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Fortsættes ...

PEA PROTEIN COMPOSITION WITH IMPROVED NUTRITIONAL QUALITY

Field of the invention

The present invention relates to a pea protein composition having improved nutritional quality, in particular its PDCAAS, to the process for preparing same and to the use of this composition in a food or pharmaceutical composition.

Background art

Daily requirements for proteins are between 12 and 20 % of food intake. These proteins are provided equally by products of animal origin (meat, fish, eggs, dairy products) and by plant-based food (cereals, leguminous plants, seaweed).

However, in developed countries, protein intake is predominantly in the form of proteins of animal origin. And yet, numerous studies show that excessive consumption of proteins of animal origin to the detriment of plant proteins is one of the causes of increases in cancer and cardiovascular diseases.

Moreover, animal proteins have many drawbacks, both in terms of their allergenicity, notably regarding proteins from milk or eggs, and in environmental terms, in connection with the harmful effects of intensive farming.

Thus, there is an increasing demand from manufacturers for proteins of plant origin having beneficial nutritional and functional properties without, however, having the disadvantages of compounds of animal origin.

Since the 1970s, the pea is the seed legume which has been the most developed in Europe, predominantly in France, especially as a protein resource intended for animal and human food. Pea contains approximately 27 % by weight of protein. The term “pea” is considered here in its broadest accepted use and includes, in particular, all the wild varieties of “smooth pea” and all the mutant varieties of “smooth pea” and “wrinkled pea”, regardless of the uses for which said varieties are usually intended (human food, animal feed and/or other uses).

International application WO 2012/047252 (D1) describes that a pea protein isolate has a content of 90 to 95 % total proteins with respect to the weight of the dry extract. This document also recalls that the soluble proteins contained in pea consist of 65 to 80 % globulins and 20 to 35 % albumins.

The document "Pea protein" (XP002788006, D2) describes a commercial pea protein isolate which has an average protein content of 83 % and which can be added to a beverage.

The document Food Research International. 2015, 76, 160-167 (D3) addresses the characterization of proteins in pea protein isolates and pea flours that can be used for the preparation of food products.

The document "Roquette Nutralys" (XP002788026, D4)describes the product Nutralys® sold by the applicant company and that can be used in specialized nutrition or food.

The document J. Sci. Food Agric 1990, 53, 107-110 (D5) addresses the evaluation of the impact of sieving on the protein composition of a pea flour. Document WO 2007/017572 (D6) describes a pea protein composition having a dry protein content of at least 60 % by weight, preferably between 60 and 95 % by weight, and an antitrypsin factor content between 2 and 5.5 TIU/mg.

Despite their undeniable qualities, pea proteins have lower nutritional power than animal and soy proteins. Indeed, several studies have demonstrated that their PDCAAS is less than 1 (see for example "Aliments à base de soja - source de protéines de haute qualité", ENSA, August 2015). PDCAAS, or protein digestibility-corrected amino acid score, is used to evaluate the quality of proteins as a function of two criteria: the essential amino acid requirements of humans and the digestibility of proteins. Since 1989, the WHO and FAO recommend using this method to determine protein quality. It is acknowledged that a perfect protein from a nutritional point of view should obtain a score of 1 (or 100 % depending on the expression of the result).

Many papers and studies have attempted to overcome this shortcoming of pea protein. The PDCAAS of less than 1 is due in part at least to the presence of antinutritional factors co-extracted with the pea protein. Mention may be made, for example, of the antitrypsin factors which, by inhibiting digestive trypsin, disrupt the digestion of the protein. The Applicant has thus developed a process for preparing a pea protein composition comprising predominantly globulins and having a low content of antinutritional factors (see WO2007017572). The PDCAAS of this composition is clearly improved, reaching a value of 93 % (or 0.93) (see "Vegetable Protein: A winner?", C.Lefranc-Millot, 2014). In order to achieve the PDCAAS value of 1, a well-known solution consists in adding to this composition a mixture of proteins extracted from wheat (see also in "Vegetable Protein: A winner?", C.Lefranc-Millot, 2014). This elegant solution nevertheless has several disadvantages, such as the need to sequence several steps in order to obtain the desired protein and the use of wheat proteins likely to contain traces of gluten.

Furthermore, if it is possible to envisage obtaining a protein with a PDCAAS of 1 by mixing proteins other than pea, a mixture is obtained which loses the functional properties of said pea protein, in particular its emulsifying capacity. The preservation of an emulsifying power, while increasing its PDCAAS, in order to be able to easily be formulated in food recipes is a major requirement for certain industrial applications, in particular in the food, pharmaceutical and cosmetic fields.

There is therefore still an unsatisfied need for a protein extracted only from pea, whose nutritional quality is equivalent to those of animal proteins and whose functional properties, in particular its emulsifying activity, remain high

It is to the Applicant's credit to have undertaken work to meet these needs and to have developed the present invention. In particular, the Applicant has developed a composition having a PDCAAS of 1 comprising only proteins extracted from pea by mixing the protein composition of application WO2007017572, the pea globulin fraction, with an albumin-rich pea extract. This solution would not have been considered by a person skilled in the art since albumin-rich pea extracts also exhibit a high antitrypsin factor content. The antitrypsin factors are albumins with a

low molecular weight, from 8 to 10 kDa, which are capable of irreversibly binding to active sites of trypsin. They thus prevent the gastrointestinal hydrolysis of proteins ingested by the proteases and therefore reduce the digestibility of proteins and their PDCAAS. By managing to lower the antitrypsin factor content of albumin-rich pea extracts and combining them with globulin-rich pea extracts, the applicant was able to obtain a pea protein composition having excellent nutritional quality.

