-25°C, preferably at from 15 to 20 °C.

The permeate, in step 2 above, must be concentrated before spray drying. This is preferably done by reverse osmosis to take out water and concentrate the protein up to a level 20-40 % dry matter. An alternative is concentration in high vacuum evaporators at low temperature (below 20 °C).

The above example of the application of a further purification technique have been included for illustrative purposes only. It will be obvious to the skilled man that other techniques for protein purification that are known in the art may be applied as well.

According to a process of the present invention the soy material, preferably soy flour, is extracted in an aqueous solution, preferably water, at a concentration of less than 40 % w/v, such as less than 30% w/v, e.g. less than 25% w/v, such as less than 20% w/v, e.g. less than 15% w/v. In a further preferred embodiment of the invention a combination of sodium and calcium chloride is dissolved in enough water to make a maximum 0.1 molar solution in order to increase the denaturation temperatures of both 7S and 11S part of the soy protein.

According to a process according to the present invention a first extraction is then performed using gentle mixing for at least part of the extraction period. According to a presently preferred embodiment of the present invention the pH is adjusted up to 6,0-9.5, preferably 6,5-9.0, more preferably 7-8,5 and most preferably around 8,0 by e.g. addition of KOH or NaOH and the temperature is adjusted to a maximum of 68 °C, preferably lower than 66°C, e.g. lower than 64°C, preferably lower than 62°C, e.g. lower than 60°C preferably lower than 58°C, e.g. lower than 56°C preferably lower than 54°C, e.g. lower than 52°C preferably lower than 50°C, e.g. lower than 48°C preferably lower than 46°C, e.g. lower than 44°C preferably lower than 42°C, e.g. lower than 40°C preferably lower than 38°C, e.g. lower than 36°C, preferably lower than 34°C, e.g. lower than 32°C, preferably lower than 30°C, e.g. lower than 28°C, preferably lower than 26°C, e.g. lower than 24°C, preferably lower than 22°C, e.g. lower than 20°C, preferably lower than 21°C, e.g. lower than 20°C, preferably lower than 19°C, e.g. lower than 18°C, preferably lower than 17°C, e.g. lower than 16°C, preferably lower than 15°C, e.g. lower than 14°C, preferably lower than 13°C, e.g. lower than 12°C, preferably lower than 11°C, e.g. lower than 10°C, preferably lower

than 9°C, e.g. lower than 8°C, preferably lower than 7°C, e.g. lower than 6°C preferably lower than 5°C, e.g. lower than 4°C.

According to a preferred embodiment of the present invention the extraction time should be restricted to a maximum of 180 minutes, e.g. be less than 150 minutes, such as 120 minutes, e.g. be less than 90 minutes, such as 75 minutes, e.g. be less than 60 minutes, such as 45 minutes, e.g. be less than 44 minutes, such as 43 minutes, e.g. be less than 42 minutes, such as 41 minutes, e.g. be less than 40 minutes, such as 39 minutes, e.g. be less than 38 minutes, such as 37 minutes, e.g. be less than 36 minutes, such as 35 minutes, e.g. be less than 34 minutes, such as 33 minutes, e.g. be less than 32 minutes, such as 31 minutes, e.g. be less than 30 minutes, such as 29 minutes, e.g. be less than 28 minutes, such as 27 minutes, e.g. be less than 26 minutes, such as 25 minutes, e.g. be less than 24 minutes, such as 23 minutes, e.g. be less than 22 minutes, such as 21 minutes, e.g. be less than 20 minutes, such as 19 minutes, e.g. be less than 18 minutes, such as 17 minutes, e.g. be less than 16 minutes, such as 15 minutes, e.g. be less than 14 minutes, such as 13 minutes, e.g. be less than 12 minutes, such as 11 minutes, e.g. be less than 10 minutes, such as 9 minutes, e.g. be less than 8 minutes, such as 7 minutes, e.g. be less than 6 minutes, such as 5 minutes.

Following the above extraction the un-dissolved part of the soy bean material is according to a process of the present invention separated from the extract by decantation, filtering or centrifugation or a combination of these.

Optionally a second extraction from the un-dissolved material may be carried out in an analogous manner to produce a second extract. These two extracts may be further processed in parallel or processed together.

According to the present invention, following the removal of un-disolved soy bean material the pH from the extract(s) is reduced to 4.8-5.5 and kept at a temperature below 25°C as short as possible and less than 3.5 hours.

According to a presently preferred embodiment of the present invention the pH of the solution is reduced to 4.9-5.5, e.g. 5.1-5.5, such as 5.3-5.5, for example 5.4.

According to a presently preferred embodiment of the present invention the solution is cooled to a temperature below 20°C, such as below 15°C, e.g. below 10°C, such as between 3-8°C. According to a presently preferred embodiment of the present invention the solution is kept at the conditions specified for 0-3 hours, such as 1-2 hours, e.g. 1,5 hours.

According to a presently preferred embodiment of the present invention, sedimentation of the material may be obtained by e.g. centrifugation and the obtained ISP fractions (ISP1 and 2) may then be dried as described below.

According to a process of the present invention the pH of the solution is then further reduced to 2,5-4,5 and kept at a temperature of below 10°C for less than 2 hours.

According to a presently preferred embodiment of the present invention the pH of the solution is reduced to 3-4 for example 3.5.

According to a presently preferred embodiment of the present invention the solution is kept at a temperature below 8°C, such as below 7°C, e.g. below 6°C, such as below 5°C, for example 4°C.

According to a presently preferred embodiment of the present invention, sedimentation of the material may be obtained by e.g. centrifugation and the obtained ISP fractions (ISP 3 and 4) may then be dried as described below.

According to a process of the present invention the pH of the ISP fractions are optionally increased to 5-8 prior to drying.

According to a process of the present invention the curd is then dried carefully by an applicable drying technology. According to a presently preferred embodiment of the present invention this drying is done by freeze or spray drying or any other conventional drying method such as for example vacuum drying, drum drying, air drying in a zigzag drier, on continous belt driers, cabinet tray driers or rotational driers. According to a presently most preferred embodiment of the present invention this drying is done by spray drying at an air temperature below 120 °C, such as below 115 °C, e.g. below 110°C, such as below 105 °C, e.g. below 100°C, such as below 95 °C,

e.g. below 90°C, such as below 85 °C, e.g. below 80°C, such as below 75 °C, e.g. below 70°C, such as below 65 °C, e.g. below 60°C.

According to an especially preferred embodiment of the present invention the product temperature does not exceed 60 °C.

As may be noted the above parameters are set to ensure that the soy protein structure is only denatured to a minimal extent i.e. meaning that preferably at least 50%, such as at least 52%, preferably at least 54%, such as at least 56%, preferably at least 58%, such as at least 60%, preferably at least 62%, such as at least 64%, preferably at least 66%, such as at least 68%, preferably at least 70%, such as at least 72%, preferably at least 74%, such as at least 76%, preferably at least 78%, such as at least 80%, preferably at least 82%, such as at least 84%, preferably at least 86%, such as at least 88%, preferably at least 90%, such as at least 92%, preferably at least 94%, such as at least 96%, preferably at least 98%, such as at least 99%, of the 7S and 11S subunits are preserved in their undenatured form, thereby referring either to the individual subunits of 7S and 11S alone or all subunits of 7S and 11S combined. It is especially preferred that the globular structure is mainly folded with the hydrophobic part of the molecules inside the globular structure (a disk with room for all fat soluble substances inside the molecule including the isoflavons, which are linked to the hydrophobic side of the proteins by hydrogen bonds).

In one aspect the soy protein products comprise more than 50% protein of dry matter, such as at least 55%, e.g. at least 60%, e.g at least 65%, e.g. at least 70%, such as 75%, e.g. at least 80%, such as at least 85%, e.g. at least 90%, such as at least 95% protein of dry matter.

The amount of intact 7S subunits (α + α' + β) and 11S subunits (A + B) in soy protein products according to the invention preferably constitute more than 5 % of the total soy protein content, such as more than 10 %, for example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for

example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 51 %, such as more than 52 %, for example more than 53 %, such as more than 54 %, for example more than 55 %, such as more than 56 %, for example more than 57 %, such as more than 58 %, for example more than 59 %, such as more than 60 %, for example more than 61 %, such as more than 62 %, for example more than 63 %, such as more than 64 %, for example more than 65 %, such as more than 66 %, for example more than 67 %, such as more than 68 %, for example more than 69 %, such as more than 70 %, for example more than 71 %, such as more than 72 %, for example more than 73 %, such as more than 74 %, for example more than 75 %, such as more than 77 %, for example more than 79 %, such as more than 81 %, for example more than 83 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunits (α + α' + β) in soy protein products according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α in soy protein products according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α' in soy protein products according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for

example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The 7S enthalpy peak in a soy protein product to be incorporated in a food product according to the invention preferably constitute at least 0.1 J/g protein, such as 0.2, e.g. 0.3, such as 0.4, e.g. 0.5, such as 0.6, e.g. 0.7, such as 0.8, e.g. 0.9, such as 1.0, e.g. 1.1, such as 1.2, e.g. 1.3, such as 1.4, e.g. 1.5, such as 1.6, e.g. 1.7, such as 1.8, e.g. 1.9, such as 2.0 e.g. 2.2, such as 2.4, e.g. 2.6, such as 2.8, e.g. 3.0, such as 3.5, e.g. 4.0, such as 4.5, e.g. 5.0, such as 6.0, e.g. 7.0, such as 8.0, e.g. 9.0, such as 10.0, e.g. 11.0, such as at least 12.0 J/g protein.

In a soy protein product according to the invention the soluble proteins preferably constitute more than 55 %, such as more than 60%, e.g. more than 65%, such as more than 70%, e.g. more than 75%, such as more than 80%, e.g. more than 82%, such as more than 84%, e.g. more than 86%, such as more than 88%, e.g. more than 90%, such as more than 92%, e.g. more than 94%, such as more than 95%, e.g. more than 96%, e.g more than 97%, such as more than 98%, e.g. more than 99%.

Phytoestrogen compounds according to the present invention are defined as naturally occurring plant substances, said substances being either structurally or functionally similar to 17-estradiol or generating estrogenic effects. Phytoestrogens consist of a number of classes including isoflavones, coumestans, lignans and resorcylic acid lactones. Examples of isoflavones according to the present invention are genistein, daidzein, equol, glycitein, biochanin A, formononetin, and O-desmethylangolesin. The phytoestrogen compounds of a soy protein product according to the present invention are preferably isoflavones, more preferably genistein, daidzein, glycitein and/or equol, yet more preferably genistein and/or daidzein, and even more preferably genistein. A preferred soy protein product according to the present invention may accordingly comprise a single isoflavone, such as genistein, daidzein, glycitein or equol, or it may comprise at least one isoflavone selected from the group comprising at least genistein, daidzein, glycitein and equol. When present in the plant the isoflavones are mainly in a

glucoside form, i.e. attached to a sugar molecule. This glucoside form can be deconjugated to yield a so-called aglycone form, which is the biologically active species. A soy protein product according to the present invention may comprise isoflavones in glucoside and/or aglycone forms regardless of whether the deconjugation to the aglycone form has taken place biologically, in vitro or by any other means whereby the isoflavones are included in a soy protein product according to the present invention or if the aglycone forms are the native form of the isoflavones.