In addition, the mixture of the protein composition of application WO2007017572, the pea globulin fraction, with an albumin-rich pea extract is carried out:

- By using a particular albumin-rich pea extract, in that its emulsifying activity is greater than 600 ml of oil per gram of proteins, preferentially greater than 800 ml of oil per gram of proteins, even more preferentially greater than 1000 ml of oil per gram of proteins.

- And by performing this mixture by wet process then by drying the solution thus obtained, preferentially by atomization.

Detailed description

A first subject matter of the present invention is a pea protein composition comprising globulins and albumins characterized in that the dry extract of the composition:

- comprises at least 80 % by weight, preferably from 80 to 99 % by weight, more preferentially from 85 to 98 % by weight, from 90 to 97 % by weight, from 92 to 95 % by weight of proteins with respect to the weight of the dry extract;

- has a mass ratio of globulins to albumins of 65/35 to 85/15, preferably of 70/30 to 82/18, more preferentially of 75/25 to 80/20,

the albumins having an emulsifying activity greater than 600 ml of corn oil per gram of albumins,

and the dry extract of the composition has an antitrypsin factor content of 1 to 5 TIU/mg.

Preferably, the composition is characterized by an emulsifying activity greater than 300 ml of oil per gram of proteins, preferentially between 300 and 500 ml of oil per gram of proteins, preferentially between 350 and 450.

A second subject matter of the present invention is a process for preparing a pea protein composition according to the invention, comprising the following steps:

a) extracting the globulins and the albumins from pea to obtain a protein fraction;

b) separating the globulins from the albumins to obtain a globulin-enriched fraction and an albumin-enriched fraction;

c) reducing the antitrypsin factor content of the albumin-enriched fraction to obtain an albumin-enriched fraction treated with a process comprising the following steps:

c-i) performing a microfiltration or centrifugation of the albumin-enriched fraction to obtain a microfiltration permeate or a centrifugation supernatant, and

c-ii) performing an ultrafiltration of said microfiltration permeate or of said centrifugation supernatant in order to obtain an ultrafiltration retentate, corresponding to the treated albumin-enriched fraction;

d) adjusting the pH to a value between 6.5 and 7.5 and then heating the treated albumin-enriched fraction to a temperature between 130 °C and 150 °C, preferentially 140 °C, for a period between 5 and 15 seconds, preferentially 10 seconds in order to obtain a thermized albumin-enriched fraction, the emulsifying activity of which is greater than 600 ml of oil per gram of proteins, preferentially greater than 800 ml of oil per gram of proteins, even more preferentially greater than 1000 ml of oil per gram of proteins;

e) mixing the globulin-enriched fraction and the thermized albumin-enriched fraction in the presence of water so that the dry extract of the mixture has a mass ratio of globulins to albumins of 65/35 to 85/15, preferably of 70/30 to 82/18, more preferentially of 75/25 to 80/20.

f) drying the solution thus obtained.

Preferably, step d) is carried out by adjusting the pH between 6 and 8, preferentially between 6.5 and 7.5 and by heating between 130 °C and 150 °C, preferentially 140 °C, with a treatment time of between 5 and 15 seconds, preferentially 10 seconds.

In a preferred manner, step f) is carried out by atomization, preferentially by so-called “multiple-effect” atomization

A third subject matter of the present invention is the use of the pea protein composition according to the invention in a food or pharmaceutical composition.

The term “pea” should be understood in the present application as all the wild varieties of “smooth pea” and all the mutant varieties of “smooth pea” and “wrinkled pea”.

The term “protein” should be understood in the present application to mean the macromolecules formed from one or more polypeptide chains consisting of a sequence of amino acid residues bonded to one another by peptide bonds. In the particular context of pea proteins, the present invention relates more particularly to globulins (about 50-60 % by weight of the pea proteins) and albumins (20-25 % by weight of the pea proteins). Pea globulins are mainly subdivided into three sub-families: legumins, vicilins and convicilins. Pea albumins are mainly subdivided into two families, referred to as PA1 and PA2.

The term “antitrypsin factors” should be understood in the present application as all the compounds having an activity inhibiting digestive proteases, in particular trypsin. The method for calculating the antitrypsin factor content of the composition according to the invention is detailed in the examples of the present application.

The term “PDCAAS” should be understood in the present invention as the Protein Digestibility-Corrected Amino Acid Scoring or Score. This method is most commonly used nowadays to estimate the protein quality of food intended for human nutrition. The PDCAAS evaluates the quality of the proteins based upon two criteria: the essential amino acid requirements of humans according to the recommendations of the FAO and the digestibility of proteins. Since 1989, the WHO and FAO recommend using this method to determine protein quality. It is acknowledged that a perfect protein from a nutritional point of view should obtain a score of 1 (or 100 % depending on the expression of the result). The method for calculating the PDCAAS of the composition according to the invention is detailed in the examples of the present application.

The term “emulsifying activity” should be understood as the maximum amount of oil that can be dispersed in an aqueous solution containing a defined amount of emulsifier before the emulsion breaks or reverses phase (Sherman, P., 1995. A critique of some methods proposed for evaluating the emulsifying capacity and emulsion stabilizing performance of vegetable proteins. Ital. J. Food Sci., 1: 3). In order to quantify it, the Applicant has developed a test making it possible to quantify it easily, quickly and reproducibly. This term is also well-known under the term “Critical Micelle Concentration” or “CMC”.

The various subjects of the invention will be better understood in the following detailed description of the invention.

The composition that is the subject of the present invention is a pea protein composition which comprises globulins and albumins.

Hereinafter, the terms “proteins”, “globulins” and “albumins” respectively denote proteins, globulins and albumins extracted only from pea. Thus, the proteins, globulins and albumins extracted from a plant source other than pea or from an animal source are not encompassed by these terms. Globulins can be distinguished from albumins by various methods well known to those skilled in the art, especially by their solubility in water, with albumins being soluble in pure water

whereas globulins are only soluble in salt water. It is also possible to identify the albumins and globulins present in a mixture by electrophoresis or chromatography.

The dry extract of the composition according to the invention comprises at least 80 % by weight, preferably from 80 to 99 % by weight, more preferentially from 85 to 98 % by weight, from 90 to 97 % by weight, from 92 to 95 % by weight, of proteins relative to the weight of the dry extract. Any reference assay method for quantifying the protein content well known to those skilled in the art can be used. Preferably, the total nitrogen (in %/crude) is assayed, and the result is multiplied by the coefficient 6.25. This well-known methodology in the field of plant proteins is based on the observation that proteins contain on average 16 % nitrogen. Any dry matter assay method well known to those skilled in the art may also be used.

Furthermore, the dry extract of the composition according to the invention has a mass ratio of globulins to albumins of 65/35 to 85/15, preferably of 70/30 to 82/18, more preferentially of 75/25 to 80/20.

According to one particular embodiment, the albumins contained in the composition according to the invention consist essentially of albumins of PA1 and PA2 type and lectins. Thus, the percentage by weight of the antitrypsin factors relative to the weight of the proteins of the composition according to the invention is less than the percentage by weight of the antitrypsin factors relative to the weight of the proteins in the pea in the natural state. Preferably, the dry extract of the composition according to the invention has an antitrypsin factor content of 1 to 5 TIU/mg. The antitrypsin factor content may be measured especially according to the process described hereinafter.

The albumins of the composition according to the invention exhibit an emulsifying activity greater than 600, preferably greater than 800, more preferentially greater than 1000 ml of corn oil per gram of albumins.

“Emulsifying activity” is defined as the maximum amount of oil that can be dispersed in an aqueous solution containing a defined amount of emulsifier before the emulsion breaks or reverses phase (Sherman, 1995). In order to quantify it,

the Applicant has developed a test making it possible to quantify it easily, quickly and reproducibly. This method consists in implementing the following steps:

1. 0.2 g of the product sample is dispersed in 20 ml of water;

2. the solution is homogenized with an Ultraturax IKA T25 apparatus for 30 seconds at a speed of 9,500 rpm;

3. adding 20 ml of corn oil sold under the name AMPHORA by CARGILL with homogenization under the same conditions as the preceding step 2;

4. centrifugation for 5 minutes at 3100 g;

a. if a good emulsion is obtained, the test is repeated at step 1, increasing the quantities of water and corn oil by 50 %;

b. if a bad emulsion is obtained, for example a phase shift, the test is repeated at step 1, reducing the quantities of water and corn oil by 50 %.

The maximum amount of oil (Qmax in ml) that can be emulsified is thus determined iteratively.

The emulsifying activity is therefore the maximum amount of corn oil that can be emulsified per gram of product.

$$\text{Emulsifying activity} = (Qmax / 0.2)*100$$

Albumins having an emulsifying activity greater than 600 ml of corn oil per gram of albumins can be obtained especially by heating a protein fraction comprising albumins as described in step d) of the method hereinafter.

According to one particular embodiment, the composition according to the invention has a PDCAAS equal to 1 (or 100 % depending on the expression of the results). Indeed, the low antitrypsin factor content of the composition according to the invention gives it good digestibility. The mass ratio of the globulins to the albumins in the composition according to the invention further contributes to a good equilibrium of the amino acids. The presence of albumins makes it possible

especially to enrich the composition with sulfur-containing amino acids. Thus, the composition according to the invention has excellent nutritional quality.

The composition according to the invention can be obtained especially by the method for preparing a pea protein composition described hereinafter.

The method for preparing a pea protein composition that is the subject of the present invention comprises a step a) in which the globulins and the albumins are extracted from pea to obtain a protein fraction.