A soy protein product according to the invention may optionally also contain phytoestrogens, such as isoflavons. The phytoestrogen compound is preferably present in an amount, referring to the aglycone forms, of at least about 0.10 weight percent of the soy protein content, such as at least about 0.11 weight percent, for example at least about 0.12 weight percent of the soy protein content, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

A soy protein product according to the invention may optionally have the trypsin inhibitors partly or fully destroyed or removed. The amount of ACTIVE trypsin inhibitor in a soy protein product according to the invention may preferably be less than 50% of the amount in the original soy bean, such as less than 40%, for example less than 30%, such as less than 25%, for example less than 20%, such as less than 15%, for example less than 10%, such as less than 5 %, for example less than 1%.

Researchers have shown that specific amino acids may to some extent effect serum lipid levels and potentially alleviate cardiovascular diseases. Animal studies have indicated that the amino acid lysine increases serum cholesterol levels, while arginine counteracts this effect (Kurowska et al., J. Nutr. 124, 364-370 (1994) and Sanchez et al., Med. Hypotheses 35, 324-329 (1991). This observation appears to be in correspondence with the well established influence of NO on vasodilation, since arginine may potentially be converted to citrullin and NO by NO-synthetase. Thus according to a presently preferred hyphothesis soy protein having a high arginine to lysine ratio effects serum lipid levels and alleviates symptoms of cardiovascular diseases to a greater extent than soy protein having a lower or normal arginine to lysine ratio. Consequently, isolated, potentially processed, soy protein having a high arginine to lysine ratio is a particularly preferred soy protein product according to the present invention. Preferably the soy protein product according to the present invention should have an arginine to lysine ratio of at least about 1.0, such as at least about 1.1, for example at least about 1.2, such as at least about 1.3, for example at least about 1.4, such as at least about 1.5, for example at least about 1.6, such as at least about 1.7, for example at least about 1.8, such as at least about 1.9, for example more than about 2, such as at least about 2.1, for example at least about 2.2, such as at least about 2.5, for example at least about 2.75, such as at least about 3, for example more than about 3.3, such as at least about 3.6, for example at least about 4, such as at least about 4.5, for example at least about 5, such as at least about 6, for example at least about 7, such as at least about 8, for example at least about 9, such as at least about 10, for example at least about 11, such as at least about 12, for example at least about 13, such as at least about 14.

The present invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament effective in preventing, treating, prophylactically treating, alleviating and/or eliminating a cardiovascular disease in a subject. The present invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament effective in preventing, treating, prophylactically treating, alleviating and/or eliminating arteriosclerosis or a related cardiovascular disease in a subject. The present invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament for treating diabetic subjects, said treatment being effective in lowering serum levels of glucose and/or insulin and/or lipids. The present

invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament effective in treating and/or alleviating type 2 diabetes, the metabolic syndrome as defined herein and/or cardiovascular diseases associated therewith in a subject. The present invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament effective in treating subjects diagnosed as having a pulmonary disease, said treatment being effective at least in increasing FEV1 of a subject, as measured by forced expiratory volume in the first second of expiration. The present invention also provides the use of such a soy protein product as a medicament and/or in the manufacture of a medicament effective in treating and/or alleviating pulmonary diseases in a subject. The present invention also provides the use of such a soy protein product and/or such a soy protein product for use in treating arteriosclerosis or a related cardiovascular disease in a subject. The present invention also provides the use of such a soy protein product and/or such a soy protein product for use in the treatment of diabetic subjects, said treatment being particularly effective in lowering serum levels of glucose and lipids in a subject. The present invention also provides the use of such a soy protein product and/or such a soy protein product for use in the treatment and/or alleviation of a pulmonary disease in a subject, said treatment and/or alleviation resulting in an increased FEV1 of a subject, as measured by forced expiratory volume in the first second of expiration.

A soy protein product according to the present invention may optionally comprise a carbohydrate source, flavoring agents, vitamins, minerals, electrolytes, trace elements and other conventional additives. The nutritional soy protein product according to the present invention may in one embodiment also comprise one or more flavoring agents such as cocoa, vanilla, lime, strawberry or soup flavors, such as mushroom, tomato or bouillon and/or sweeteners such as aspartame as well as other additives such as xanthan gum.

When a carbohydrate source is present in a soy protein product according to the present invention, it is preferably present in an amount of less than 30 weight percent such as less than 25 weight percent of the soy protein product. Preferably, the amount of carbohydrate amounts to at least 5 weight percent, more preferred at least 10 weight percent, and most preferred at least 15 weight percent, of the soy protein product. The preferred carbohydrates for use in a soy protein product according to the present invention are dextrose, fructose and/or maltodextrin, or glucose. Skimmed milk

and lecithinated fat reduced cacao are other possible carbohydrate sources. When a soy protein product according to the present invention is for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and associated cardiovascular diseases, lecithinated fat reduced cacao is particularly preferred. Other preferred carbohydrates for use in a soy protein product according to the present invention for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and associated cardiovascular diseases are polydextrose or saccharose, but these should be limited using other sweeteners like e.g. aspartame.

Vitamins and minerals may optionally be added to a soy protein product according to the present invention in accordance with the limits laid down by health authorities. A soy protein product according to the present invention may comprise all recommended vitamins and minerals. The vitamins will typically include A, B1, B2, B12, folic acid, niacin, panthotenic acid, biotin, C, D, E and K. The minerals will typically include iron, zinc, iodine, copper, manganese, chromium and selenium. Electrolytes, such as sodium, potassium and chlorides, trace elements and other conventional additives may also be added in recommended amounts.

A soy protein product according to the present invention may be used for special dietary use, preferably for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides in subjects such as hyperlipidemic patients or normocholesterolemic patients suffering from a cardiovascular disease, and/or for lowering serum levels of glucose and/or insulin and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or for increasing glucose tolerance and/or insulin sensitivity and/or for preventing, treating and/or alleviating impaired glucose tolerance and/or insulin secretory failure in diabetic subjects and/or for preventing, treating and/or alleviating an arteriosclerotic condition by reducing the influx of lipoproteins and/or cholesterol and/or triglycerides into the endocelium of the arterial wall of a diabetic subject suffering from a cardiovascular disease. For example, from one to three daily meals of ordinary food can be supplemented or replaced by a soy protein product according to the present invention. Hereby, significant reductions in serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides can be obtained, as well as an improvement of serum HDL/LDL-cholesterol ratio and/or an increase in serum HDL-cholesterol levels. The soy protein product may provide from about 50 to about 250 kcal per serving.

The daily dose of a soy protein product according to the present invention may comprise an energy content of from 400 to 800, in particular from 450 to 800 kcal/day, which is considered to be a very low calorie diet (VLCD), or it may comprise an energy content of from 800 to 1200 kcal/day, which is considered to be a low-calorie diet (LCD). In another medical embodiment of the present invention, the energy content may correspond to the daily energy requirement of a normal person.

The present invention also provides a soy protein product according to the invention in the form of a micronutrient. In this connection a micronutrient is a nutritional supplement and/or a pharmacological soy protein product and/or a medicament comprising i) a synthetic phytoestrogen-like compound capable of binding to an estrogen receptor or an estrogen-like receptor, and/or ii) a naturally occurring, plant-extractable compound in an amount, on a weight per weight basis, in excess of the amount of said compound, when it is present in a natural host such as a plant cell from which the compound can be extracted or isolated, iii) soy peptides obtainable from a partial hydrolysis of soy protein and iv) soy lecithin.

The naturally occurring, plant-extractable compound is preferably but not limited to compounds capable of binding to an estrogen receptor, an estrogen-like receptor, a beta-2-adrenergic receptor or a receptor belonging to the class of beta-2-adrenergic receptors. When the naturally occurring compounds are isolated from plants such as soybeans, they may be selected from the group at least containing phytoestrogens such as soybean phytoestrogens such as soybean isoflavones, soy protein or fragments thereof, e.g. peptides or amino acid sequences, soybean fibers, lecithin, linolenic acid, an antioxidant, a saponin, a lignan, a protease inhibitor, a trypsin inhibitor, and a tyrosine kinase inhibitor. Additional constituents of the micronutrient may preferably be selected among a DNA topoisomerase inhibitor, a ribosome kinase inhibitor, a growth control factor such as e.g. epidermal growth factor, transforming growth factor alpha, platelet derived growth factor, and preferably any growth control factor controllable by a tyrosine kinase activity. The micronutrient may also comprise ormeloxifene and/or levormeloxifene as described by among others Holm et al. (1997) in Arteriosclerosis, Thrombosis, and Vascular Biology 17 (10), 2264 – 2272, and in Clinical Investigation, 100 (4), 821 – 828. When the naturally occurring compound is an isoflavone, the isoflavone may have been deconjugated to the aglycone form either biologically or in vitro prior to the incorporation in the micronutrient.

In one particularly preferred embodiment the present invention provides a soy protein product or a micronutrient according to the present invention in combination with a functional food ingredient comprising a sterol, preferably an ingredient selected from the group comprising a stanol ester, a tocotrienol, a mevinolin, and a phytosterol compound such as e.g. campesterol, sitosterol or stigmasterol, or a combination thereof.

According to one preferred embodiment, a soy protein product or a micronutrient according to the present invention is for use as a functional food ingredient. A soy protein product or a micronutrient according to the present invention may also be administered as a probe or by intravenous administration, or in tablet or capsule form. The present invention also provides a pharmaceutical preparation comprising the a soy protein product or a micronutrient according to the present invention, use of the a soy protein product or a micronutrient according to the present invention in therapy and/or a diagnostic method performed on the human or animal body, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a medicament, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a medicament for treating a subject suffering from cardiovascular diseases, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a medicament for treating a subject suffering from type 2 diabetes, the metabolic syndrome and/or cardiovascular diseases associated therewith in a diabetic subject and use of a soy protein product or a micronutrient according to the present invention in the manufacture of a medicament for treating a subject suffering from pulmonary diseases.

The micronutrient is particularly useful in preventing, treating, prophylactically treating and/or alleviating hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis and/or related cardiovascular diseases and in preventing and/or treating type 2 diabetes, the metabolic syndrome and/or cardiovascular diseases associated therewith in a diabetic subject and in preventing, treating, prophylactically treating and/or alleviating a pulmonary disease such as e.g. a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

In one embodiment the present invention provides a soy protein product according to the present invention for use as a medicament or as a dietary preparation. A soy protein product according to the present invention for use as a medicament or as a dietary preparation may preferably be used in preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases such as e.g. a disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension, in a subject, preferably for use in preventing, treating, prophylactically treating and/or alleviating arteriosclerosis and/or atherosclerosis in a subject . A soy protein product according to the present invention for use as a medicament or as a dietary preparation may also preferably be used in preventing, treating, alleviating and/or eliminating type 2 diabetes. A soy protein product according to the present invention for use as a medicament or as a dietary preparation may also preferably be used in preventing, treating, alleviating and/or eliminating a cardiovascular disease, such as e.g. hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, hyperinsulinemia, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, and myocardial infarction, in a diabetic subject. A soy protein product according to the present invention for use as a medicament or as a dietary preparation may also preferably be used for preventing and/or treating pulmonary diseases, such as preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in a subject.

The present invention also provides the use of a soy protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases such as e.g. a disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension, particularly a disease selected from the group comprising arteriosclerosis and atherosclerosis, in a subject. The present invention also provides the use of a soy protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing, treating and/or alleviating type 2 diabetes and/or the metabolic syndrome in a subject and/or a cardiovascular disease in a diabetic subject. The present invention also provides the use of a soy

protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing, treating, prophylactically treating and/or alleviating pulmonary diseases such as e.g. a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in a subject.

The soy protein product according to the present invention is effective in lowering levels of cholesterol in normocholesterolemic patients by at least 2%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such as at least 20%, for example at least 25%, such as at least 30%. The soy protein product according to the present invention is effective in lowering levels of triglycerides in normocholesterolemic patients by at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such as at least 20%, for example at least 25%, such as at least 30%.

The soy protein product according to the present invention is effective in lowering levels of cholesterol in mildly hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%. The soy protein product according to the present invention is effective in lowering levels of triglycerides in mildly hypercholesterolemic patients by at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%.

The soy protein product according to the present invention is effective in lowering levels of cholesterol in severely hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%. The soy protein product according to the present invention is effective in lowering levels of triglycerides in severely hypercholesterolemic patients by at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for

example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%.

A soy protein product according to the present invention for use as a medicament and/or the use of a soy protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases in a subject may be effective in reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or causing dilation of blood vessels and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or lowering serum levels of total cholesterol and/or LDL-cholesterol and/or homocystein and/or triglycerides and/or increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of HDL-cholesterol in a subject and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction.

A soy protein product according to the present invention for use as a medicament and/or the use of a soy protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing and/or treating diabetes and/or the metabolic syndrome and/or a cardiovascular disease associated therewith in a subject may be effective in i) lowering serum glucose levels and/or ii) reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or causing dilation of blood vessels and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or iii) lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or iv) increasing glucose tolerance and/or insulin sensitivity and/or v) alleviating impaired glucose tolerance and/or insulin secretory failure and/or improving insulin secretion and/or vi) preventing, treating, alleviating,and/or eliminating cardiovascular diseases, such as e.g. hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, hypertension, hyperglycemia, and hyperinsulinemia, in a diabetic subject and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous

plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a diabetic subject contracting angina pectoris and/or reducing or eliminating the risk of a diabetic subject contracting a myocardial infarction and/or in treating a procoagulant state and/or an increased activity of clotting factors, insulin resistance, glycosidation and/or oxidation and/or chemical modification of lipoproteins, as well as impaired glucose tolerance.

A soy protein product according to the present invention for use as a medicament and/or the use of a soy protein product according to the present invention as a medicament and/or in the manufacture of a medicament for preventing, treating, prophylactically treating and/or alleviating pulmonary diseases may be effective in i) preventing, treating, prophylactically treating and/or alleviating asthma and/or ii) reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or iii) increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration and/or iv) preventing, treating, prophylactically treating, alleviating and/or reducing inflammation of the airways and/or v) preventing, treating, prophylactically treating and/or alleviating bronchoconstriction.

In another embodiment the present invention provides the use of a soy protein product according to the present invention in the treatment of cardiovascular diseases in the human or animal body in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred selected from arteriosclerosis and atherosclerosis.

In another embodiment the present invention provides the use of a soy protein product according to the present invention in the treatment of type 2 diabetes and/or the metabolic syndrome in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HLDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

In another embodiment the present invention provides the use of a soy protein product according to the present invention in the treatment of a pulmonary disease in a human or animal body, preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or small airways diseases and/or asthma and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration.

The present invention also provides a method of preventing, treating, prophylactically treating and/or alleviating by therapy a cardiovascular disease in the human or animal body such as an arteriosclerotic condition of a human or animal body, said method comprising administration of a soy protein product according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum

HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred selected from arteriosclerosis and atherosclerosis.

The present invention also provides a method of preventing and/or treating by therapy type 2 diabetes and/or the metabolic syndrome in a human or animal body, said method comprising administration to said human or animal body of a soy protein product according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HLDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

The present invention also provides a method of preventing, treating, prophylactically treating and/or alleviating by therapy a pulmonary disease in a human or animal body, preferably a disease selected from the group comprising inflammation of the airways,

bronchoconstriction, bronchitis, asthma, and small airways diseases, said method comprising administration to said human or animal body of a soy protein product according to the present invention in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or asthma and/or small airways diseases and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration.

The period of treatment is preferably in the range of from 1 to 12 months or more, such as from 2 weeks to 9 months, for example from 3 weeks to 6 months, such as from 4 weeks to 4 months, such as from 6 weeks to 3 months. However, the period of treatment shall not be limited to these periods and may e.g. be longer than 12 months, such as e.g. a lifelong treatment in order to prevent cardiovascular diseases or in order to prevent and/or alleviate type 2 diabetes and/or a cardiovascular disease in connection therewith or in order to prevent pulmonary diseases.

In one embodiment the present invention provides a pharmaceutical preparation comprising a soy protein product according to the present invention. The pharmaceutical preparation can be prepared in any way known to the skilled person.

In another embodiment the present invention provides the use of a soy protein product according to the present invention as a nutritional preparation and/or in the manufacture of a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum levels of HDL-cholesterol in a subject, including a diabetic subject, and/or for alleviating a pulmonary condition such as e.g. asthma. The nutritional preparation may take any form, which is suitable for human or animal consumption. In one preferred embodiment, the soy protein product is a powdery mixture, which is suspendable, dispersible or emulsifiable in a liquid for human or animal consumption. The liquid is preferably a water-containing liquid such as e.g. water, coffee, tea or juice, including fruit juice. For such a purpose, the soy protein product may be packed in a package intended for covering part of or the total nutritional requirement for a defined period of time, such as a period of e.g. three days or a week. The present invention also provides the nutritional preparation in the form of a dietary supplement.

The nutritional preparation in one embodiment of the present invention is preferably a functional food or drink, i.e. a readily obtainable edible or drinkable substance that is supplemented with a soy protein product according to the present invention to provide a medical or pharmaceutical effect. Accordingly, the present invention provides a soy protein product according to the present invention for use as a functional food ingredient. Functional foods and drinks are preferably selected from the group comprising dairy products, such as yoghurt and yoghurt ice cream, juice, such as orange juice or tomato juice, ready made liquids for drinking, a spreadable product such as e.g. a margarine or a vegetable or plant extracted oil, a cereal product, such as a traditional breakfast cereal product, nutritional bars, biscuits, bread, soups, such as tomato soup, a meat product, such as a hamburger, a meat substitute product, and a vegetable product. In a further embodiment, a nutritional preparation according to the present invention may be in the form of a ready made liquid or in a powder form or in the form of a troche, a solid soy protein product such as a nutritional bar, a fruit bar, a cookie, a cake, a bread or a muffin.

In another embodiment, a soy protein product according to the present invention is a liquid nutritional preparation in a water-containing liquid, in which the solid ingredients are suspended, dispersed or emulgated in an amount of from 10 to 25 weight percent. When the liquid nutritional preparation is intended for drinking, it will usually comprise a flavoring agent as discussed above. However, the liquid nutritional preparation may also be used for probe administration.

In another embodiment, the present invention relates to the use of a soy protein product according to the present invention as a partial or total diet for an overweight subject, an overweight subject suffering from an arteriosclerotic condition or an overweight subject suffering from a diabetic condition. Obesity is believed to be one of the major causes of diabetes including type 2 diabetes. Overweight subjects, including overweight diabetic subjects, often have increased serum cholesterol levels and increased triglyceride levels and are therefore more likely to develop cardiovascular diseases. However, the present invention is not limited to treating subjects with an increased risk of contracting a cardiovascular disease, i.e. subjects likely to have increased serum levels of cholesterol and/or triglycerides, or to treating obese diabetic subjects with an increased risk of contracting a cardiovascular disease, i.e. obese diabetic subjects likely to have increased serum levels of cholesterol and/or

triglycerides. A soy protein product according to the present invention also has substantial serum cholesterol, serum LDL-cholesterol and serum triglyceride lowering effects in subjects having a more normal lipid profile and in diabetic subjects that do not also suffer from overweight. The medical use of a soy protein product according to the present invention is not limited to overweight or obese subjects, including diabetic subjects, but may be used for normal weight subjects having increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides or for subjects with a cardiovascular condition such as e.g. arteriosclerosis or a related condition who have normal serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides. Such increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides may be caused by intake of a diet rich in fats or it may be genetically related.

For the purpose of the present invention, subjects having an initial total serum cholesterol level of 5.7 mmol/l or below are considered to have a normal or hypocholesterolemic level, whereas subjects having a total serum cholesterol level above 5.7 mmol/l are considered to be hypercholesterolemic. Accordingly, by treating normocholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels below a concentration of 5.7 mmol/l in subjects, including diabetic subjects, particularly sensitive to developing e.g. arteriosclerosis, or prevent further development of cardiovascular diseases in patients, including diabetic patients, with previous cardiovascular events.

By treating hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels above a concentration of 5.7 mmol/l in subjects sensitive to developing e.g. arteriosclerosis under such conditions.

More particularly, subjects having a total serum cholesterol level of from 5.7 mmol/l to 7.9 mmol/l are considered to be mildly hypercholesterolemic. Accordingly, by treating these hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels of from 5.7 to 7.9 mmol/l. Subjects having a total serum cholesterol level of more than 7.9 mmol/l are considered to be severely hypercholesterolemic. Accordingly, by treating these hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels of more than 7.9 mmol/l.