According to a preferred embodiment, in step a) the globulins and albumins are extracted from pea with a method comprising the following steps:

a-i) milling peas;

a-ii) introducing the milled peas into an aqueous solution so as to obtain a solid phase A suspended in a liquid phase B; and

a-iii) separating the liquid phase B, corresponding to the protein fraction, from the solid phase A.

Such a method is described especially in patent application EP1400537.

The peas used in step a-i) may have been previously subjected to steps that are well known to those skilled in the art, such as especially cleaning (removal of undesired particles such as stones, dead insects, soil residues, etc.) or even the removal of the external fibers of the peas (external cellulose hull) through a well-known step referred to as “dehulling”.

In step a-i), the peas may be milled in the absence of water (so-called “dry milling” process). According to an alternative embodiment, the peas may be milled in the presence of water (so-called “wet milling” method). In this case, step a-ii) is not carried out, since a solid phase A is obtained directly in suspension in a liquid phase B at the end of the wet milling step.

In step a-ii), the pH of the aqueous solution may be especially between 6.2 and 7 and the temperature of the aqueous solution may be especially between 5 and 20 °C.

Step a-iii) makes it possible to separate the liquid phase B from the solid phase A. The liquid phase B corresponds to the protein fraction and is also referred to as "soluble fraction". It contains the proteins, in particular the globulins and albumins, as well as other compounds soluble in the aqueous phase, in particular salts, amino acids and carbohydrates. Solid phase A for its part contains the pea fibers and starch.

Preferably, the liquid phase B and the solid phase A are separated by fractionation. Separation by fractionation may be carried out especially by means of centrifugal decanters or hydrocyclones.

The method according to the invention comprises a step b) wherein the globulins and the albumins are separated in order to obtain a globulin-enriched fraction and an albumin-enriched fraction.

The terms "globulin-enriched fraction" and "albumin-enriched fraction" mean a fraction having a percentage by weight of globulins, respectively albumins, relative to the weight of the proteins in said fraction which is greater than the percentage by weight of globulins, respectively albumins, relative to the weight of the proteins in the pea in the natural state. The enrichment therefore corresponds to the percentage increase in the proportion of globulins, respectively albumins, between the pea in the natural state and the enriched fraction. In particular, the enrichment of the enriched fraction is of at least 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, or 50 % relative to the pea in the natural state. The enrichment may especially be obtained by purification and/or concentration of the protein fractions of interest with globulins and with albumins. These fractions, according to the method applied, may be in hydrated or dry form.

Step b) can especially be implemented by precipitation of the proteins at their isoelectric pH or by membrane separation, for example by ultrafiltration.

According to a preferred embodiment, in step b), the globulins are separated from the albumins with a method comprising the following steps:

b-i) flocculating globulins of the protein fraction to obtain a solid phase C suspended in a liquid phase D; and

b-ii) separating the liquid phase D, containing the albumins, from the solid phase C, containing the globulins.

Preferably, in step b-i) the flocculation of the globulins is carried out by bringing the pH of the protein fraction to the isoelectric pH of the globulins. More preferentially, the pH of the protein fraction is adjusted to 4.5. The flocculation of the proteins can be carried out especially by heating the protein fraction to a temperature of 30 to 70 °C, in particular for 5 to 30 minutes, more particularly between 10 and 30 minutes.

In step b-ii), the solid phase C (also referred to as “floc”) is preferably separated from the liquid phase D by centrifugation. The solid phase C corresponds to the globulin-enriched fraction and comprises the globulins. The liquid phase D corresponds to the albumin-enriched fraction and comprises the albumins as well as other compounds soluble in the aqueous phase, in particular salts, amino acids and carbohydrates.

The method according to the invention comprises a step c) wherein the antitrypsin factor content of the albumin-enriched fraction is reduced in order to obtain a treated albumin-enriched fraction.

According to a preferred embodiment, in step c), the treated albumin-enriched fraction has an antitrypsin factor content in the dry extract of 20 to 80 TIU/mg, preferably of 30 to 50 TIU/mg.

In step c), the antitrypsin factor content of the albumin-enriched fraction is reduced with a method comprising the following steps:

c-i) performing a microfiltration or centrifugation of the albumin-enriched fraction to obtain a microfiltration permeate or a centrifugation supernatant, and

c-ii) performing an ultrafiltration of said microfiltration permeate or of said centrifugation supernatant in order to obtain an ultrafiltration retentate, corresponding to the treated albumin-enriched fraction.

The microfiltration of step c-i) leads to a permeate and a microfiltration retentate. It is the permeate that is then treated in the subsequent step c-ii) of ultrafiltration. The centrifugation of step c-i) leads to a supernatant and a centrifugation sediment. It is the supernatant that is then treated in the subsequent step c-ii) of ultrafiltration.

When step c-i) is a microfiltration, it is preferentially a cross-flow membrane microfiltration. More particularly, cross-flow microfiltration is preferentially performed with a ceramic membrane having a porosity of 0.01 µm to 1 µm, preferentially 0.05 µm to 0.5 µm.

The ultrafiltration step c-ii) is carried out on the microfiltration permeate or on the centrifugation supernatant. It makes it possible to obtain, on the one hand, an ultrafiltration retentate rich in albumins, and on the other hand a permeate rich in salts, in amino acids and in carbohydrates. More particularly, it is recommended to perform ultrafiltration with a membrane having a cut-off between 0.1 and 0.5 µm, the transmembrane pressure being kept below 4 bars.