It has also been shown that a soy protein product according to this invention has a potentiating effect to the effect of medications such as e.g. statins and/or niacin. By combining a soy protein product according to the present invention with e.g. statins, such as HMG-CoA-reductase-inhibitors, niacin, bile acid resins, fibrates, nicotinic acid derivatives, oat products, such as oat meal, rye products, such as rye meal and various fish oil concentrates with a high content of □-3-fatty acids, it is possible to achieve a further 5 to 15% reduction in serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides. The present invention also provides a soy protein product according to the present invention in combination with a statin, preferably an HMG-CoA-reductase-inhibitor, niacin, bile acid resins, fibrates, oat products, rye products, nicotinic acid derivatives and various fish oil concentrates with a high content of omega-3-fatty acids.

EXAMPLES

Example 1

Preparation of a soy protein product with a high content of intact 7S (α + α' + β) and/or 11S subunits (A + B)

Starting material: G.M.O. free Canadian Soybeans

Methods:

Beans were dehulled and ground. Coarse powder was extracted three times with petroleum spirit (1:4 ratio). Ground with solid carbon dioxide to pass through a 0.5mm sieve. The flour was suspended in a 100mM Tris-HCl buffer of pH 8.0 in a 1:10 ratio (w/v) and stirred for 1 h at room temperature. Centrifuged (30 min: 9000Xg;10°C) and the supernatant was brought to pH 4.8 with 2 M HCl to induce precipitation. After 2 h at 4°C the dispersion was centrifuged (30 min; 9000Xg: 10°C). The precipitate obtained was washed twice with 10 mM sodium acetate buffer at pH 4.8 in a 1:8 ratio (W/v) and freeze-dried.

EXAMPLE 2

Preparation of a soy protein product with a high content of intact 7S (α + α' + β) and/or 11S subunits (A + B)

After dehulling the activity of the Trypsin inhibitors, Bowman-Birk inhibitor and Kunitz Trypsin inhibitor will be reduced by subjecting the dehulled beans to almost boiling water for less than 15 minutes. In order to obtain a final isolate with a level of active Trypsin inhibitors between 5-20 % of the original activity in the beans, a combination with the NADP/thioredoxin system, can be used (from Escherichia coli or wheat germ) (described by Jin-an Jiao et al, J. Agric.Food Chem, 40:2333-2336). An alternative is to combine the initial heat treatment with small quantities of Sodium Sulphite (Na_2SO_3). After crushing and pressing, the rest of the oil will be removed by the traditional treatment with hexane. The soy beans, preferably in the form of flakes will be added to water in an amount less than 15 % w/v. An amount of a combination of sodium and calcium chloride is dissolved in the water in order to make a solution of no more than 0.1 molar. This will increase the denaturation temperatures of both 7S and 11S part of the soy protein. The extraction will be carried out with gentle mixing. The pH is adjusted up to 5.5-7.5 and the temperature should be maximum 68 °C, preferable lower. The time will be restricted to maximum 45 minutes. The un-dissolved material from the beans will be removed by filtration or by centrifugation. The pH is reduced to 4.8-5.5 and the solution is slowly cooled to 5-10 °C during a 1.5 –2 hours period. The solute is removed from the curd. Carbohydrates will be removed by washing once with water. The curd can now be dried carefully by different drying technologies e.g. spray drying/ air temperature below 120 °C. The end temperature of the product should not exceed 60 °C regardless of which drying method is applied.

EXAMPLE 3

Preparation of ISP by precipitation at pH 5.4 and/or 3.5

Preparations of ISP's from commercial soy flour were carried out as outlined in table 1.

Table 1: Separation scheme for isolates

Step	Action	Fractions	Time, min
1	Disperse material (20g) in water (200ml) at pH 8	-	
2	Stand at ambient	-	60
3	Centrifuge	-	30
4	Separate supernatant and precipitate at pH 5.4 at 4°C or 20 °C	-	90-210
	Centrifuge and recover solids IS1	IS1	30
5	Wash 1st solids (S1) with water at pH 8 (100ml)		30
	Centrifuge and recover solids (S2)		30
6	Precipate the supernatant at pH 5.4 (IS2) at 4°C	IS2	90
7	Precipitate the supernatant at pH 3.5 at 4°C for 2 h	IS3	60
8	Dry all materials in freeze drier - 1-2 days		

Three similar procedures were carried out in which the first precipitation of the isolate at pH 5.4 were varied with respect to time and temperature according to Table 2.

Table 2: Time/temperature variations

Procedure	Time	Temperature, °C
1	3.5	20
2	3.0	4
3	1.5	4

The Flow-sheet for Procedure 3 is shown in figure 1.

In the full process 7 materials are produced, the washed residue called Fibre, pure isolates, IS1& IS2 and TI-rich isolate IS3& IS4 and soluble solids SS1& SS2.

The samples were examined by chemical analysis for protein, fat and fibre, Trypsin Inhibitor (TI) analysis, Differential Scanning Calorimetry (DSC) and Polyacrylamide electrophoresis (PAGE).

Analysis of fractions for protein

Only small differences in recovery of fractions and proteins were found between the three procedures. The precipitated protein fractions IS1-3 contained 60-65% of the total protein from the flour.

Analysis for Trypsin Inhibitor

TI analysis is International standard for soya products. It is expressed in mg of Bovine trypsin (such as Merck No. 24579) inhibited /mass in g of test sample. TIU trypsin inhibitor units (TIU/mg of sample) 1.9 TIU equals 1 mg of trypsin inhibited.

The separation according to procedure 3 gave a recovery of 60-62% of the protein in fractions IS 1-3 with only 13% of the original TI. In comparison, gave procedures 1 and 2 higher values of respectively 29% and 25% TI in the IS 1-3 fractions. It is therefore clear that procedure 3 offers the best separation of the globular soy proteins from TI.

DSC analysis

The DSC measures the heat of denaturation in proteins as they are heated over a regime from 25 to 180°C. The DSC results showed that the isolate formed at pH 5.4 were recovered from soya flour without causing any further denaturation to the proteins. It was furthermore found that the 7 and 11S proteins were partially fractionated by the precipitations, with a relatively higher yield of 11S at pH 5.4 and of 7S at pH 3.5.

DSC measurements of fractions from procedure 3.

Samples	7S enthalpy peak J/g protein	11S enthalpy peak J/g protein
IS1/procedure 1	0.09	11.51
IS2/procedure 1	0.58	4.87
IS3/procedure 1	3.55	0
IS1/procedure 2	0.3	10.9
IS2/procedure 2	0.23	4.92
IS3/procedure 2	4.36	0.06

IS1/procedure 3	0.65	10.62
IS2/procedure 3	0.47	5.39
IS3/procedure 3	3.78	0.04

Analysis of protein composition by SDS PAGE

The 7 fractions from each process were run at an equivalent protein level on a separate gel with standards for molecular size. All the tracks on the gels were stained with a blue dye and also analysed by densitometry. This technique is very sensitive and reveal traces of 7 and 11 S globulins that can not be measured by DSC.

The 11S bands were stronger in the IS1 and IS2 fractions and the 7S in the IS3 fraction, in agreement with the DSC findings.

The protein pattern for all sample fractions were quite similar for all three gels. The values for the relevant peaks obtained from the densitometry analysis was used for a quantitative analysis of the distribution of the globular soy proteins in the different fractions. The results are summarised in the following table. Two commercail soy protein isolates Suprosoy and Samprosoy are included for comparison:

Product/ preparation	Content of 7S alpha	Content of 7S alha'	Content of 7S	Content of 7S+11S
Suprosoy	8,6	7,4	26,9	61,2
Samprosoy	9,1	7,7	30,3	59,3
Procedure 1 first precipitation at pH 5.4 (ISP1)	8,6	6,5	29,4	64,6
Procedure 1 second precipitation at pH 5.4 (ISP2)	11,0	7,3	32,0	61,6

Procedure 1 precipitation at pH 3.5 (ISP3)	16.7	9.6	39.2	48.8
Procedure 2 first precipitation at pH 5.4 (ISP1)	9,4	7,0	30,6	65,2
Procedure 2 second precipitation at pH 5.4 (ISP2)	12,0	8,2	34,1	62,9
Procedure 2 precipitation at pH 3.5 (ISP3)	15.4	8.5	34.5	45.5
Procedure 3 first precipitation at pH 5.4 (ISP1)	8,9	6,8	29,3	63,0
Procedure 3 second precipitation at pH 5.4 (ISP2)	11,2	7,6	32,2	60,0
Procedure 3 precipitation at pH 3.5 (ISP3)	15.7	8.8	34.0	46.0

EXAMPLE 4 - Pilot scale preparation of new ISP's

Processing Conditions

The process and process conditions of this example were based on Procedure 3 from EXAMPLE 3. One important difference was that no second extraction at pH 5.4 was carried out. The preparation of the ISP was carried out in a number of batches which were combined to give the products as the Final Mix (FM).

Procedure

Table 3: Separation Scheme for Isolate

Step	Action	Fractions	Time (min)
1	Soy flour (40 kg) was dispersed in water (300l) at 20 to 21°C in Tank 1 with a portion of anti-foam FDP (75 ml).		~ 10
2	The mixture was adjusted to pH 8.0 with 2 M KOH (~3.6 l)		~ 5.0
3	Extraction with continuous stirring (30 min.) was followed by holding without stirring (30 min.)		60
4	The slurry was centrifuge to remove solids with feed rate of 726 l/h and a pressure of 4.25 kg/cm^2 into Tank 2. The partially cleared slurry was re-centrifuged and returned to Tank1. The fibre-rich solids collected by the centrifuge were discarded.	Fibre + high Mwt. proteins	~ 90
5	Small ice pieces (48kg) were added to bring temperature ~ 7.0° C		~ 5.0
6	The pH of the solution was reduced to 5.4 with 5.5 M HCl (~1.7 l)		~ 5.0
7	Precipitation was allowed to occur without stirring		90
8	Isolate was separated by centrifuging at 726 l/h and a pressure of 4.25 kg/cm^2 from Tank1 to Tank 2 and then back to Tank 1. The precipitate recovered from	ISP, fraction 1	~ 90

	the centrifuge. Antifoam FDP (75 ml) was added during the separation.		
9	Small ice pieces (12 kg) were added to the solution to bring temperature ~ 10° C		~ 3.0
10	The pH was reduced to 3.5 with 5.5 M HCl (approximately 3.7l)		~ 5.0
11	Precipitation was allowed to occur without stirring		60
12	The isolate was separated by centrifugation at a feed a rate of 726 l/h and a pressure of 4.25 kg/cm^2 in a single pass from Tank 1 to Tank 2. The insoluble isolate was collected and the liquor of solubles discarded.	ISP, fraction 2	~ 40
13	Both isolates were combined in a 25l-vessel and mixed with a Silverson. Samples were collected for microbiology, protein and moisture.		~ 10
14	The pH was raised to between 6.0 to 6.5 with a concentrated solution of KOH (415g in approx. 500 ml water).		~ 10
15	Portions (6.0 kg) of the slurry were deposited in freezing trays (1000mm x 495mm) lined with plastic sheets and frozen at -21°C in walk-in freezer.		~ 60

RESULTS

The results from the analysis if the FM for moisture, fibre, ash, and protein content.

Table 4. Analysis of freeze-dried isolate

	Moisture (%)	Fibre	Fibre dry basis	Ash	Ash (dry basis)	Protein (as is)	Protein (dry basis)	Fibre Ash+Prot
Final mix FM	**5.3**	**4.30**	**4.5**	**6.64**	**7.0**	**77.0**	**81.3**	**92.8**

Physical analysis of free-dried isolate

Differential thermal analysis

Table 5: DSC data for flour, isolate and some commercial isolates

	Weight used in DSC pan (mg)	Enthalpy Peak 1 (J/g dry protein)	Enthalpy Peak 2 (J/g dry protein)
Commerciall soy flour	46.3	1.48	5.64
Soya isolate final mix	32.5	1.10	5.41
A - FXP H0161	52.8	0	0
D - XT 10	34.6	0	0

The two figures for soy flour and soy isolate FM are plotted on the same graph with the same scale for the peaks. The difference in baseline due to sample mass has been reduced by shifting the flour graph downwards by 7.0 units.

Figure 2: Comparison of DSC graphs of soya flour and isolate FM on same protein basis, showing the 7S (75-85°C) and 11S (95-105°C) enthalpy peaks.

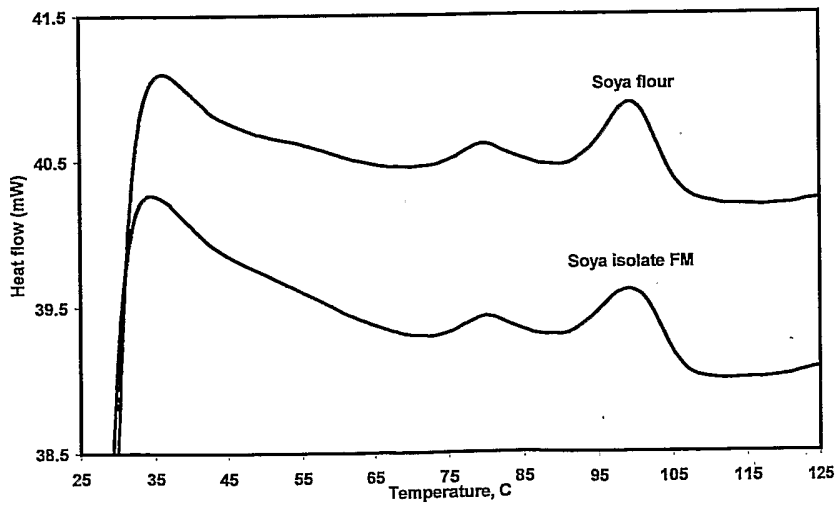
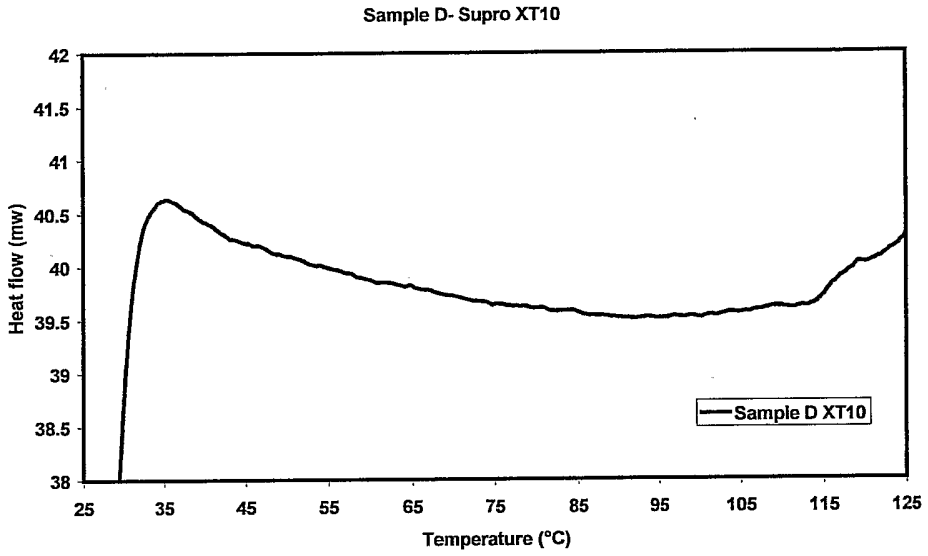
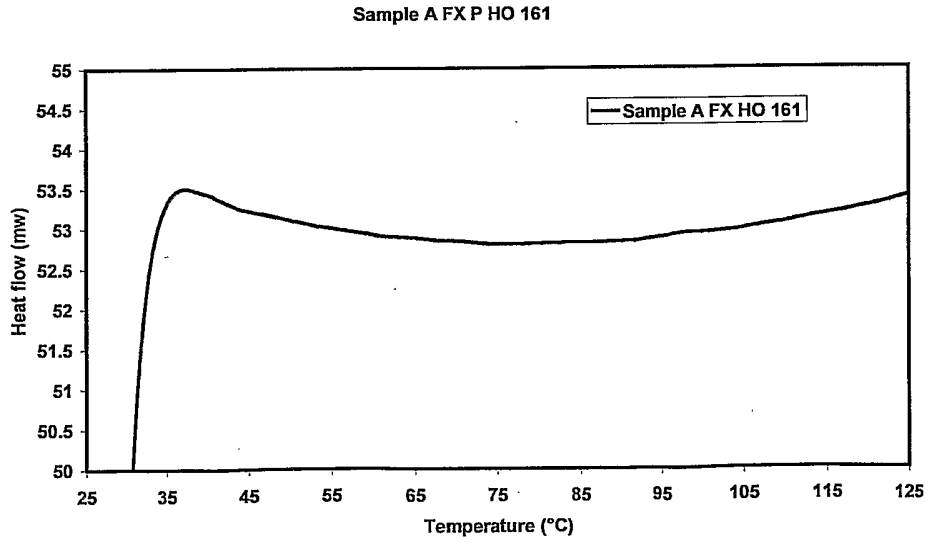


Figure 3. DSC analysis for two commercial available qualities of ISP from Du Pont Protein Technologies.

The curves are without peaks, indicating that there is not any un-denatured globular protein structure left in the products.





The PAGE data showed strong bands for the 7 and 11 S proteins that were more pronounced than for the flour, supporting the DSC finding.

Table 6: Values of the peak areas shown in graph of densitometry in the soy flour and the FM isolate at equivalent protein levels for loading the PAGE.

Mol. weight in Da	79.5	71.7	64.6	43.1	29.3	14	9.6
Protein type	7S	7S	7S	7S	11S	11S	PEPTIDES
Soya flour OD value	49	81	125	118	160	176	23
Isolate FM OD value	50	105	135	180	210	210	20

OD is the optical density reading of the scanning device used to "read" the gels.

Solubility of isolated precipitated at 5.4 and 3.5

Table 7: Results of analysis of commercial isolates for %protein solubility

Samples		Total protein, %	Soluble protein, %		% soluble
A	FX H0 161	84.5	46.5		55
B	Supro 760	84.5	23		27.2
C	661	87.5	19		21.7
D	XT10	82	34		41.5
E	FX H0 159	77.5	40		51.6
F	219	83	43.5		52.4
G	219D	82.56	44		53.3
H	LH (Bunge)	85	35.5		41.8
I	NB (Bunge)	86.5	24		27.8
J	Profam 940 (ADM)	84	28		33.3
K	Fibrim 1020	8.5	3		35.3
L	Supro ST	79	16		20.3
M	Supro 770LN	84.5	25		29.6
N	Supro XT34	80	22		27.5
Isolate from		**85.2**	**80.9**		**95.0**

Example 3					
Isolates from Example 4, batches 18/20		**82.1** **80.1**	**78.8** **77.7**		**96.0** **97.1**

The solubility of the isolates prepared by the large-scale method were approximately 96-97% after freeze-drying showing no denaturation had occurred

Isoflavon analysis

Table 8: Selected values from Table 13 for comparison on total isoflavon level

Identification	Genistein	Daidzein	Glycitein	Total as Aglycon mcg/g	Total as Glycoside mg/g
Commercial soy flour used for preparing ISP	770	654	13	1437	2.44
ISP	667	641	94	1402	2,38

From the above data it is clear that the process according to the present invention allows for the preparation of soy protein products retaining almost all the isoflavones present in the starting material. This is in line with the undenatured state of the soy protein as the isoflavones are associated with the interior of the proteins and would thus remain associated with the proteins when the globular structure is intact.

PAGE densitograms:

The isolate was run at 3 concentrations of material added to the gel. In general terms the O.D. values are linear from 0.01 to 0.8 and it is unwise to use higher values. The most diluted sample gave the best separation of the peaks and the densitogram from this was consequently used for further analysis. This was for the isolate obtained by mixing 0.025ml of the extract with 0.375ml of Laemmli buffer.

The same was true for the densitograms for the soy flour. The lowest concentration of material added to the gel gave a better separation.

Table 9: 7 and 11S protein content in Flour and ISP

Product	7S	11S	Other, soy proteins
Flour, %	28	29	43
ISP, %	34	39	27

Table 10: Composition of the basic components in 7S and 11S of the ISP

	7 S			11S	
Components	α'	α	β	A	B
Amounts % of total prot.	9	12	13	19	20

The amounts of 7 and 11S proteins are considerably increased in concentration during the processing. This is because the highest molecular weight proteins, the 15 S fraction will be separated out together with the insoluble fibres and much of the 2S fraction is more water soluble and will be a part of the water solution and removed.

The SDS-PAGE data from Example 3, indicated that the second washing and precipitation of ISP at pH 5.4 would have contributed to higher yield of ISP, but also to

an increased amount of 7S as this ISP fraction showed a different relation between 7 and 11S than from the first precipitation. The result would most probably have been a result of more equal quantities of 7 and 11S than shown in the Table 9 above.

Materal and Equipment.

1. Two deliveries (1000 kg) of soy flour were purchased from Cargill as lots 1 and 2. Lot 1 was processed as batches No.14 to 31 and lot 2 as batches No.32 to 40.
2. Reagent grade Hydrochloric acid (specific gravity 1.18) and
3. Potassium hydroxide (56.1g/mol.) was purchased from BDH and tap water was used to make the slurry.
4. A foam depressing reagent, antifoam FDP, was obtained from Basildon Chemical Company Ltd., Kimber Road Abingdon Oxon., OX14 1RZ.