The method according to the invention comprises a step d) wherein the treated albumin-enriched fraction is subjected to a pH adjustment and then to heating in order to obtain a thermized albumin-enriched fraction. This step makes it possible especially to obtain albumins having an emulsifying activity greater than 600 ml of corn oil per gram of albumins.

In step d) the pH of the treated albumin-enriched fraction is adjusted to a value between 6 and 8, preferably between 6.5 and 7.5. The pH of the treated albumin-enriched fraction may especially be adjusted by adding a base selected from sodium hydroxide, potassium hydroxide or ammonia, preferentially sodium hydroxide. It should be noted that the use of carbonate is to be avoided since it has a detrimental action on the taste of the albumin fraction obtained.

Heating the treated albumin-enriched fraction after the pH adjustment is a heating of UHT type, that is heating at a very high temperature for a short time.

In step d), the treated albumin-enriched fraction is heated at a temperature between 130 °C and 150 °C, preferentially 140 °C, for a period between 5 and 15 seconds, preferentially 10 seconds.

The method according to the invention comprises a step e) wherein the globulin-enriched fraction and the thermized albumin-enriched fraction are mixed so that the dry extract of the mixture has a mass ratio of globulins to albumins of 65/35 to 85/15, preferably of 70/30 to 82/18, more preferentially of 75/25 to 80/20.

The globulin-enriched fraction and the thermized albumin-enriched fraction are mixed in a wet medium and said mixture is then dried. The mixing in a wet medium can be carried out especially in any container suitable for this purpose, preferably equipped with a suitable stirring system such as a mobile shaft equipped with blades or turbines. After obtaining a homogeneous mixture, the latter is dried using techniques that are well-known to those skilled in the art, such as atomization, preferentially so-called “multiple-effect” atomization, or else lyophilization.

The method according to the invention may further comprise one or more optional steps before and/or after one of the steps a), b), c), d) or e). According to one particular embodiment, the method according to the invention may comprise a step of enzymatic hydrolysis of the globulin-enriched protein fraction and/or of the treated albumin-enriched protein fraction between step c) and step e). The type of enzyme used in the enzymatic hydrolysis reaction is preferably an enzyme from the protease group.

Without being bound by any theory, the applicant noticed that it is the choice

- of a particular fraction rich in albumins in that its emulsifying activity is greater than 600 ml of oil per gram of proteins, preferentially greater than 800 ml of oil per gram of proteins, even more preferentially greater than 1000 ml of oil per gram of proteins

- then mixing it by wet process with a globulin-rich fraction followed by drying the solution thus obtained, preferentially by atomization

which makes it possible to obtain the unique properties of the composition that is the matter of the present invention.

The present invention also relates to the use of the pea protein composition according to the invention in a food or pharmaceutical composition.

Indeed, because of its excellent nutritional quality and its low allergenicity, such a pea protein composition is of particular interest in numerous industrial applications, in particular in the agri-food or pharmaceuticals industry, and in animal feed.

"Food composition" is intended to mean a composition intended for human or animal food. The term food composition encompasses food products and food supplements. "Pharmaceutical composition" is intended to mean a composition intended for therapeutic use.

The following examples help to illustrate the application, but do not limit its scope.

Example 1: Production of pea flour and fractions enriched with pea globulins and albumins, respectively.

Pea flour is initially prepared by milling dehulled field peas on an ALPINE hammer mill equipped with a 100 µm grid. This flour will be referred to as "pea flour".

300 kg of pea flour with a solids content of 87 % by weight are then soaked in water at a final concentration of 25 % by weight of solids, at a pH of 6.5. 1044 kg of flour suspension containing 25 % by weight of solids (thus 261 kg of dry flour) are then introduced with 500 kg of water into a hydrocyclone array formed of 14 stages. It is fed the flour suspension on stage No. 5. This separation leads to the obtaining of a light phase which corresponds to the output of stage No 1. It consists of a mixture of proteins, fibers and solubles. This light phase at the hydrocyclone outlet contains as a mixture (142 kg of solids in total): the fibers (about 14.8 % by weight, that is 21 kg of solids), the proteins (about 42.8 % by weight, that is 60.8 kg of solids) and the solubles (about 42.4 % by weight, that is

60.2 kg of solids). This fraction has a solids content of 11.4 % by weight. The fibers are separated out on WESTFALIA decanter centrifuges used in an industrial potato starch processing unit. The light phase at the outlet of the centrifugal decanter contains a mixture of proteins and of solubles, while the heavy phase contains the pea fibers. The heavy phase contains 105 kg of fibers with a solids content of 20 %. It is noted that virtually all of the fibers are indeed found in this fraction. The protein-and-solubles fraction contains 1142 kg of a dissolved mixture of soluble matter and proteins (6 % solids fraction). The proteins are flocculated at their isoelectric point by adjusting the light phase at the outlet of the decanter centrifuge to a pH of 4.5 and heating to 50 °C. The proteins thus flocculated are left for 10 minutes in a maturation tank. After precipitation of the proteins, centrifugal decantation is carried out, which makes it possible to recover the sediment containing 56 kg of proteins (86 % of N x 6.25 to dry) at 20 % by weight of solids and a supernatant containing, inter alia, the protein fraction containing the albumins. This sediment corresponds to the globulin-enriched protein fraction and will be referred to as “globulin-enriched fraction”. The supernatant corresponds to the albumin-enriched protein fraction and will be referred to as “albumin-enriched fraction”.