The table below show the main data for the two batches of Soy Flour used as raw material for the process and the pilot scale production:

Table 11. Soy flour - starting material

Detail		
Name	De-fatted soya Provabis 200/80	De-fatted soya Provabis 200/80
Batch number	820423	8198859
Production	18/04/02	18/04/02
Protein, %	> 52	> 52
Fibre, %	3.0-3.5	3.0-3.5
Oil, %	0.7-1.2	0.7-1.2
Moisture, %	< 10	< 10
Total plate count, ln	max. 5.2	max. 5.2
Enterobacteriacae, ln	max. 4.0	max. 4.0
Salmonella	nil	nil
Granulation %	< 6	< 6
Used in batches	1-13	14-40

Equipment

1. Centrifugal separators from Westphalia Separators, Model SA 7-06-476 with self-cleaning bowl and a set of 69 separating cones, and

2. A paddle mixer was used to make the flour/water slurry and for mixing during pH adjustment.

1. A Silverson mixer (without shearing element) model D, Silverson Machine Ltd, Waterside, Chesham, Bucks, HP5 1PQ was used to mix the isolates during pH adjustment.
2. APV 454 litres capacity steam jacketed kettles were used as holding tanks.
3. A NORD, model SK 20R150U90L/433, CIP lobe pump was used to feed slurry to the separator.
4. An Edwards freeze Dryer Modulyo was used for small-scale drying of isolates.
5. Commercial Freeze Drying was performed with N° Three and N° Seven freeze dryers.

Freeze Drying:

Comment:

Freeze drying was chosen as a suitable method in this case. To avoid microbiological growth in all produced ISP in the established pilot process was frozen on trays immediately after production. Instead of having to defrost all material again with increased microbiological risk, we decided to use freeze drying in this particular case. Freeze drying is also a rather gentle drying method.

Generally all kind of drying methods can be used, but the drying conditions must in all cases be chosen carefully to avoid further denaturation. The standard spray drying method is preferred for full production scale operations. But in such cases it is an advantage not to dry to low water level as this might influence the dispersion and flavour properties of the final product.

Laboratory scale to obtain analytical values

Samples were frozen at -21°C. Edwards freeze Dryer Modulyo was used for drying the isolate at lab scale. Samples were kept at -55°C and a vacuum pressure of 10^{-1} mbar (0.1mm Hg) was used. It took 4 to 5 days to dry even very small samples.

Commercial Freeze Drying:

The process was undertaken in two dryers, N° Three and N° Seven designed and built by Commercial Freeze Drying Ltd. Both dryers were CFD. N° Three has 28 trays of approximately 1000mm x 495mm and N° Seven holds 102 trays of the same size.

The frozen product was received by Commercial Freeze Dry Ltd. was held in cold storage at -20°C. Clean trays were then lined and the product weighed onto them at 5 kg/tray. The trayed-product was then held overnight at -20°C to stabilise and the freeze dryer was cleaned with Commercial Freeze Dry Ltd. normal cleaning procedures and cooled to -20°C ready for loading. After loading a vacuum was created in the freeze dryer and it was run at a vacuum pressure < 2 mbar. Since the product was heat sensitive, a heat profile was run which prevented the product from reaching a temperature of greater than +30°C. This considerably extended the drying time.

First batch of 140 kg of wet product was dried In N° Three dryer on 5th September through 7th September. Dried product was then ground with an Apex comminuting mill model 114 type S2 at a fast hammer rotation speed and a screen aperture of 0.125". After examination of the powder sample, it was too coarse and to reduce the particle size further, the finished product screen aperture was reduced to 0.107"for subsequent milling of the product. The product was then dispatched to CCFRA.

The remaining wet product was dried in five lots of 140 kg each in N° Three dryer and two lots of 510 kg each in N°Seven dryer. The dried product was then ground using 0.107" screen and dispatched to CCFRA in sealed in plastic sacks for further processing. Grinding screens were restricted by concerns for the temperature sensitivity of the product. A fine screen necessary to obtain particles < 150 um caused a build up of heat in the product, so a larger screen was used.

EXAMPLE 9

Preparation of chocolate milk with Abacor or placebo with subsequent treatment at very high temperature

The Verum trial substance (Abacor, reg.trade mark, see table below) or the placebo trial substance (see table below) was stirred into long-life chocolate milk (defatted milk, fat content 0.2%). The resulting chocolate milk preparations contain 25 g of soy protein respectively casein per liter.

The treatment at very high temperature was performed as follows: The chocolate milk preparations were preheated to 80°C by a plate heat exchanger for approximately 1-2 minutes. The preheated preparations were then treated at very high temperature by direct steam injection to 142°C and kept at this temperature for 4-8 seconds. Following cooling by vacuum to approximately 80°C the preparations were homogenized at 30 bar and subsequently at 150 bar for a maximum of 30 seconds followed by cooling to approximately 20°C over 60-90 seconds.

Table 12.

Content	Amount in 100 g Verum substance (Abacor Reg.)	Amount in placebo substance
Total protein	37.9 g	35.62 g
Soy protein	33.8 g	-
Casein	-	25.95 g
Fat	10.3 g	2.71 g
CHO	30.7 g	48.03
Sodium	0.13 g	-
Energy, kcal	359	348
Energy, kJ	1501	1455

EXAMPLE 10

Preparation of milk with Abacor without subsequent treatment at very high temperature

Half a liter of low fat (max. 0.3% fat) long-life milk obtained by wholesale was diluted 1:1 with water and 77.5 g of the Verum trial substance (Abacor, Registered trade mark, see table below) was stirred into the liter of thinned milk. The resulting milk preparation contain 25 g of soy protein/L.

Table 13.

Ingredient	Amount in 100 g Verum trial substance (Abacor REG)
Total protein	37.9 g
Soy protein	33.8 g
Fat	10.3 g
CHO	30.7 g
Sodium	0.13 g
Energy, kcal	359
Energy, kJ	1501

EXAMPLE 11

The effect of treatment at very high temperature on protein subunit composition as observed by PAGE.

An analysis was performed on the soy protein product (Supro 760), used for the preparation in examples 9 and 10. This was compared to an analysis of the preparation from example 9 which had been treated at very high temperature, in order to investigate whether the soy protein subunit composition was changed following heat treatment. The PAGE was performed under reducing conditions.

Figure 4. Supro 760 analysed by gel electrophoresis.
The profile shows distinct bands for the 7S and 11S globular proteins.

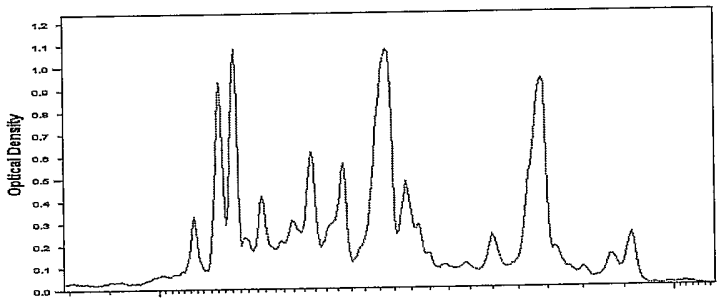
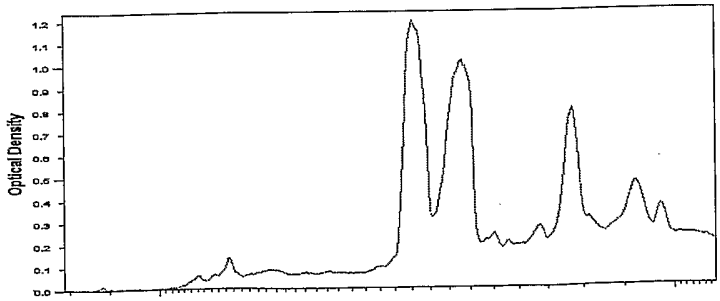


Figure 5. Supro 760 added to milk and treated at very high temperature according to example 9.
The distinct bands for the globular soy proteins have been considerably reduced. When considering the amount of soy protein applied to each of the lanes, it can be concluded that the bands for the globular soy proteins have been reduced more than half.



EXAMPLE 12

Clinical results from ingesting preparations according to EXAMPLE 9

A study was performed to determine the clinical effects of ingesting the soy protein preparation described in EXAMPLE 9, with 80 trial subjects (51 female, 29 male, ages 30 to 70 years) with hypercholesterolemia (inclusion range: 5.2 – 7.8 mmol total cholesterol/l ~200 – 300 mg/dl) in two dosage groups that were each tested against a placebo group. The trial subjects each drank one liter (dosage group 1), or half a liter (dosage group 2) of a preparation according to EXAMPLE 9, corresponding to 25 g and 12.5 g of soy protein respectively. For both dosage groups a placebo group was included in which each subject ingested a corresponding amount of the placebo substance according to EXAMPLE 9.

Blood samples were taken from the trial subjects at the on-set of the experiment (week 0), at 2 weeks and at 4 weeks (end of the experiment), and total serum cholesterol, LDL cholesterol and HDL cholesterol determined. For the trial parameters the first and third examination comparisons are interpreted by means of the t-test for paired observation and description on the level of a 5% probability level.

The data were recorded according to GCP requirements with double data entry and simultaneous value comparison in dBase IV. Study design, performance and documentation of the study was according to the guidelines "good clinical practice" CPMP/ICH/135/95. The primary objective was the reduction of total cholesterol in serum – secondary objectives were LDL and HDL cholesterol.

Independent of the daily dosage and examination group, in the pre/post comparison it was found that:

- no effect on the total and HDL cholesterol levels be determined,
- there was a clear elevation of (16.5 – 18.7 %) the LDL cholesterol levels in serum

The resulting lack of effect of the tested soy preparations in chocolate milk on the total and LDL cholesterol was unexpected.

Results

For both dosage groups 40 subjects each were included. Because there were no premature terminations, the full analysis was performed according to the protocol analysis.

Total cholesterol (primary objective)

In both dosage groups the verum and placebo groups did not differ in their original values (p = 0.48 relatively 0.91). During the course of the study there was only marginal change in these values. For dosage group 1 the total serum cholesterol levels were, at the end of the study, 1.0 mg/dl (0.4 %) for verum relatively 4.0 mg/dl (1.6 %) for placebo lower than at the beginning of the study (difference: 3.0 mg/dl; 95 % confidence interval: - 8.2 to +14.2 mg/dl), in dosage group 2 the verum values were 7.9 mg/dl (3.0 %) lower relatively for placebo with 1.6 mg/dl (0.6 %) higher (difference: 9.5 mg/dl; 95 % confidence interval: -33.1 to +14.1mg/dl). This last value was, in the placebo group, strongly influenced by subject Nr. 37 (increase of 180 mg/dl). If this value is removed from the evaluation, the mean value of the placebo group at the 3rd examination was 253.9 ± 30.4 mg/dl and the difference to the 1st examination was (261.7 ± 27 mg/dl for n = 19) 7.8 mg/dl (3.0 %), thereby there is no difference between the verum and placebo groups.

LDL cholesterol (secondary objective)

The original situation was homogeneous (p = 0.68 relatively 0.81, s. a. Tab. 9). In both dosage groups the LDL value increased during the course of the study. They were higher in comparison to the original values for dosage group 1 for verum by the end with approximately 24.9 mg/dl (18.6 %) and for placebo 22.7 mg/dl (16.5 %). In dosage group 2 there was an increase of approximately 26.4 mg/dl = 17.4 % (verum) and approximately 31.0 mg/dl = 20.7 % (placebo). The last named value was (as with the total cholesterol) again by subject Nr. 37 (increase of approximately 81 mg/dl) strongly influenced. Without this value, the mean values at the 3rd examination were 179.4 ± 22.6 mg/dl, and the difference to the 1st examination (151.1 ± 19.8 for n = 19) was 28.3 mg/dl (18.7 %).

Therefore, in both dosage groups there was no difference between verum and placebo. It must also be mentioned that for none of the 80 subjects included was there a reduction in LDL serum cholesterol levels, in pre/post comparison.

HDL cholesterol (secondary objectives)

With a homogeneous original situation between verum and placebo ($p = 0.38$ relatively 0.28) the HDL values in the course of the study did not change or only changed minimally (range: - 0.3 to + 2.6 %). The HDL value of subject Nr. 37 was not notable.

Discussion

The changes in cholesterol parameters found in this study obviously have nothing to do with the effects of soy protein, but in the cases of total and HDL cholesterol lead back to laboratory practice and biological variation. The LDL increased for 78 of 80 subjects (2 subjects had no pre/post change), cannot be explained without further investigation. Because the subjects were in age, weight, concurrent diseases, concurrent medication, in lifestyle and in the original cholesterol parameters not much different, the unexpected effect could only be due to the production/preparation of the chocolate milk. The low fat milk (fat content approximately 0.3 %) was, together with the additional protein, heated to a high temperature. To clarify this possible cause, a follow up study is being performed, in which the subjects mix the protein into the milk themselves before use.

Regarding HDL cholesterols, it is consistent with the cited literature that no change is to be expected. Determination of isoflavon content in serum must follow a technique validation. The serum was therefore stored in a frozen state.

Conclusion

The study result showed no effect for soy proteins.

EXAMPLE 13

Clinical effect of ingesting preparations according to example 10

A study was performed to determine the clinical effects of ingesting the soy protein preparation described in EXAMPLE 10, with 20 trial subjects (7 female, 13 male, ages 29 to 70 years) with hypercholesterolemia (inclusion range: 5.2 – 7.8 mmol total cholesterol/l ~200 – 300 mg/dl). The 20 trial subjects each drank one liter of a preparation according to EXAMPLE 10 (25 g of soy protein) daily, for a period of 4 weeks, half a liter with the morning meal and half a liter with the evening meal.

Blood samples were taken from the trial subjects at the on-set of the experiment (week 0), at 2 weeks and at 4 weeks (end of the experiment), and total serum cholesterol, LDL cholesterol and HDL cholesterol determined. For the trial parameters the first and third examination comparisons are interpreted by means of the t-test for paired observation and description on the level of a 5% probability level.

The data were recorded according to GCP requirements with double data entry and simultaneous value comparison in dBase IV. The results are shown in the following table:

Table 14.

Examination	Total cholesterol	LDL cholesterol	HDL cholesterol
Week 0	252.4±26.3	164.7±22.8	58.0±14.9
Week 2	240.9±23.5	135.2±18.3	55.9±14.2
Week 4	241.6±20.9	148.4±19.0	56.1±14.5
Difference week 2-week 0	-11.5±21.3	-29.5±16.3	-2.1±8.2
p-value	0.031	<0.001	0.27
Difference (%) Week 2-week 0	-4.6	-17.9	-3.6
Difference week 4-week 0	-10.8±18.3	-16.3±16.9	-1.9±5.3
p-value	0.020	0.001	0.13
Difference (%) Week 4-week 0	-4.3	-9.9	-3.3

Cholesterol levels are in mg/dl unless otherwise indicated.

EXAMPLE 14

Comparison of the clinical effect of ingesting undenatured soy protein according to the present invention or a commercial ISP with a high isoflavone content.

Two Isolated soy protein products A (SuproSoy), and B (Undenatured ISP) were studied with a placebo C (casein) in a randomized placebo-controlled trial according GCP with respect to their lipid lowering effects.

Study details: The patients took for 8 weeks daily 25g of the 3 products in 2 dosages in the morning and in the evening. The products were dissolved in water. Intermediate visits occurred after 2 and 4 weeks.

Patients: 120 patients of both sexes (73 women and 47 men in the age of 32 to 70 were included if the fulfilled the inclusion criteria of 200-300mg/dl or 5.2-7.8 mmol/l total cholesterol.

Statistics: For the primary (total cholesterol) and secondary parameters (LDL- and HDLcholesterol) the mean differences between the first and 4th visit weretested using the group comparison analysis of variance.

Drop-outs: During the study the following drop outs occurred: Group A: 8 cases, group B: 10 cases and group C: 11 cases, from those only partial results are available. Therefore the analysis was done for the per protocol group, in addition a intention-to-treat analysis was performed.

Side effects: No severe side-effects occurred.

Description of patients: Included: 120 patients, finished per protocol: 91

Results: Of the protocol analysis:
The mean age was 55.1 years

weight: the weight increase during the study was 0.2-0.6 kg

1) total cholesterol:

Difference visit 4 vs visit 1:

A: - 12.8 mg/dl -5.0%
B: - 24.3 mg/dl -9.4%
C: + 1.1 mg/dl +0.4%

Percentage changes Active vs Placebo:

A-C: -5.4%
B-C: -9.8%

Significances:

A:B O.017
A:C 0.013
B:C 0.001

2) LDL cholesterol

Difference visit 4 vs visit 1:

A: -12.3 mg/dl - 7.5%
B: -19.4 mg/dl -11.8%
C: - 5.8 mg/dl - 3.6%

Percentages changes Active vs Placebo:

A-C: 3.9 %
B-C: 8.2 %

Significances:

A:B 0.081

A:C 0.159

B:C 0.006

3) HDL cholesterol

No significant changes occurred

Summary:

The new ISP formulation of Nutri Pharma has proven to be significantly more effective to reduce total cholesterol than SuproSoy ISP, LDL cholesterol was significantly lowered with Nutri Pharma´s ISP but not for SuproSoy, in 91 patients with hypercholesterolemia in 8 weeks in a randomized placebo-controlled trial.

The percentage improvement of total cholesterol of Nutri Pharmas ISP was 9.8% compared to SuproSoy ISP with 5.4%, an improvement of over 80%.

The improvement of LDL cholesterol was 8.2% for Nutri Pharmas ISP compared to 3.9% for SuproSoy, an improvement of over 100%.

Experimental

Analytical techniques applied in the above examples are outlined in the following:

Protein solubility

2g of isolate was dissolved in 100ml water. The pH was in all cases adjusted to 7.0. Samples were mixed for 1 hour. For total protein samples were collected and for soluble protein, centrifuged at 32,000 rpm, or 18,000 rpm in another centrifuge in the Chemistry Department. The supernatant was used for soluble proteins and solubility was determined as: Dissolved protein x 100% / total protein.

The method is described in detail by Renkema, J.M.S in J. Biochemistry 79,(3) 223-230, (1976).

DSC

Samples for DSC were prepared by weighing x mg of solids and adding 4x mg of water to give the equivalent of 5mg of protein in the DSC pans, and allowed to equilibrate for 60 minutes after mixing the two with a spatula. The total amount of wet sample used in each test is given in the table below. The samples were scanned in the temperature range of 25 to 150°C at a scanning rate of 10°C per minute.

Phytoestrogen Aglycone Analysis

Portions of the samples (50 mg) were extracted with aqueous ethanol at 60°C for 1h with shaking. Phytoestrogen glycone extracts were defatted with hexane and then hydrolysed to the aglycons with dilute HCL. The hydrolysed aglycons were extracted with ether. Analysis of aglycons was performed by liquid chromatography with isotope dilution mass spectrometric detection (LC-MS). All data are reagent blank and spike recovery corrected.

SDS-PAGE

Definitions for Densitometric Analysis:

Band :	Protein peak with a single lane
Volume:	Area under a peak
Peak(OD):	Optical Density or intensity/height of a peak
Band%:	% area of that peak/band in relation to total area of all the peaks/bands detected within a single lane. I have averaged the duplicate values of the corresponding lanes

MW(kd): Molecular weight obtained by using a calibration with standards of known values

Rf: Retardation Factor, which means the relative mobility of a peak/band within a lane. The top of the gel lane is 0, and the bottom of the lane is 1.00. This gives you a value for the position of a peak/band in the lane. So the 10 MW stds have individual Rf values, which are dependent on their molecular size. When you look at the tables, each of the Isolate or flour components have Rf values telling the mapped position within a lane, and a calculated MW in the next column. Both these parameters help you to identify the peak/band and compare them to the corresponding components within the other lanes.

Method

10mg of each powdered samples was extracted using a vortex mixer for 1 hour with 1ml of 0.086M Tris, 0.09M Glycine, 0.004M EDTA, 8M Urea pH8.0 (Sorgentini *et al.*, 1991). After I hour, an additional 1 ml of the same extraction solution was added and mixed. Extracts were mixed with Laemmli buffer in total 0.4 ml. The dispersions were boiled for 5 minutes and spun in a micro-centrifuge for 10 minutes. This procedure results in the solubilisation of almost all of the protein fraction within the sample.

10µl of each sample extract was loaded onto all gels. SDS-PAGE gels were run using the BioRad Mini-Protean II system. These are 8 x 10 cm gels containing a 7.5-25% polyacrylamide linear gradient in 0.38 M Tris-Cl, pH 8.8 buffer. The method, including gel and tank buffer composition, is based on that reported by U.K. Laemmli (1970, Nature **227**, 680). The gels were stained using Brilliant Blue G-Colloidal Concentrate (Sigma Chemical Company), according to the method of Neuhoff, *et al.*, 1988, Electrophoresis **9**, 255.

The gels were documented as tif images using a video camera documentation system. The densitometric analysis was performed with the Phoretix 1D Advanced software. A 14-step Optical Density Calibration strip was included alongside each gel image, to convert pixel readings of band intensities to optical density, and to correct for variations in the image capture process. A background subtraction step is included within the densitometric analysis and molecular weights of protein bands are determined using a calibration with standard protein markers run on each gel.

Over-loading distorts the migration of protein bands and lowers the apparent MW value of the protein. Peak height is expressed in Optical Density (OD) units, and Lane % is the area of a particular band relative to the total area of all the bands in that lane, i.e. a percentage of the total protein fraction.