The refining of the albumin-enriched fraction is then carried out. Its pH is adjusted to 7.0 by adding 50 % sodium hydroxide. The temperature of the suspension thus obtained was brought to 70 °C. The solution is pumped through a microfiltration unit equipped with Inside Ceram® type ceramic membranes with a cut-off of 0.14 µm (19 channels of 4.5 mm). Throughout the filtration process, the temperature is regulated at 60 °C and the transmembrane pressure is maintained at a value between 0.4 and 0.6 bar. 707 liters of microfiltration permeate and 1768 liters of microfiltration retentate are thus recovered. 550 liters of the permeate are pumped through an ultrafiltration unit. The ultrafiltration unit is equipped with KERASEP® BX type ceramic membranes marketed by NOVASEP and having a cut-off of 15 kDa (7 channels each 6 mm). Throughout the filtration process, the temperature is regulated at 60 °C and the transmembrane pressure is maintained at a value between 1 and 3 bar. 467 liters of ultrafiltration permeate and 33 liters of retentate containing 75 % by weight of proteins at 7.2 % by weight

of solids are thus recovered. This ultrafiltration retentate corresponds to the treated albumin-enriched protein fraction and will be referred to as “treated albumin-enriched fraction”.

The pH of the albumin-enriched fraction is then rectified under stirring to pH 6.8 by adding 50 % sodium hydroxide solution. A UHT thermal treatment is then applied by passing the treated albumin-enriched fraction through a VOMATEC skid, at a temperature of 140 °C for a contact time of about ten seconds, then by flashing under vacuum at about 90 °C. The final product will be referred to as “thermized albumin-enriched fraction”.

Example 2: Preparation of pea protein compositions according to the invention

A stainless-steel tank equipped with a motorized stirrer is used. 1.89 kg of “globulin-enriched fraction” and 2 kg of “thermized albumin-enriched fraction” are introduced into this tank. This mixture makes it possible to obtain a ratio expressed as a relative percentage of dry matter between the “globulin-enriched fraction” and the “thermized albumin-enriched fraction” of 75/25. The motorized stirrer is then started and the product is homogenized for 15 to 30 minutes. The mixture is then sent to a single-acting atomization tower in order to be dried. A powder titrating 95 % by weight of solids is thus recovered. The product will be referred to as “75/25 pea protein composition”.

The preceding stainless-steel tank equipped with a motorized stirrer is used again. 2.5 kg of “globulin-enriched fraction” and 2 kg of “thermized albumin-enriched fraction” are introduced into this tank. This mixture makes it possible to obtain a ratio expressed as a relative percentage of dry matter between the “globulin-enriched fraction” and the “thermized albumin-enriched fraction” of 80/20. The motorized stirrer is then started and the product is homogenized for 15 to 30 minutes. The mixture is then sent to a single-acting atomization tower in order to be dried. A powder titrating 96 % by weight of solids is thus recovered. The product will be referred to as “80/20 pea protein composition”.

Example 2 bis: Preparation of pea protein compositions outside the invention, using "treated albumin-enriched fraction"

A stainless-steel tank equipped with a motorized stirrer is used. 1.89 kg of "globulin-enriched fraction" and 2 kg of "treated albumin-enriched fraction" are introduced into this tank. As described hereinbefore in example 2, this fraction is not neutralized to 6.8 and does not undergo UHT treatment. This mixture makes it possible to obtain a ratio expressed as a relative percentage of dry matter between the "globulin-enriched fraction" and the "treated albumin-enriched fraction" of 75/25. The motorized stirrer is then started and the product is homogenized for 15 to 30 minutes. The mixture is then sent to a single-acting atomization tower in order to be dried. A powder titrating 95 % by weight of solids is thus recovered. The product will be referred to as "75/25 pea protein composition".

Example 3: Methodology for calculating digestibility and PDCAAS

The measurement of protein digestibility in rats is described in the following FAO article: "Protein Quality Evaluation. Report of a Joint FAO/WHO Expert Consultation. Rome, Italy."

For this, the experimental food is composed of 10 % by weight of the proteins to be tested, 1 % by weight of a vitamin mixture AIN93, 3.5 % by weight of a mineral mixture AIN93, 0.2 % by weight of choline bitartrate, 5 % by weight of cellulose, 10 % by weight of corn oil. The food is completed to 100 % with corn starch.

This same food containing no protein will be used as a control of the test. For this, the proteins will be substituted by corn starch.

Growing Sprague-Dawley rats (weight between 50 and 70 g at the start of the test) will be hosted individually in metabolism cages at a temperature between 18 and 24 °C and a humidity between 40 and 70 %. The rats will be fed with a standard food at least 2 days before the start of the test. They are then fed with the experimental diets for a minimum period of 9 days consisting of a first period of acclimatization to the diets of 4 days followed by a period of 5 days of feces

collection. Water is given ad-libitum throughout the duration of the study. The feces thus collected daily are weighed, lyophilized for 24 hours and milled. The measurement of the nitrogen contained in the food and in the feces will make it possible to calculate protein digestibility. The measurement method used is the Kjeldahl method.

The ingested nitrogen and the excreted nitrogen is obtained by multiplying the food intake or the weight of the feces by the respective nitrogen contents. The level of basal nitrogen is obtained by measuring fecal nitrogen from animals fed by the diet not containing proteins.

The protein digestibility is obtained as follows:

Digestibility = [Ingested nitrogen - (Fecal nitrogen-Basal nitrogen)]/Ingested nitrogen * 100

The aminogram or total amino acid profile is established using the official method NF EN ISO13903:2005.

The amino acid score is determined as being the limiting essential amino acid relative to the reference profile determined in adults. To obtain this, the following ratio must be calculated: [mg of the amino acid contained in 1 g of the test protein]/[mg of the amino acid of the reference profile]. The smallest value represents the amino acid score.

The PDCAAS (protein digestibility-corrected amino acid score) is obtained by multiplying this limiting amino acid score by the protein digestibility determined in rats.

The reference profile in adults is described by the FAO in its article: “Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU expert consultation. Geneva, Switzerland. 2007.

Essential amino acid	g/g of protein
Histidine	15

Isoleucine	30
Leucine	59
Lysine	45
Methionine	16
Cysteine	6
Methionine + Cysteine	22
Phenylalanine + Tyrosine	30
Threonine	23
Tryptophan	6
Valine	39

Example 4: Methodology for measuring the antitrypsin factor content

The method for measuring the antitrypsin factor content consists in extracting the trypsin inhibitors by sodium hydroxide. Increasing volumes of the diluted sample are placed in contact with an excess of trypsin in the presence of N-alpha-benzoyl-DL-arginine p-nitroanilide (BAPNA) which will then be hydrolyzed in the form of p-nitroaniline, an absorbing compound at 410 nm. After blocking the reaction with acetic acid, the increase in coloration is measured with a spectrophotometer at 410 nm. The inhibitor content is then calculated from the rate of decrease in coloration. A trypsin unit is arbitrarily defined as the amount of enzyme necessary to cause an increase of 0.01 units of the absorbance at 410 nm for 10 ml of reaction mixture under the conditions of the AOCS Ba 12-75 method. The antitrypsin factor content is expressed as a trypsin inhibition unit per mg of sample to be tested (TIU/mg).

Example 5: Comparison of the various fractions

The table hereunder summarizes the analyses of the PDCAAS (according to example 3), the antitrypsin factor content (according to example 4) and the emulsifying capacity (according to the method explained in the description).

Reference	Antitrypsin factor content (TIU/mg)	Digestibility	PDCAAS	Emulsifying capacity (in ml of oil per g of proteins)

Pea flour	Not done	77.3	0.63	
Globulin-enriched fraction	3	97.3	0.93	
Treated albumin-enriched fraction	41	96.9	0.56	500
Thermized albumin-enriched fraction	9.8	97.1	Not done	1300
75/25 pea protein composition according to the invention	4	97.0	1	400
80/20 pea protein composition according to the invention **pea protein compositions outside the invention, using** **"treated albumin-enriched fraction"**	5	7.2	1	450 100

These examples clearly demonstrate the concentration of the antitrypsin factors in the albumin-enriched fraction, which therefore confirms the general teaching of the technical field indicating that these water-soluble fractions of low molecular weight are rich in antitrypsin factors. A person skilled in the art would therefore have been dissuaded from reusing this fraction with the intent of improving the PDCAAS of the globulin-enriched fraction. They would rather have chosen to use additional sources such as wheat proteins as taught in the prior art. The Applicant went beyond this teaching and has developed a solution making it possible to obtain a

protein having a PDCAAS equal to 1 based only on protein fractions derived from pea.

Example 8: Production of a “Ready To Drink” beverage

The various compositions are compared by their use in the formulation of so-called “Ready To Drink” beverages. The various components are summarized in the following table:

	100 % milk control	Conventional isolate control	Invention 1	Invention 2	Outside the invention
water	60				
Glucidex IT19 maltodextrin (ROQUETTE)	18.74	18.34	18.51	18.51	18.51
MPI Prodiet 85b milk protein	10.8	5.53	5.53	5.53	5.53
Nutralys S85F pea isolate	0	5.67	0	0	0
75/25 pea protein composition according to the invention	0	0	5.5	0	0
80/20 pea protein composition according to the invention	0	0	0	5.5	0
Pea protein composition outside the invention, using “treated albumin-enriched fraction”	0	0	0	0	5.5
rapeseed oil	3.78				

sucrose	3.4
sunflower oil	2.52
soy lecithin	0.4
vanilla flavor	0.36

The method for producing the beverages is as follows:

- Dry mixing the powders (proteins, maltodextrins and sucrose),
- Heating the water to 50 °C, adding the powders, dispersing with a Silverson stirrer for 30 min at 50 °C, 3500 rpm, adding vanilla aroma,
- Separately mixing and melting soy lecithin and oil at 50 °C,
- After 30 min, adding the lecithin/oil mixture to the aqueous solution, mixing under fast stirring for 5 min at 10,000 rpm,
- Heating to 75 °C
- Homogenizing at 200 bar in 2 steps
- Cooling and storing at 4 °C.

In order to quantify the quality of the emulsion in the beverages, the size of the particles is measured using a Particle Size Analyzer 3000 from MALVERN. The Dmode is the average size of the emulsified particles.

in microns	100 % milk control	Conventional isolate control	Invention 1	Invention 2	Outside the invention
D10	0.186	0.565	0.203		3.61
D50	0.546	43.1	0.482		9.34
D90	1.66	105	6.2		103
D mode	0.577	69.1	0.444		6.19
D 4.3	3.48	47.9	3.08		35.1

Only the beverages produced with the invention make it possible to obtain particles that are as well emulsified as with the reference 100 % milk control.

PATENTKRAV

1. Ærteproteinsammensætning, der omfatter globuliner og albuminer, **kendetegnet ved, at** tørstofekstraktet af sammensætningen:

– omfatter mindst 80 vægt-%, fortrinsvis fra 80 til 99 vægt-%, mere præferentielt fra 85 til 98 vægt-%, fra 90 til 97 vægt-%, fra 92 til 95 vægt-% proteiner i forhold til tørstofekstraktets vægt;

– har et masseforhold mellem globuliner og albuminer fra 65/35 til 85/15, fortrinsvis fra 70/30 til 82/18, mere præferentielt fra 75/25 til 80/20,

idet albuminerne har en emulgerende aktivitet, der er større end 600 ml majsolie pr. gram albuminer,

og tørstofekstraktet af sammensætningen har et indhold af antitryptiske faktorer på 1 til 5 TIU/mg.

2. Sammensætning ifølge krav 1, **kendetegnet ved, at** albuminerne har en emulgerende aktivitet, der er større end 800, præferentielt større end 1000 ml majsolie pr. gram albuminer.

3. Sammensætning ifølge et hvilket som helst af kravene 1 eller 2, **kendetegnet ved, at** den har en PDCAAS, der er lig med 1.

4. Fremgangsmåde til fremstilling af en ærteproteinsammensætning ifølge et hvilket som helst af de foregående krav, hvilken fremgangsmåde omfatter følgende trin:

a) ekstrahering af globulinerne og albuminerne fra ærter med henblik på at opnå en proteinfraktion;

b) separering af globulinerne fra albuminerne med henblik på at opnå en globulinberiget fraktion og en albuminberiget fraktion;

c) reduktion af indholdet af antitryptiske faktorer i den albuminberigede fraktion med henblik på at opnå en albuminberiget fraktion, som er behandlet med en fremgangsmåde, der omfatter følgende trin:

c-i) udførelse af en mikrofiltrering eller en centrifugering af den albuminberigede fraktion med henblik på at opnå et mikrofiltreringspermeat eller en centrifugeringssupernatant; og

c-ii) udførelse af en ultrafiltrering af mikrofiltreringspermeatet eller af centrifugeringssupernatanten med henblik på at opnå et ultrafiltreringsretentat, svarende til den behandlede albuminberigede fraktion;

d) indstilling af pH-værdien til en værdi mellem 6,5 og 7,5, derefter opvarmning af den behandlede albuminberigede fraktion ved en temperatur mellem 130 °C og 150 °C, fortrinsvis 140 °C, i et tidsrum på mellem 5 og 15 sekunder, præferentielt 10 sekunder, med henblik på at opnå en varmebehandlet albuminberiget fraktion med en emulgerende aktivitet, der er større end 600 ml majsolie pr. gram albuminer;

e) blanding, i nærværelse af vand, af den globulinberigede fraktion og den varmebehandlede albuminberigede fraktion, således at tørstofekstraktet af blandingen har et masseforhold mellem globuliner og albuminer fra 65/35 til 85/15, fortrinsvis 70/30 til 82/18, mere præferentielt fra 75/25 til 80/20;

f) tørring af den således opnåede opløsning.

5. Fremgangsmåde ifølge krav 4, **kendetegnet ved, at** globulinerne, i trin b), separeres fra albuminerne med en fremgangsmåde, der omfatter følgende trin:

b-i) flokkulering af globuliner i proteinfraktionen med henblik på at opnå en fast fase C, der er suspenderet i en flydende fase D; og

b-ii) separering af den flydende fase D, der indeholder albuminerne, fra den faste fase C, der indeholder globulinerne.

6. Fremgangsmåde ifølge et hvilket som helst af kravene 4 eller 5, **kendetegnet ved, at** den behandlede albuminberigede fraktion, i trin c), har et indhold af antitryptiske faktorer i tørstofekstraktet på 20 til 80 TIU/mg, fortrinsvis 30 til 50 TIU/mg.

7. Anvendelse af ærteproteinsammensætningen som defineret i et hvilket som helst af kravene 1 til 3 i en fødevaresammensætning eller en farmaceutisk sammensætning.