CLAIMS

1. A processed soy protein product, in which more than 84 % of the total protein is soluble in water.

2. A processed soy protein product, in which the denaturation enthalpy for the 7S globular proteins is at least 1.0 J/g protein.

3. A processed soy protein product, in which the content of intact 7S subunits (α + α' + β) and 11S subunits (A + B) constitute more than 62 % of the total protein content.

4. A processed soy protein product, in which the content of intact 7S subunits (α+ α' + β) constitute more than 31 % of the total protein content.

5. A processed soy protein product, in which the content of intact 7S subunit α subunit constitute more than 10 % of the total protein content.

6. A processed soy protein product, in which the content of intact 7S subunit α' subunit constitute more than 8 % of the total protein content.

7. A processed soy protein product according to any of claims 1 to 6 wherein the protein content is more than 60 % of dry matter.

8. A processed soy protein product according to any of claims 1 to 6 wherein the protein content is more than 70 % of dry matter.

9. A processed soy protein product according to any of claims 1 to 6 wherein the protein content is more than 80 % of dry matter.

10. A processed soy protein product according to any of claims 1 to 6 wherein the protein content is more than 90% of dry matter.

11. A processed soy protein product according to any of claims 1-10 in which the content of active trypsin inhibitor has been reduced to less than 20% of the amount present in the original soy bean material.

12. A processed soy protein product according to any of claims 1-11 in which the arginine:lysine ratio is at least 1.

13. A processed soy protein product according to any of claims 1-12 in which the isoflavone content as aglycons, is at least 0.1 % (w/w) of the total soy protein.

14. A process for the manufacture of a soy protein product comprising the steps of

a) removal or inactivation of trypsin inhibitors from a soybean-based raw material,
b) aqeuous extraction of soy proteins from the soybean-based raw material to aqueous solution at a concentration of less than 40% (w/v) of the soy-based raw material at a pH of 6-9.5 and a temperature below 60°C,
c) reducing the pH and the temperature of the aqueous protein solution from b), thereby obtaining a protein curd which is separated from the aqueous solution and
d) drying the protein curd.

15. A process according to claim 14 in which the inactivation of the trypsin inhibitors in step a), comprises a combination with the NADP/thioredoxin system or a combination of an initial heat treatment with small quantities of sodium sulphite.

16. A process according to any of claims 14 or 15 in which the aqeuous extraction in step b) is carried out with water or a 0.1 M aqueous solution of $NaCl/CaCl_2$.

17. A process according to any of claims 14-16 in which the aqeuous extraction in step b) is carried out for less than 180 minutes.

18. A process according to any of claims 14-17 in which a second extraction is carried out to produce a second extract.

19. A process according to any of claims 14-18 in which the pH of the protein extract or extracts is reduced to 4.8-5.5 and the temperature is reduced to below 25°C for a less than 3,5 hours in step c).

20. A process according to any of claims 14-19 which comprise in step c) a further step of reducing the pH of the protein extract or extracts to 2.5-4.5 and kept at a temperature below 10°C for less than 2 hours.

21. A process according to any of claims 14-20 which comprise in step c) a further step of neutralising the curd to a pH of 6-8.

22. A process according to any of claims 14-21 in which protein curd is dried in step d) by freeze or spray drying, vacuum drying, drum drying, air drying in a zigzag drier, on continous belt driers, cabinet tray driers or rotational driers.

23. A process according to 22 in which the spray drying is carried out at an air temperature below 60°C.

24. A process according to any of claims 14-23 in which the product temperature does not exceed 60°C.

25. A process according to any of claims 14-24 in which the soy proteins are furthermore partially degraded.

26. A soy protein product produced by a process according to any of claims 14-25.

27. A soy protein product produced by a process according to any of claims 14-26 wherein the protein content is more than 60% of dry matter.

28. A soy protein product produced by a process according to any of claims 14-26 wherein the protein content is more than 70% of dry matter.

29. A soy protein product produced by a process according to any of claims 14-26 wherein the protein content is more than 80% of dry matter.

30. A soy protein product produced by a process according to any of claims 14-26 wherein the protein content is more than 90% of dry matter.

31. The use of a soy protein product according to any of claims 1-13 and 26-30 as a food ingredient.

32. Use according to claim 31 wherein added soy protein comprises 0-10 wt% of the dry matter in the food.

33. Use according to claim 31 wherein added soy protein comprises more than 10 wt% of the dry matter in the food.

34. Use according to any of claims 31-33 for food products comprising soy particles with a size between 500 to 10000 microns and a bulk density of 200 to 1300 gram per liter.

35. Use according to any of claims 31-24 for fermented food products.

36. Use according to any of claims 31-24 of food products comprising bread.

37. Use according to any of claims 31-24 of food products comprising bars.

38. Use according to any of claims 31-24 of food products comprising drinks.

39. Use of a soy protein product according to any of claims 1-13 and 26-30 in a food product for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease.

40. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a food product for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease.

41. Use according to claim 39 or 40 where said cardiovascular disease is selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension

42. Use according to claim 41 where said cardiovascular disease is arteriosclerosis.

43. Use according to claim 41 where said cardiovascular disease is atherosclerosis.

44. Use according to any of claims 39 to 43 where the food product is effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of HDL-cholesterol and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation in a subject and/or in reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction.

45. Use of a soy protein product according to any of claims 1-13 and 26-30 in a food product for treating a subject suffering from type 2 diabetes and/or the metabolic syndrome.

46. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a food product for treating a subject suffering from type 2 diabetes and/or the metabolic syndrome.

47. Use of a soy protein product according to any of claims 1-13 and 26-30 in a food product for treating a cardiovascular disease in a diabetic subject.

48. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a food product for treating a cardiovascular disease in a diabetic subject.

49. Use according to any of claims 45 to 48 where the food product is effective in lowering serum levels of glucose and/or of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or glucose tolerance and/or insulin sensitivity and/or alleviating impaired glucose tolerance and/or insulin secretory failure and/or improving insulin secretion.

50. Use of a soy protein product according to any of claims 1-13 and 26-30 in a food product for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a subject.

51. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a food product for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a subject.

52. Use according to claim 50 or 51, where the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

53. Use according to any of claims 50-52 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating asthma.

54. Use according to any of claims 50-52 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways.

55. Use according to any of claims 50-52 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating bronchoconstriction.

56. Use according to any of claims 50-52 where the food product is effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma.

57. Use according to any of claims 50-52 where the food product is effective in increasing FEV_1 as measured by forced expiratory volume in the first second of expiration.

58. Use of a soy protein product according to any of claims 1-13 and 26-30 as a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a subject.

59. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a subject.

60. Use of a soy protein product according to any of claims 1-13 and 26-30 as a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a diabetic subject.

61. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a diabetic subject.

62. 63. Use of a soy protein product according to any of claims 1-13 and 26-30 as a nutritional preparation for alleviating a pulmonary condition.

63. 64. Use of a soy protein product according to any of claims 1-13 and 26-30 in the manufacture of a nutritional preparation for alleviating a pulmonary condition.

64. Use according to any of claims 56 to 61 where the nutritional preparation is in the form of a dietary supplement.

65. Use of a soy protein product according to any of claims 1-13 and 26-30 as a partial or total diet for an overweight subject.

66. Use of a soy protein product according to any of claims 1-13 and 26-30 as a partial or total diet for an overweight subject suffering from an arteriosclerotic condition.

67. Use of a soy protein product according to any of claims 1-13 and 26-30 as a partial or total diet for an overweight subject suffering from a diabetic condition.

68. Use of a soy protein product according to any of claims 1-13 and 26-30 for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease in the human or animal body in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection.

69. Use according to claim 68 where the cardiovascular disease is selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension.

70. Use according to claim 69 where the cardiovascular disease is arteriosclerosis.

71. Use according to claim 69 where the cardiovascular disease is atherosclerosis.

72. Use of a soy protein product according to any of claims 1-13 and 26-30 for preventing and/or treating type 2 diabetes in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial

infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

73. Use of a soy protein product according to any of claims 1-13 and 26-30 for preventing and/or treating the metabolic syndrome in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction.

74. Use of a soy protein product according to any of claims 1-13 and 26-30 for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a human or animal body in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or small airways diseases and/or asthma and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV_1 of a subject as measured by forced expiratory volume in the first second of expiration.

75. Use according to claim 74 where the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

76. Use according to claim 74 or 75 in an amount effective in preventing, treating, prophylactically treating and/or alleviating asthma.

77. Use according to claim 74 or 75 in an amount effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma.

78. Use according to claim 74 or 75 in an amount effective in increasing FEV_1 of a subject as measured by forced expiratory volume in the first second of expiration.

79. Use according to claim 74 or 75 in an amount effective in reducing inflammation of the airways.

80. A method of preventing, treating, prophylactically treating and/or alleviating by therapy a cardiovascular disease in a human or animal body, said method comprising administration to said human or animal body of a soy protein product according to any of claims 1-13 and 26-30 in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction and/or alleviating the clinical condition of patients contracting a myocardial infection.

81. A method according to claim 80 wherein the cardiovascular disease is an arteriosclerotic condition of the human or animal body.

82. A method according to claim 80 wherein the cardiovascular diseases is selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension.

83. A method according to claim 82 wherein the cardiovascular disease is arteriosclerosis.

84. A method according to claim 82 wherein the cardiovascular disease is atherosclerosis.

85. Method of preventing and/or treating by therapy type 2 diabetes in a human or animal body, said method comprising administration to said human or animal body of a soy protein product according to any of claims 1-13 and 26-30 in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a diabetic subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a diabetic subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

86. Method of preventing and/or treating by therapy the metabolic syndrome in a human or animal body, said method comprising administration to said human or animal body of a soy protein product according to any of claims 1-13 and 26-30 in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina

pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction.

87. A method of preventing, treating, prophylactically treating and/or alleviating by therapy a pulmonary disease in a human or animal body, said method comprising administration to said human or animal body of a soy protein product according to any of claims 1-13 and 26-30 in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or asthma and/or small airways diseases and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV_1 of a subject as measured by forced expiratory volume in the first second of expiration.

88. A method according to claim 87 wherein the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

89. A method according to claim 87 or 88 wherein the soy protein product is effective in preventing, treating, prophylactically treating and/or alleviating asthma.

90. A method according to claim 87 or 88 wherein the soy protein product is effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma.

91. A method according to claim 87 or 88 wherein the soy protein product is effective in increasing FEV_1 of a subject as measured by forced expiratory volume in the first second of expiration.

92. A method according to claim 87 or 88 wherein the soy protein product is effective in reducing inflammation of the airways.

FIGURE 1 Flow-sheet, production procedure: