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

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
09 January 2025 (09.01.2025)

WIPO | PCT

(10) **International Publication Number**
WO 2025/008469 A1

(51) **International Patent Classification:**
A23J 1/14 (2006.01) *A23K 20/147* (2016.01)
A23J 3/34 (2006.01) *A23L 33/185* (2016.01)
A23K 10/14 (2016.01) *C12N 9/42* (2006.01)
A23K 10/30 (2016.01)

(21) **International Application Number:** PCT/EP2024/068892

(22) **International Filing Date:** 04 July 2024 (04.07.2024)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
23183682.6 05 July 2023 (05.07.2023) EP

(71) **Applicant: DSM IP ASSETS B.V.** [NL/NL]; Wilhelminasingel 39, 6221 BE Maastricht (NL).

(72) **Inventors: GUILONARD, Lambertus Jacobus Otto**; c/o DSM Intellectual Property, P.O. Box 4, 6100 AA ECHT (NL). **QIU, Weixi**; c/o DSM Intellectual Property, P.O. Box 4, 6100 AA ECHT (NL). **ZHA, Ying**; c/o DSM Intellectual Property P.O. Box 4, 6100 AA ECHT (NL).

(74) **Agent: DSM-FIRMENICH IP**; DSM-Firmenich AG Wurmisweg 576, 4303 Kaiseraugst (CH).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** AUGMENTING PROTEIN CONTENT OF RAPESEED PROTEIN MEAL

(57) **Abstract:** The invention relates to a process for augmenting the protein content of rapeseed protein meal, and to a composition useful in a process for improving the protein content of rapeseed protein meal.

WO 2025/008469 A1

AUGMENTING PROTEIN CONTENT OF RAPESEED PROTEIN MEAL

Field

The invention relates to a process for augmenting the protein content of plant protein meal, and to a composition useful in a process for improving the protein content of plant protein meal.

Background

Protein is a main feature of human and animal nutrition. This may be sourced from animals (e.g. meat, fish, egg, dairy) or from plants, such as vegetables. There is a general desire to reduce the amount of animal based protein. The use of egg protein is often undesirable. For example, due to problems with egg allergies, medical problems associated with cholesterol levels in eggs, religious restrictions/convictions, culinary preferences (such as, for example, a vegetarian or a vegan diet), cost fluctuations in the price of eggs, use of antibiotics and hormones in poultry production, and diseases associated with poultry (such as, for example, bird flu), the use of alternative proteins may be desired. The use of plant based protein in human nutrition is known, for example WO 2008/094434 discloses the use of wheat protein isolates as an alternative to the use of egg yolk protein in compositions. However, the use of wheat protein isolates may not be desirable for those with gluten allergies. The use of soy based protein instead of whey protein has been described for example in WO 2014/018922. Soy protein is widely used, however in view of some intolerances to soy products there is a need to find other sources of plant proteins.

Suitable alternatives include pea protein and rapeseed protein. Rapeseed seeds are rich in oil and contain considerable amounts of protein that accounts for 17 to 25% of seed dry weight. Processing rapeseed for oil for human consumption produces rapeseed meal (60%) as a by-product which contains about 30 to 40% protein. The rapeseed used for this purpose is usually of the varieties *Brassica napus* and *Brassica juncea*. These varieties contain only low levels of erucic acid and glucosinolates, and are also known as Canola. Canola is a contraction of Canada and "ola" (for "oil low acid"), but is now a generic term defined as rapeseed oil comprising <2% erucic acid and < 30 mmol/g glucosinolates. The resultant rapeseed meal is currently used as a high-protein animal feed.

Proteins are available as hydrolysates, concentrates and isolates. Hydrolysates are proteins that have been partially broken down by exposing the protein to heat, acid or enzymes that break apart the bonds linking amino acids. This makes it taste more bitter, but also allows it to be absorbed more rapidly during digestion than a native (non-hydrolyzed) protein. Isolates are purer than concentrates, meaning other non-protein components have been partially removed to "isolate" the protein. Many concentrates are around 80% protein, which means that on a dry basis, 80% of the total weight is protein. Isolates are typically around 90% protein (dry basis). This is calculated using the Kjeldahl method.

The predominant storage proteins found in rapeseed are cruciferins and napins. Cruciferins are globulins and are the major storage protein in the seed. A cruciferin is composed of 6 subunits and has a total molecular weight of approximately 300 kDa. Napins are albumins and are low molecular weight storage proteins with a molecular weight of approximately 14 kDa. Napins are more easily solubilized and in for example EP 1715752 B1 a process is disclosed to separate out the more soluble napin fraction, preferably to at least 85 wt.%. Napins are primarily proposed for use in applications where solubility is key.

Rapeseed proteins can also be divided into various fractions according to the corresponding sedimentation coefficient in Svedberg units (S). This coefficient indicates the speed of sedimentation of a macromolecule in a centrifugal field. For rapeseed proteins, the main reported fractions are 12S, 7S and 2S. Cruciferin and napin are the two major families of storage proteins found in rapeseed. Napin is a 2S albumin, and cruciferin is a 12S globulin. Furthermore, Schwenke and Linow (A reversible dissociation of the 12S globulin from rapeseed (Brassica napus L.) depending on ionic strength, Nahrung (1982) 26, K5-K6) state that the cruciferin complex is present as a 300 kDa 12S hexamer when exposed to higher ionic strength ($\mu > 0.5$ mS/cm), and reversibly dissociates into 7S trimeric molecules of 150 kDa when exposed to low ionic strength conditions.

It has been found that high purity rapeseed protein isolate has a broadly-based functionality in food products, unique among proteinaceous materials. The ability to utilize a protein which is plant in origin in food products enables truly vegetarian food products to be provided in instances where egg white and/or animal-derived protein have been used in the absence of any available substitute.

The rapeseed protein isolate may be used in conventional applications of protein isolates, such as protein fortification of processed foods, emulsification of oils, body formers in baked foods and foaming agents in products which entrap gases. The rapeseed protein isolate also has functionalities not exhibited by the source material and isoelectric precipitates. The rapeseed protein isolate has certain functionalities including the ability to be formed into protein fibers and to be used as a protein substitute or extender in food products where animal protein or other plant proteins are used. As described herein, the rapeseed protein isolate has additional functionalities.

EP 1389921 B1 relates to a process of forming a food composition, which comprises extracting rapeseed oil seed meal with an aqueous food-grade salt solution at a temperature of at least 5°C to cause solubilization of protein in the rapeseed oil seed meal and to form an aqueous protein solution having a protein content of 5 to 30 g/l and a pH of 5 to 6.8, and subsequently two protein fractions are separated out via micelles. This is done to improve solubility as the 12S fraction is usually considered as less soluble over a wide pH range when not in the presence of a salt. The resultant protein isolate is incorporated in said food composition in substitution for egg white, milk protein, whole egg, meat fibres, or gelatin.

A similar micelle fractionation approach is disclosed in US 2010/041871 leading to separate fractions of cruciferin and napin.

DE 10 2014 005466 A1 also describes a process for obtaining purified cruciferin and napin fractions. During the process, also a protein mixture of the two with 55-60% napins and 40-45% cruciferins is obtained. The solubility of this protein mixture is approximately 75%.

The use of enzymes is known in the field to increase protein extraction yield and to remove unwanted substances.

WO 2013/000066 and Rodriguez et al, 2016 teach rapeseed protein products having a protein content of at least about 60 wt.% with a low phytic acid content.

Sari et al, 2013 teaches protease treatment to increase protein extraction yield (from 15-30% to 50-80%).

CN1884572 teaches preparation of peptides from rapeseed meal using protease treatment.

CN10346350 teaches oil extraction and protein recovery from rapeseed meal using cellulase and protease treatment.

KR20140094238 teaches pectin production from rapeseed meal using using cellulase and protease treatment.

CN108441536 teaches increasing of the emulsifying properties of rapeseed protein by protease treatment.

JP2021137001 teaches production of high protein rapeseed meal combined with ethanol production from rapeseed meal using treatment by a glucanase and a cellulase enzyme.

EP4079161A1 teaches a rapeseed meal that is depleted from protein by a protein extraction process.

WO2021/123049 teaches rapeseed meal with high protein quality (high in indispensable amino acids) using a heat treatment process.

WO2018007492 teaches preparation of a rapeseed protein isolate using a precipitation process.

WO2012/135955 describes an aqueous process for the preparation of soluble protein products, such as a protein isolate. The oil seed does not require the use of solvents to provide a meal sufficiently reduced in oil content suitable for further processing. The oilseed meal is then further processed without the use of a solvent other than an aqueous solution (water). As a result the protein products do not suffer from contamination of organic solvents, salts or other extraction media. The remaining oil seed meal is said to have an oil content of about 12-18% oil. Said oil seed meal is the further processed without the use of a solvent.

Rodrigues et al, 2010 teaches an increase of protein content of rapeseed meal by use of carbohydrase enzymes mixtures. Most successful was Viscozyme treatment for 24 hours at 45 ºC and pH 3.5, where the protein content increased from 41% to 68%. Mixing of Viscozyme with other enzymes such as a pectinase did not increase the protein content further. There is a need to further improve the protein content and quality of rapeseed meal.

Typical protein content of hexane extracted rapeseed meal is 35-45%, and it is desired to increase this protein content. Preferably, the protein content is increased to at least 50% and more preferably, to at least 55%. Increasing the protein content of hexane extracted rapeseed meal is considered to be difficult.

Description of the Figures

Figure 1 depicts a typical method for processing of rapeseed into an oil stream and rapeseed meal.

Figure 2 depicts a preferred process according to the invention for augmenting the protein content of a plant meal from dried and cooled rapeseed meal.

Figure 3 depicts a preferred process according to the invention for augmenting the protein content of a plant meal starting from wet and toasted rapeseed meal at an elevated temperature.

Summary

The invention relates to a process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal.

The invention further relates to a plant protein meal obtainable by the process according to the invention.

The invention further relates to a food- or feed product comprising a plant protein meal according to the invention.

The invention further relates to the use of a plant protein meal according to the invention in the preparation of a food- or feed product.

The invention further relates to a process for the production of a food- or feed product, comprising contacting a plant protein meal according to the invention with a food- or feed product.

The invention further relates to an enzyme composition comprising a pectinase and a cellulase, wherein:

a) the ratio of the pectinase activity / cellulase activity is between: 50 AVJP units - 500 AVJP units pectinase / 1CXU units - 10CXU units cellulase; and wherein optionally,

b) the ratio of arabinofuranosidase activity / cellulase activity is between: 0.7 ARF units - 7 ARF units arabinofuranosidase / 1CXU units - 10CXU units cellulase.

Detailed description

The inventors have developed several surprising improvements over the prior art. The process according to the invention can advantageously result in an increased protein content. In addition improvements in respect of functionality can be achieved, such as an improved solubility, bio-availability, dispersibility, emulsification, foamability, gelation, taste and/or texture, formability, and/or gelation. More in specific, the process according to the invention results in an increased protein content in hexane extracted rapeseed meal. Increasing the protein level in hexane treated rapeseed meal is difficult. Surprisingly and as shown herein within the experimental part, the inventors show that the protein content in hexane extracted rapeseed meal can be increased substantially. It is thought that the pectinase and cellulase as used in the claimed subject matter increase fiber solubility, in other words bring more fiber into the liquid phase; by doing so, less fiber will be in the pellet, therefore, the protein content in the pellet increases. The majority (for example over 80 %, 85%, 90% or 95%) of the proteins in hexane extracted rapeseed meal is denatured, i.e. the majority of proteins in the hexane extracted rapeseed meal are non-native.

Accordingly, in a first aspect, there is provided for a process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal.

By a plant protein is herein understood a protein from a plant source. A plant may be a vegetable. A vegetable is a herbaceous plant, i.e., a plant with a soft stem. Vegetables can be distinguished from the edible nuts and fruits produced by plants with woody stems such as shrubs and trees. All such plants are herein included as a possible source for a protein meal. Preferred plant sources for such plant proteins include rice, cereals, potato, seeds, such as oilseeds, nuts, beans and pulses. More preferred plant sources are soy beans, beans, peas, faba, wheat, maize, potato, rapeseed, sunflower seed, barley, rye and rice. Most preferred plant sources are soy, peas and rapeseed. However, also brewer's spent grain can be a good source of plant proteins. Preferred plant proteins include rice protein, seed protein, nut protein, barley protein, bean protein and pulse protein. Also protein from brewer's spent grain is preferred. More preferred plant proteins are soy protein, pea protein and rapeseed protein, most preferred is rapeseed protein. A preferred source of plant based proteins are so-called plant meals or vegetable meals, such as soy meal, peas meal (also called *pea flour*) or rapeseed meal, most preferred is rapeseed meal. The terms rapeseed and canola are used interchangeably herein.

The claimed method uses hexane extracted rapeseed meal which is the meal which is the waste stream after oil extraction with hexane has been performed on rapeseed. Hexane extracted rapeseed meal has low oil content, typically less than 5% and even more typically less than 2%. Hence, the hexane extracted rapeseed meal used as a starting material in the claimed method has an oil content of less than 5%, more preferably less than 2%.

In the embodiments herein, the protein content of the plant protein meal is preferably augmented by at least about 1%, about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 3%, 32%, 33%, 34%, 35%, 36%, 37%. 38%. 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, and most preferably by at least about 100%.

The augmentation protein content is preferably indicated as percentage increase in protein content of the dry meal material. The amount of protein or protein concentration can be determined by any suitable means known to the person skilled in the art, see e.g. the assay used in the examples herein. The person skilled in the art knows to select to most convenient and suitable assay.

The incubation of the plant protein meal in the presence of enzyme activities is preferably performed in an aqueous mixture or composition, comprising water, the plant protein meal and the enzyme activities. If the plant protein meal is in a dry form, it may be brought into an aqueous mixture. The aqueous mixture may further comprise a suitable buffering agent to keep the pH within a desired range. The plant protein meal may be added to the mixture first, subsequently or simultaneously with the enzyme activities.

In the embodiments herein, the plant protein meal may be provided as such or may be prepared from plant protein by any means known to the person skilled in the art, such as milling.

When preparing the plant protein meal from oil seeds, the process may comprise the steps of:

- milling of the oil seeds;
- extraction of the oil by pressing or by solvent extracting of the plant oil from the milled oil seeds to prepare a liquid comprising the plant oil and a solid residue comprising the plant protein, wherein preferably the solvent is an alkane, more preferably hexane;
- separating of the liquid and the solid residue, preferably by means of a solid-liquid separation, optionally followed by stripping of residual solvent from the solid residue by further means, resulting in hexane extracted rapeseed meal (which is the starting material for the presently claimed subject matter);
- optionally milling of the solid residue.

A preferred process for the preparation of plant protein meal from oil seeds is depicted in Figure 1. The use of hexane to extract oil from rapeseed results in the denaturation of the proteins. Additionally, hexane extraction is performed at high temperatures which also denatures the proteins. As a result, the proteins in the hexane extracted rapeseed meal are denatured, Surprisingly, the herein described process shows that the amount of protein in the hexane extracted rapeseed meal can be increased when compared to non-enzymatically treated material.

The hexane extracted rapeseed meal (also referred to as hexane extracted canola meal) has an oil content of less than 2% (based on weight, i.e. (w/w), oil content is less than 2 g / 100 g rapeseed meal).

The starting material hexane extracted rapeseed meal has an oil content of less than 2% and the majority (for example over 80 %, 85%, 90% or 95%) of the protein is denatured.

In the embodiments herein, a pectinase is an enzyme that is capable of breaking down pectin by hydrolysis, transelimination and/or deesterification reactions, thus breaking down the ester bond that holds together the carboxyl and methyl groups in pectin.

In the embodiments herein, a cellulase is an enzyme that is capable of breaking down cellulose and related polysaccharides into smaller polysaccharides, oligosaccharides and monosaccharides.

In the embodiments herein, the pectinase may be present in an amount of at least 1500 AVJP units per gram of protein of the dry plant protein meal starting material and the cellulase may be present in an amount of at least 30 CXU units per gram of protein of the dry plant protein meal starting material.

In the embodiments herein, at least 1500 AVJP units per gram of protein of the dry plant protein meal starting material is preferably at least 1500, 3000, 4500, 6000, 7500, 9000, 10500, 12000, 13500, 15000, 30000 or at least 45000 AVJP units per gram of protein of the dry plant protein meal starting material.

In the embodiments herein, at least 30 CXU units per gram of protein of the dry plant protein meal starting material is preferably at least 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 600 or at least 900 CXU units per gram of protein of the dry plant protein meal starting material.

In the embodiments herein, the ratio of the pectinase activity / cellulase activity is preferably between: 50 AVJP units - 500 AVJP units pectinase / 1CXU units - 10CXU units cellulase, such as 50 AVJP units / 1CXU units, 100 AVJP units / 1CXU units, 150 AVJP units / 1CXU units, 200 AVJP units / 1CXU units, 250 AVJP units / 1CXU units, 300 AVJP units / 1CXU units, 350 AVJP units / 1CXU units, 400 AVJP units / 1CXU units, 450 AVJP units / 1CXU units, or 500 AVJP units / 1CXU units, or such as 50 AVJP units / 2CXU units, 50 AVJP units / 3CXU units, 50 AVJP units / 5CXU units, 50 AVJP units / 6CXU units, 50 AVJP units / 7CXU units, 50 AVJP units / 8CXU units, 50 AVJP units / 9CXU units, or 50 AVJP units / 10CXU units.

In the embodiments herein, the pectinase may comprise arabinofuranosidase activity. The arabinofuranosidase activity is preferably present in an amount of at least 20 ARF units per gram of protein of the dry plant protein meal starting material. In the embodiments herein, an amount of at least 20 ARF units per gram of protein of the dry plant protein meal starting material is preferably at least 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 400 or at least 600 ARF units per gram of protein of the dry plant protein meal starting material.

AVJP, CXU and ARF units are defined elsewhere herein in the Definitions section.

In the embodiments herein, the cellulase may be any cellulase or cellulase preparation known to the person skilled in the art, such as a cellulase comprising one or more of activities selected from the group consisting of: a galactosidase and a glucanase. The cellulase may comprise galactosidase or glucanase activity. The cellulase may comprise glucanase activity. The cellulase may comprise galactosidase activity. The cellulase may comprise galactosidase and glucanase activity.

In the embodiments herein, the pH of the incubation mixture at the start of the incubation is at least 3.6. Preferably, the pH is at least 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, or at least pH 4.5. Preferably the pH at the end of the incubation is at least 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, or at least pH 4.5.

Preferably, the pH of the incubation mixture is at least 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, or at least pH 4.5 at the start and at the end of the incubation.

In the embodiments herein, the temperature of the incubation mixture may be between 35 ºC and 60 ºC. Preferably, the pH is between 40 ºC and 60 ºC, between 45 ºC and 60 ºC, between 45 ºC and 55 ºC, between 48 ºC and 55 ºC. More preferably, the temperature of the incubation mixture is about 50 ºC, such as 48, 49, 50, 51, or 52 ºC; most preferred is 50 ºC.

In the embodiments herein, the incubation time may be between 1 and 72 hours. Preferably, the incubation time is between 2 and 48 hours, between 4 and 48 hours, between 8 and 48 hours, between 12 and 48 hours and more preferably between 4 and 24 hours.

In the embodiments herein, the plant protein meal may be subjected to a process according to the embodiments are set forward herein after any stage in the preparation of the raw plant protein meal. In an embodiment, the raw plant protein meal is processed substantially immediately after oil extraction and solvent evaporation, preferably without a drying step. Two preferred preparations of starting plant protein meals are depicted in Figure 1, including their methods of preparation.

In the embodiments herein, an additional enzyme may be present in the incubation mixture. Preferably, such enzyme is a hemicellulase and/or an endoglucanase. Accordingly, either a hemicellulase, an endoglucanase or both may be present. Such hemicellulase may e.g. be a mannanase or a xylanase.

In the embodiments herein, the additional enzyme may also be a protease, such as an acid protease, preferably an acid endoprotease, preferably a fungal acid endoprotease, preferably an aspergillopepsin I protease. Preferably, the protease is derived from an *Aspergillus*, such as *Aspergillus niger*.

The process as claimed herein comprises a step of separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal. The person skilled in the art knows many means to separate the dry matter from the liquid matter. One of such means is centrifugation or decanting, which is a preferred means herein.

Alternatively phrase, the claimed process thus comprises a step of separating the enzyme incubated rapeseed meal with a solid/liquid separation to form

- a liquid phase (comprising mainly soluble fiber and soluble sugar), and
- a solid phase (comprising mainly protein).

The solid phase (herein also referred to as pellet or dry matter) comprises the desired rapeseed meal with augmented protein content. As described above, the majority (for example over 80 %, 85%, 90% or 95%) of the proteins are denatured.

The invention thus provides a process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

further comprising a step of separating the enzyme incubated rapeseed meal with a solid/liquid separation to form

- a liquid phase, and
- a solid phase,

and collecting the solid phase to obtain an augmented plant protein meal.

The process as set forward in the embodiments herein may be performed as such, but may also comprise further process steps such as mixing hexane extracted rapeseed meal with water to form a slurry and/or washing the pellet and/or drying the pellet.

Provided herein are thus

- a process for augmenting the protein content of a plant protein meal, comprising mixing hexane extracted rapeseed meal with water to form a slurry and incubating the obtained slurry in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal.

- a process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal and washing the obtained dry matter.

- a process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the solid phase from the liquid matter and collecting the solid phase to obtain an augmented plant protein meal and drying the obtained solid phase.

- a process for augmenting the protein content of a plant protein meal, comprising mixing hexane extracted rapeseed meal with water to form a slurry and incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal and washing and drying the obtained dry matter.

- a process for augmenting the protein content of a plant protein meal, comprising mixing hexane extracted rapeseed meal with water to form a slurry incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

further comprising a step of separating the enzyme incubated rapeseed meal with a solid/liquid separation to form

- a liquid phase, and
- a solid phase,

and collecting the solid phase to obtain an augmented plant protein meal, further comprising washing and drying the solid phase.

The end result of the claimed process is a protein augmented plant protein meal with more than 50% (w/w) protein content.

A further step that may be performed is extraction of the protein. The person skilled in the art knows many means for protein extraction. A preferred method is set forward in EP22213449.6, which is herein incorporated by reference.

In the embodiments herein, the plant protein meal used a starting material may be any plant protein meal known to the person skilled in the art, such as but not limited to soy meal, peas meal or rapeseed meal. Preferably, the plant protein meal is rapeseed meal. Preferably, the rapeseed meal is prepared according to the process in Figure 1. The rapeseed meal may be dried and cooled rapeseed meal. A preferred process to augment the protein content of such dried and cooled rapeseed meal is depicted in Figure 2. The rapeseed meal may be wet and toasted rapeseed meal. A preferred process to augment the protein content of such wet and toasted rapeseed meal is depicted in Figure 3.

As a consequence of the process as set forward in the embodiments herein, the plant protein meal obtained by the process as set forward in the embodiments herein will have an augmented protein content. Accordingly, in a second aspect, there is provided for a plant protein meal obtainable by the process as set forward in the embodiments of the first aspect herein. The features in this second aspect are preferably the features of the first aspect herein. More in specific, provided is a hexane extracted protein rapeseed meal comprising a protein content of at least 50% (w/w), more preferably at least 51, 52, 53, 54 or 55 % (w/w). As described above, the majority (for example over 80 %, 85%, 90% or 95%) of the proteins in the hexane extracted rapeseed meal is denatured.

Further provided herein is a hexane extracted rapeseed meal comprising a protein content of at least 50% (w/w), more preferably at least 51, 52, 53, 54 or 55 % (w/w). As described above, the majority (for example over 80 %, 85%, 90% or 95%) of the proteins in the hexane extracted rapeseed meal is denatured.

The protein meal obtained can conveniently be used in a food- or feed product. Accordingly, in a third aspect, there is provided for a food- or feed product comprising a plant protein meal protein of the second aspect herein or comprising a plant protein meal obtained by the process as set forward in the embodiments of the first aspect herein. The features in this third aspect are preferably the features of the first and/or second aspect herein.

Further provided in this aspect is the use of al plant protein meal protein of the second aspect herein or a plant protein meal obtained by the process as set forward in the embodiments of the first aspect herein, in the preparation of a food- or feed product. The food- or feed product may be prepared by any means known to the person skilled in the art as long as the final obtained food- or feed product comprises the plant protein meal protein with augmented protein content of the embodiments herein. Such process may comprise contacting a plant protein meal protein of the second aspect herein or a plant protein meal obtained by the process as set forward in the embodiments of the first aspect herein, with a food- or feed product.

In a fourth aspect, there is provided for an enzyme composition comprising a pectinase and a cellulase, wherein:

a) the ratio of the pectinase activity / cellulase activity is between: 50 AVJP units - 500 AVJP units pectinase / 1CXU units - 10CXU units cellulase; and wherein optionally,

b) the ratio of arabinofuranosidase activity / cellulase activity is between: 0.7 ARF units - 7 ARF units arabinofuranosidase / 1CXU units - 10CXU units cellulase.

In this aspect, these features are preferably those as set forward in the embodiments of the first aspect herein.

In the embodiments herein, the pectinase may be a pectinase derived from a filamentous fungus, such as an *Aspergillus*, such as *Aspergillus niger*.

In the embodiments herein, the cellulase may be a cellulase derived from a filamentous fungus, such as a *Trichoderma*, such as *Trichoderma longibrachiatum* (*T. reesei*).

Further provided herein, is the use of a pectinase and a cellulase for increasing the protein content (% w/w) in hexane extracted rapeseed meal.

Definitions

Unless defined otherwise or clearly indicated by context, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

Throughout the present specification and the accompanying claims, the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e. to one or at least one) of the grammatical object of the article. By way of example, “an element” may mean one element or more than one element. When referring to a noun (e.g. a compound, an additive, etc.) in the singular, the plural is meant to be included. Thus, when referring to a specific moiety, e.g. "gene", this means "at least one" of that gene, e.g. "at least one gene", unless specified otherwise.

The word “about” or “approximately” when used in association with a numerical value (e.g. about 10) preferably means that the value may be the given value (of 10) more or less 10% of the value.

When referring to a compound of which several isomers exist (e.g. a D and an L enantiomer), the compound in principle includes all enantiomers, diastereomers and cis/trans isomers of that compound that may be used in the particular aspect of the invention; in particular when referring to such as compound, it includes the natural isomer(s).

Unless explicitly indicated otherwise, the various embodiments of the invention described herein can be cross-combined.

The terms "polypeptide", "peptide" and "protein" are preferably used herein to refer to a polymer of amino acid residues. The terms preferably apply to amino acid polymers in which one or more amino acid residue(s) is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms "polypeptide", "peptide" and "protein" are preferably also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulphation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation.

The term “enzyme” preferably refers herein to a polypeptide (i.e. a protein) having a catalytic function.

Pectinase activity

UNIT DEFINITION

The activity is expressed in ‘**a**ctivité **v**iscosimétrique de **j**us de **p**ommes’ (AVJP) units. One unit is defined as the amount of enzyme that that causes a change in viscosity of the substrate with a speed giving a slope of 0.00027 per minute under the conditions of the test (50°C, pH3.85).

PRINCIPLE OF ABSOLUTE ENZYME ASSAY

The AVJP assay is a method where the reduction of the viscosity of a methylated pectin solution of pH 3.85 and 50°C, caused by pectinase enzyme activity, is measured using an Ubbelohde viscometer. The reduction in viscosity is a measure for the enzyme activity.

Substrate

Pectin from apple with a high degree of methylation (>85%)

Conditions of Incubation

Temperature: 50 °C

pH 3.85

Measurement

The time it takes for the substrate to pass between two calibrated marks on an Ubbelohde viscosimeter (https://en.wikipedia.org/wiki/Ubbelohde_viscometer) is a measure for the viscosity of the solution.

Arabinofuranosidase activity

UNIT DEFINITION

The activity is expressed in ARabinoFuranosidase (ARF) units. One unit is defined as the amount of enzyme required to release 1nmol/s of p-nitrophenol from p-nitrophenyl-a-L-arabinofuranoside under the assay conditions.

PRINCIPLE OF ABSOLUTE ENZYME ASSAY

The ARF Assay is based on the measurement of p-nitrophenol released from the substrate p-nitrophenyl-a-L-arabinofuranoside.

Substrate

p-nitrophenyl-a-L-arabinofuranoside

Conditions of Incubation

Temperature: 40 °C

pH 4.4

Measurement

p-Nitrophenol is measured via the increase in absorbance at 405 nm after reaction termination with an alkaline solution.

Cellulase activity

UNIT DEFINITION

The activity is expressed in CXU. One CXU is defined as the amount of enzyme required to release an amount of reducing sugar equal to 0.5 mg glucose in 1 hour from carboxymethylcellulose under the assay conditions.

PRINCIPLE OF ABSOLUTE ENZYME ASSAY

The CXU Cellulase Assay is based on the increase in reducing sugar content arising from the hydrolysis of glycosidic bonds within carboxymethylcellulose.

Substrate

Carboxymethylcellulose

Conditions of Incubation

Temperature: 37 °C

pH 4.6

Measurement

After incubating enzyme with substrate for 60 minutes, the reaction is stopped and reducing sugar content is determined by developing a colored complex with dinitrosalicylic acid.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

Unless otherwise indicated each embodiment as described herein may be combined with another embodiment as described herein.

The examples herein are offered for illustrative purposes only and are not intended to limit the scope of the present invention in any way.

Further embodiments of the invention

1. A process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

   a) a pectinase,

   b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6.

2. A process according to embodiment 1, wherein:

   a) the pectinase is present in an amount of at least 1500 AVJP units per gram of protein of the dry plant protein meal starting material, and

   b) the cellulase is present in an amount of at least 30 CXU units per gram of protein of the dry plant protein meal starting material.

3. A process according to embodiment 1 or 2, wherein the pectinase comprises arabinofuranosidase activity, and wherein the arabinofuranosidase activity is present in an amount of at least 20 ARF units per gram of protein of the dry plant protein meal starting material.

4. A process according to embodiment 3, wherein the cellulase comprises one or more of activities selected from the group consisting of: a galactosidase, a glucanase, a mannanase, and a xylanase.

5. A process according to any one of the preceding embodiments, wherein the pH of the incubation mixture at the start of the incubation is at least 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, or at least pH 4.5.

6. A process according to any of the preceding embodiments, wherein the temperature of the incubation mixture is between 35 ºC and 60 ºC.

7. A process according to any one of the preceding embodiments, wherein the incubation time is between 1 and 72 hours, preferably between 2 and 28 hours, more preferably between 4 and 24 hours.

8. A process according to any one of the preceding embodiments, wherein the plant protein meal is processed substantially immediately after oil extraction and solvent evaporation, preferably without a drying step.

9. A process according to any one of the preceding embodiments, wherein an additional enzyme is present, preferably a hemicellulase and/or an endoglucanase.

10. A process according to any one of the preceding embodiments, wherein the additional enzyme is a protease, preferably an acid protease, preferably an acid endoprotease, preferably a fungal acid endoprotease, preferably an aspergillopepsin I protease, preferably derived from an *Aspergillus*, such as *Aspergillus niger*.

11. A process according to any one of the preceding embodiments, further comprising separating the dry matter from the liquid matter.

12. A process according to anyone of the preceding embodiments, further comprising extraction of the protein.

13. A process according to any one of the preceding embodiments, wherein the plant protein meal is soy meal, peas meal or rapeseed meal, preferably rapeseed meal.

14. A plant protein meal obtainable by any one of embodiments 1 to 13.

15. A food- or feed product comprising a plant protein meal according to embodiment 14.

16. Use of a plant protein meal according to embodiment 14 in the preparation of a food- or feed product.

17. A process for the production of a food- or feed product, comprising contacting a plant protein meal according to embodiment 14 with a food- or feed product.

18. An enzyme composition comprising a pectinase and a cellulase, wherein:

a) the ratio of the pectinase activity / cellulase activity is between: 50 AVJP units - 500 AVJP units pectinase / 1CXU units - 10CXU units cellulase; and wherein optionally,

b) the ratio of arabinofuranosidase activity / cellulase activity is between: 0.7 ARF units - 7 ARF units arabinofuranosidase / 1CXU units - 10CXU units cellulase.

19. An enzyme composition according to embodiment 17, wherein the pectinase is a pectinase derived from a filamentous fungus, preferably an *Aspergillus*, preferably *Aspergillus niger*.

20. An enzyme composition according to embodiment 17, wherein the cellulase is derived from a filamentous fungus, preferably a *Trichoderma*, preferably *Trichoderma longibrachiatum* (*T. reesei*).

Examples

Example 1: Rapeseed meal hydrolysis with Pectinase and Cellulase combination

Hydrolysis of rapeseed meal using Pectinase preparation A and Cellulase preparation B combination is shown in this example. The process and analytical methods are also described.

Enzyme mixtures:

Pectinase preparation A contains pectinase and arabinofuranosidase with the following activities

- Pectinase: 150000 AVJP/g
- Arabinofuranosidase: 2000 ARF/g

Cellulase preparation B contains cellulase, xylanase with the following activities:

- Cellulase: 3000 CXU/g
- Xylanase: 100 XVU/g

A commercially available example of pectinase preparation A is Rapidase Power and can be obtained from dsm-firmenich.

A commercially available example of cellulase preparation B is Validase TRL and can be obtained from dsm-firmenich.

Dry matter analysis

The Halogen Moisture Analyzer was used for determining the dry matter of either the raw material or the liquid fraction. The instrument works on the thermogravimetric principle. At the start of the measurement the Moisture Analyzer determines the weight of the sample, the sample is then quickly heated by the integral halogen heating module and the moisture vaporizes. During the

drying process the instrument continually measures the weight of the sample and displays the reduction in moisture. Once drying has been completed, the moisture or solids content of the sample is displayed as the final result.
The dry matter of liquid sample is determined at 120°C as percentage of the final weight in the initial weight

Protein content (Kjeldahl) analysis

The analysis of the determination of Kjeldahl nitrogen involves two steps. The sample is first digested in sulfuric acid (95-97%) after addition of a catalyst (1.5 g K2SO4 and 7.5 mg Se) during 90 minutes at 360°C. Then, sample is injected in San++ Continuous Flow Analyzer System. It means the sample containing ammonium is mixed with a continuously streaming flow of a buffer solution.

$NH_4^+ + OH^- \rightarrow NH3\uparrow + H_2O$

The ammonia formed is separated in a diffusion cell from the solution over a hydrophobic semipermeable membrane and taken up by a streaming recipient flow containing an indicator. Due to the resulting pH shift, the buffer solution will change its colour which is measured continuously in the flow photometer. The absorbance at 660nm is recorded continuously as Nitrogen peak. The Total Kjeldahl Nitrogen is defined as sum of present organic and ammonium Nitrogen. And the protein content is calculated as 6.25 times Kjeldahl Nitrogen value.

Experiment

The raw material used in all Examples was rapeseed meal resulted from crude rapeseed oil processing from a European supplier. Rapeseed was harvested and subjected to pressing to obtain pressed rapeseed oil and pressed rapeseed cake; the pressed rapeseed cake was then subjected to hexane extraction to obtain extracted rapeseed oil and wet rapeseed meal; the wet rapeseed meal was then subjected to solvent removal and drying to obtain rapeseed meal, while the extracted rapeseed oil was mixed with pressed rapeseed oil to form crude rapeseed oil. The herein used hexane extracted rapeseed meal typically contains 35-40% protein, 15-20% carbohydrates, 25-30% fiber, ~10% moisture, ~2% ash, and less than 5% oil.

The experiment was conducted at 30 gram scale with dried rapeseed meal. A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding the enzymes, the incubation was carried out for 2, 4 or 24h. The samples were subsequently heat up to 95°C for 10min. Solid-liquid separation was done by centrifugation at 4300G for 25min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in the liquid phase are depicted in **Table 1**. The Pectinase preparation A and Cellulase preparation B combination treatment results in a significantly hydrolysis of the fibre fraction, releasing it into the liquid phase.

Table 1. Dry matter and Protein content analysis results of the liquid phase with and without enzyme treatment

Enzyme dosage	**Dry matter of liquid fraction [%]**			**Protein content of liquid fraction [%]**		
	2h	**4h**	**24h**	**2h**	**4h**	**24h**
No enzyme	5.14	5.17	5.12	0.56	0.56	0.69
5% Pectinase preparation A + 5% Cellulase preparation B	6.52	7.19	9.09	0.69	0.81	1.13

<u>Protein yield in the pellet calculation</u>

A. The composition of the raw material is listed in **Table 2.** In the 30gram scale incubation, 6gram of raw material was used, which has 5.26gram dry matter and contains 1.95gram protein. The liquid portion of 30gram raw material suspension was 24.7gram.

Table 2. Dry matter and protein content of the raw material

	Dry matter [%]	**Protein content [%]**
Dried rapeseed meal from European supplier	87.7	32.5

B. The protein yield in the pellet was calculated as following

$$\text{protein yield in the pellet} = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet was calculated as following

$$protein\ content\ in\ the\ pellet = 1.95g - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times 24.7g$$

The weight of insoluble fraction was calculated as following

$$weight\ of\ insoluble\ fraction = 5.26g - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times 24.7g$$

The protein yield in pellet after S/L separation is depicted in **Table 3**. The protein yield in the pellet, which was treated with Rapidase Power and Validase TRL combination, was significantly higher as compared to the raw material and increased to 55.5%.

Table 3. Protein content in the pellet after S/L separation

Enzyme dosage	**Protein content in the pellet [%]**

	2h	4h	24h
No enzyme	45.4	45.5	44.6
5% Pectinase preparation A + 5% Cellulase preparation B	48.8	50.2	55.5

Example 2: Rapeseed meal hydrolysis with Rapidase Press and Validase TRL combination

The hydrolysis of rapeseed meal using Rapidase Press and Validase TRL combination is shown in this example. The process and analytical findings are described.

Rapidase Press contains pectinase with the following activity:
- Pectinase: 180000 AVJP/g

Validase TRL contains cellulase, xylanase with the following activities:
- Cellulase: 3000 CXU/g
- Xylanase: 100 XVU/g

Rapidase Press can be obtained from dsm-firmenich.

The experiment was conducted at 30 g scale with wet rapeseed meal from a European supplier (material is described in Example 1). A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding enzymes, the incubation was carried out for 2, 4 or 24h. The samples were heat up to 95°C for 5min. Solid-liquid separation was done by centrifugation at 4300G for 20min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content (Kjeldahl) analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in liquid phase are given in **Table 4**. The Rapidase Press and Validase TRL combination treatment results in a significantly hydrolysis fiber fraction, releasing it into liquid phase.

Table 4. Dry matter and Protein content analysis results of liquid phase with and without enzyme treatment

Enzyme dosage	**Dry matter of liquid fraction [%]**			**Protein content of liquid fraction [%]**		
	2h	**4h**	**24h**	**2h**	**4h**	**24h**
No enzyme			4.9			0.69
5% Rapidase Press + 5% Validase TRL	6.3	6.9	8.2	0.75	0.76	1.23

Protein yield in the pellet calculation

A. The composition of the raw material was listed in **Table 5.** In the 30g scale incubation, 6g of raw material was used, which has 5.11g dry matter and contains 1.88g protein. The liquid volume of 30g suspension is 24.89g

Table 5. Dry matter and protein content of the raw material

	Dry matter [%]	**Protein content [%]**
Wet rapeseed meal from European supplier	85.1	31.25

B. The protein yield in the pellet is calculated as following

$$\text{protein yield in the pellet } = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet is calculated as following

$$protein\ content\ in\ the\ pellet = 1.88g - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The weight of insoluble fraction is calculated as following

$$weight\ of\ insoluble\ fraction = 5.11g - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The protein yield in pellet after S/L separation is given in **Table 6**. The protein yield in the pellet, which treated with Rapidase Press and Validase TRL combination, are significantly higher and it reached 51.4%.

Table 6. Protein content in the pellet after S/L separation

Enzyme dosage	**Protein yield in the pellet [%]**		
	2h	**4h**	**24h**
No enzyme			43.9
5% Rapidase Press + 5% Validase TRL	47.8	49.8	51.3

Example 3: Rapeseed meal hydrolysis with Rapidase Press and Validase TRL combination in large scale

The hydrolysis of rapeseed meal in large scale using Rapidase Press and Validase TRL combination is shown in this example. The process and analytical findings are described.

Rapidase Press contains pectinase with the following activity.

- Pectinase: 180000 AVJP/g

Validase TRL contains cellulase, xylanase with the following activities:

- Cellulase: 3000 CXU/g
- Xylanase: 100 XVU/g

The experiment was conducted at 1L scale with wet rapeseed meal from a European supplier (material is described in Example 1). A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding enzymes, the incubation was carried out for 24h. The suspension was, then, heated up to 85°C for 5min for enzyme inactivation. Solid-liquid separation was done by centrifugation at 4300G for 20min. The solid phase was collected, and then water was added to wash the pellet. The second solid-liquid separation was done by centrifugation at 4300G for 20min. The washing step was repeated three times. The liquid phase during washing was discarded and the solid phase was collected for next step. The final pellet was further dried by freeze drying. Its dry matter and protein content were determined.

The dry matter analysis determines the total mass of the pellet, the protein content (Kjeldahl) analysis determines the protein content of the pellet. The dry matter and protein content of wet rapeseed meal and the pellet are given in **Table 7**.

Table 7. Dry matter and protein content in wet rapeseed meal and the pellet with and without enzyme treatment

Enzyme dosage	**Protein content of pellet [%]**	**Dry matter of pellet [%]**
Wet rapeseed meal from European supplier	31.3	85.1
No enzyme	39.7	95.6
5% Rapidase Press + 5% Validase TRL	49.1	97.9

The protein content in dry base is calculated as following:

$$\text{protein content in dry base } = \frac{protein\ content\ in\ the\ pellet\ [\%]}{Dry\ matter\ of\ pellet\ [\%]} \times 100$$

As shown in **Table 8**, the pellet from Rapidase Press and Validase TRL combination treatment contains significantly higher amount of protein. It reaches 50.2% protein in dry base. It is concluded that the process can also successfully be performed at larger scale.

Table 8. Protein content in dry base of wet rapeseed meal and pellet with and without enzyme treatment

Enzyme dosage	**Protein content in dry base [%]**
Wet rapeseed meal from European supplier	36.8
No enzyme	41.5
5% Rapidase Press + 5% Validase TRL	50.2

Example 4: Dose response of Rapidase Power and Validase TRL combination in rapeseed meal hydrolysis.

The hydrolysis of rapeseed meal using different dosages of Rapidase Power and Validase TRL combination is shown in this example. The process and analytical findings are also described.

Rapidase Power contains pectinase and arabinofuranosidase with the following activities

- Pectinase: 150000 AVJP/g
- Arabinofuranosidase: 2000 ARF/g

Validase TRL contains cellulase, xylanase with the following activities:

- Cellulase: 3000 CXU/g
- Xylanase: 100 XVU/g

The experiment was conducted at 30 g scale with wet rapeseed meal from a European supplier (material is described in Example 1). A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding enzymes, the incubation was carried out for 24h. The samples were heat up to 95°C for 5min. Solid-liquid separation was done by centrifugation at 4300G for 20min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in liquid phase are given in **Table 9**. When different dosages of Rapidase Power and Validase TRL combination were used, the amount of fiber fraction releasing into liquid phase differs.

Table 9. Dry matter and Protein content analysis results of liquid phase with and without enzyme treatment

Enzyme dosage	**Dry matter of liquid fraction [%]**	**Protein content of liquid fraction [%]**
No enzyme	4.74	0.75
5% Rapidase Power + 5% Validase TRL	9.17	1.50
3% Rapidase Power + 3% Validase TRL	8.36	1.25
2% Rapidase Power + 2% Validase TRL	7.83	1.13

1% Rapidase Power + 1% Validase TRL	6.81	0.94

<u>Protein yield in the pellet calculation</u>

A. The composition of the raw material was listed in **Table 10.** In the 30g scale incubation, 6g of raw material was used, which has 5.11g dry matter and contains 1.88g protein. The liquid volume of 30g suspension is 24.89g

Table 10. Dry matter and protein content of the raw material

	Dry matter [%]	Protein content [%]
Dried rapeseed meal from European supplier	85.1	31.25

B. The protein yield in the pellet is calculated as following

$$\text{protein yield in the pellet } = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet is calculated as following

$$protein\ content\ in\ the\ pellet = 1.88g - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The weight of insoluble fraction is calculated as following

$$weight\ of\ insoluble\ fraction = 5.11g - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The protein yield in pellet after S/L separation is given in **Table 11**. Reducing dosages of Rapidase Power and Validase TRL combination results in a lower protein yield in pellet.

Table 11. Protein content in the pellet after S/L separation

Enzyme dosage	Protein yield in the pellet [%]
No enzyme	43.1
5% Rapidase Power + 5% Validase TRL	53.3
3% Rapidase Power + 3% Validase TRL	51.8
2% Rapidase Power + 2% Validase TRL	50.6
1% Rapidase Power + 1% Validase TRL	48.2

Example 5: Rapeseed meal hydrolysis with different ratios of Rapidase Press and Validase TRL combination.

The hydrolysis of rapeseed meal using different ratios of Rapidase Press and Validase TRL combination is shown in this example. The process and analytical findings are described.

Rapidase Press contains pectinase with the following activity:

- Pectinase: 180000 AVJP/g

Validase TRL contains cellulase, xylanase with the following activities:

- Cellulase: 3000 CXU/g
- Xylanase: 100 XVU/g

The experiment was conducted at 30 g scale with wet rapeseed meal from a European supplier (material is described in Example 1). A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding enzymes, the incubation was carried out for 24h. The samples were heat up to 95°C for 5min. Solid-liquid separation was done by centrifugation at 4300G for 20min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content (Kjeldahl) analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in liquid phase are given in **Table 12**. Modifying the ratio in the Rapidase Press and Validase TRL combination results in slightly fiber fraction hydrolysis.

Table 12. Dry matter and Protein content analysis results of liquid phase with and without enzyme treatment

Enzyme dosage	**Dry matter of liquid fraction [%]**	**Protein content of liquid fraction [%]**
No enzyme	4.89	0.69
5% Rapidase Press + 5% Validase TRL	8.23	1.23
5% Rapidase Press + 4% Validase TRL	8.14	1.19
5% Rapidase Press + 3% Validase TRL	8.12	1.17
5% Rapidase Press + 2% Validase TRL	7.96	1.17
5% Rapidase Press + 1% Validase TRL	7.82	1.13
5% Rapidase Press + 0.5% Validase TRL	7.57	1.21

<u>Protein yield in the pellet calculation</u>

A. The composition of the raw material was listed in **Table 13.** In the 30g scale incubation, 6g of raw material was used, which has 5.11g dry matter and contains 1.88g protein. The liquid volume of 30g suspension is 24.89g

Table 13. Dry matter and protein content of the raw material

	Dry matter [%]	Protein content [%]
Wet rapeseed meal from European supplier	85.1	31.25

B. The protein yield in the pellet is calculated as following

$$\text{protein yield in the pellet } = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet is calculated as following

$$protein\ content\ in\ the\ pellet = 1.88g - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The weight of insoluble fraction is calculated as following

$$weight\ of\ insoluble\ fraction = 5.11g - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The protein yield in pellet after S/L separation is given in **Table 14**. Reducing dosage of Validase TRL in the combination up to 2% results in a similar protein yield in the pellet, which is around 51%.

Table 14. Protein content in the pellet after S/L separation

Enzyme dosage	Protein yield in the pellet [%]
No enzyme	43.9
5% Rapidase Press + 5% Validase TRL	51.4
5% Rapidase Press + 4% Validase TRL	51.4
5% Rapidase Press + 3% Validase TRL	51.4
5% Rapidase Press + 2% Validase TRL	50.8
5% Rapidase Press + 1% Validase TRL	50.5
5% Rapidase Press + 0.5% Validase TRL	48.9

Example 6: Rapeseed meal hydrolysis with different industrial benchmark products.

The hydrolysis of rapeseed meal using different industrial benchmark products is shown in this example. The process and analytical findings are described.

- Viscozyme L from Novozymes contains both pectinase and glucanases activity

- Pectinex Ultra SPL from Novozymes contains pectinase activity
- Rohament CL from AB enzymes contains both pectinase and cellulase activity

The experiment was conducted at 30 g scale with wet rapeseed meal from a European supplier (material is described in Example 1). A 20% w/w rapeseed meal suspension was made by adding tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding enzymes, the incubation was carried out for 24h. The samples were heat up to 95°C for 5min. Solid-liquid separation was done by centrifugation at 4300G for 20min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content (Kjeldahl) analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in liquid phase are given in **Table 15**. Different enzymes products clearly results in different amount of fiber fraction and protein hydrolysis.

Table 15. Dry matter and Protein content analysis results of different enzyme treatments

Enzyme dosage	**Dry matter of liquid fraction [%]**	**Protein content of liquid fraction [%]**
No enzyme	4.74	0.75
5% Rapidase Power + 5% Validase TRL	8.83	1.31
5% Viscozyme L	7.99	1.81
10% Viscozyme L	9.24	2.13
5% Pectinex Ultra SPL	8.22	2.19
10% Pectinex Ultra SPL	9.07	2.44
5% Rohament CL	5.97	1.00
10% Rohament CL	6.33	1.19

Protein yield in the pellet calculation

A. The composition of the raw material was listed in **Table 16.** In the 30g scale incubation, 6g of raw material was used, which has 5.11g dry matter and contains 1.88g protein. The liquid volume of 30g suspension is 24.89g

Table 16. Dry matter and protein content of the raw material

	Dry matter [%]	**Protein content [%]**
Wet rapeseed meal from European supplier	85.1	31.25

B. The protein yield in the pellet is calculated as following

$$\text{protein yield in the pellet } = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet is calculated as following

$$protein\ content\ in\ the\ pellet = 1.88g - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The weight of insoluble fraction is calculated as following

$$weight\ of\ insoluble\ fraction = 5.11g - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times 24.89g$$

The protein yield in pellet after S/L separation is given in **Table 17**. Comparing with all tested industrial benchmark products, The Rapidase Power and Validase TRL combination results in the highest protein yield in the pellet, which is 53.4%.

Table 17. Protein content in the pellet after S/L separation

Enzyme dosage	**Protein yield in the pellet [%]**
No enzyme	43.1
5% Rapidase Power + 5% Validase TRL	53.4
5% Viscozyme L	45.8
10% Viscozyme L	48.0
5% Pectinex Ultra SPL	43.6
10% Pectinex Ultra SPL	44.6
5% Rohament CL	45.0
10% Rohament CL	44.8

Example 7: Rapeseed meal hydrolysis with Rapidase Press and Validase TRL combination in different suspension concentration.

The hydrolysis of rapeseed meal using Rapidase Press and Validase TRL combination in different suspension concentration is shown in this example. The process and analytical findings are described.

Rapidase Press contains pectinase with the following activity:

- Pectinase: 180000 AVJP/g

Validase TRL contains cellulase, xylanase with the following activities:

- Cellulase: 3000 CXU/g

- Xylanase: 100 XVU/g

The experiment was conducted at 30 g scale with wet rapeseed meal from a European supplier (material is described in Example 1). Various concentrations of the rapeseed meal suspension was made by adding different amount of tap water. The pH of the suspension was adjusted to 4.5 using 4N HCl. It was then heated up to 50°C. After adding 5% Rapidase Press and 5% Validase TRL, the incubation was carried out for 24h. The samples were heat up to 95°C for 5min. Solid-liquid separation was done by centrifugation at 4300G for 20min. The liquid phase, which contains the soluble protein, was collected and used for dry matter and protein content (Kjeldahl) analysis.

The dry matter analysis determines the total mass of soluble fraction, the protein content (Kjeldahl) analysis determines the protein content in the liquid phase. The dry matter and protein content in liquid phase are given in **Table 18**. The Rapidase Press and Validase TRL combination shows clear fiber fraction hydrolysis in different suspension concentration.

Table 18. Dry matter and Protein content analysis results of different enzyme treatments

Suspension concentration [w/w]	**Enzyme**	**Dry matter of liquid fraction [%]**	**Protein content of liquid fraction [%]**
20%	Blank	4.89	0.69
30%	Blank	7.29	1.14
40%	Blank	9.77	1.78
20%	5% Rapidase Press and 5% Validase TRL	8.23	1.23
30%	5% Rapidase Press and 5% Validase TRL	12.27	1.95
40%	5% Rapidase Press and 5% Validase TRL	15.38	3.31

<u>Protein yield in the pellet calculation</u>

A. The composition of the raw material was listed in **Table 19.** In the 30g scale incubation, 6g to 12g of raw material was used. The solid weight and protein weight of used material, and the liquid volume are listed in **Table 20**

Table 19. Dry matter and protein content of the raw material

	Dry matter [%]	**Protein content [%]**
Wet rapeseed meal from European supplier	85.1	31.25

Table 20. Solid weight and protein weight of used material, and the liquid volume of the suspension

Suspension concentration [w/w]	**Raw material [g]**	**Solid weight [g]**	**Protein weight [g]**	**Liquid volume [g]**
20%	6	5.11	1.88	24.89
30%	9	7.66	2.81	22.34
40%	12	10.21	3.75	19.79

B. The protein yield in the pellet is calculated as following

$$\text{protein yield in the pellet } = \frac{protein\ content\ in\ the\ pellet\ [g]}{weight\ of\ insoluble\ fraction\ [g]} \times 100$$

The protein content in the pellet is calculated as following

$$protein\ content\ in\ the\ pellet = protein\ weigt\ [g] - protein\ content\ in\ liquid\ fraction\ [\%] \div 100 \times Liquid\ volume\ [g]$$

The weight of insoluble fraction is calculated as following

$$weight\ of\ insoluble\ fraction = Solid\ weight\ [g] - dry\ matter\ of\ liquid\ fraction\ [\%] \div 100 \times liquid\ volume[g]$$

The protein yield in pellet after S/L separation is given in **Table 21**. The Rapidase Power and Validase TRL combination shows high protein yield even in 30% suspension concentration.

Table 21. Protein content in the pellet after S/L separation

Suspension concentration [w/w]	**Enzyme**	**Protein yield in the pellet [%]**
20%	Blank	43.8
30%	Blank	42.4
40%	Blank	41.0
20%	5% Rapidase Press and 5% Validase TRL	51.3
30%	5% Rapidase Press and 5% Validase TRL	48.3
40%	5% Rapidase Press and 5% Validase TRL	43.2

CLAIMS

1. A process for augmenting the protein content of a plant protein meal, comprising incubating the plant protein meal in the presence of enzyme activities, wherein the enzyme activities comprise:

a) a pectinase,

b) a cellulase,

and wherein the pH of the incubation mixture is at least 3.6 and wherein the plant protein meal is hexane extracted rapeseed meal,

said method further comprising separating the dry matter from the liquid matter and collecting the dry matter to obtain an augmented plant protein meal.

2. A process according to claim 1, wherein:

a) the pectinase is present in an amount of at least 1500 AVJP units per gram of protein of the dry plant protein meal starting material, and

b) the cellulase is present in an amount of at least 30 CXU units per gram of protein of the dry plant protein meal starting material.

3. A process according to claim 1 or 2, wherein the pectinase comprises arabinofuranosidase activity, and wherein the arabinofuranosidase activity is present in an amount of at least 20 ARF units per gram of protein of the dry plant protein meal starting material.

4. A process according to claim 3, wherein the cellulase comprises one or more of activities selected from the group consisting of: a galactosidase, a glucanase, a mannanase, and a xylanase.

5. A process according to any one of the preceding claims, wherein the pH of the incubation mixture at the start of the incubation is at least 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, or at least pH 4.5.

6. A process according to any of the preceding claims, wherein the temperature of the incubation mixture is between 35 ºC and 60 ºC, and/or wherein the incubation time is between 1 and 72 hours, preferably between 2 and 28 hours, more preferably between 4 and 24 hours.

7. A process according to any one of the preceding claims, wherein the plant protein meal is processed substantially immediately after oil extraction and solvent evaporation, preferably without a drying step.

8. A process according to any one of the preceding claims, wherein an additional enzyme is present, preferably a hemicellulase and/or an endoglucanase and/or wherein the additional enzyme is a protease, preferably an acid protease, preferably an acid endoprotease, preferably a fungal

acid endoprotease, preferably an aspergillopepsin I protease, preferably derived from an *Aspergillus*, such as *Aspergillus niger*.

9. A process according to any one of the preceding claims, further comprising mixing rapeseed meal with water to form a slurry and/or washing the pellet and/or drying the pellet. separating the dry matter from the liquid matter.

10. A plant protein meal obtainable by any one of claims 1 to 9.

11. A food- or feed product comprising a plant protein meal according to claim 10.

12. Use of a plant protein meal according to claim 10 in the preparation of a food- or feed product.

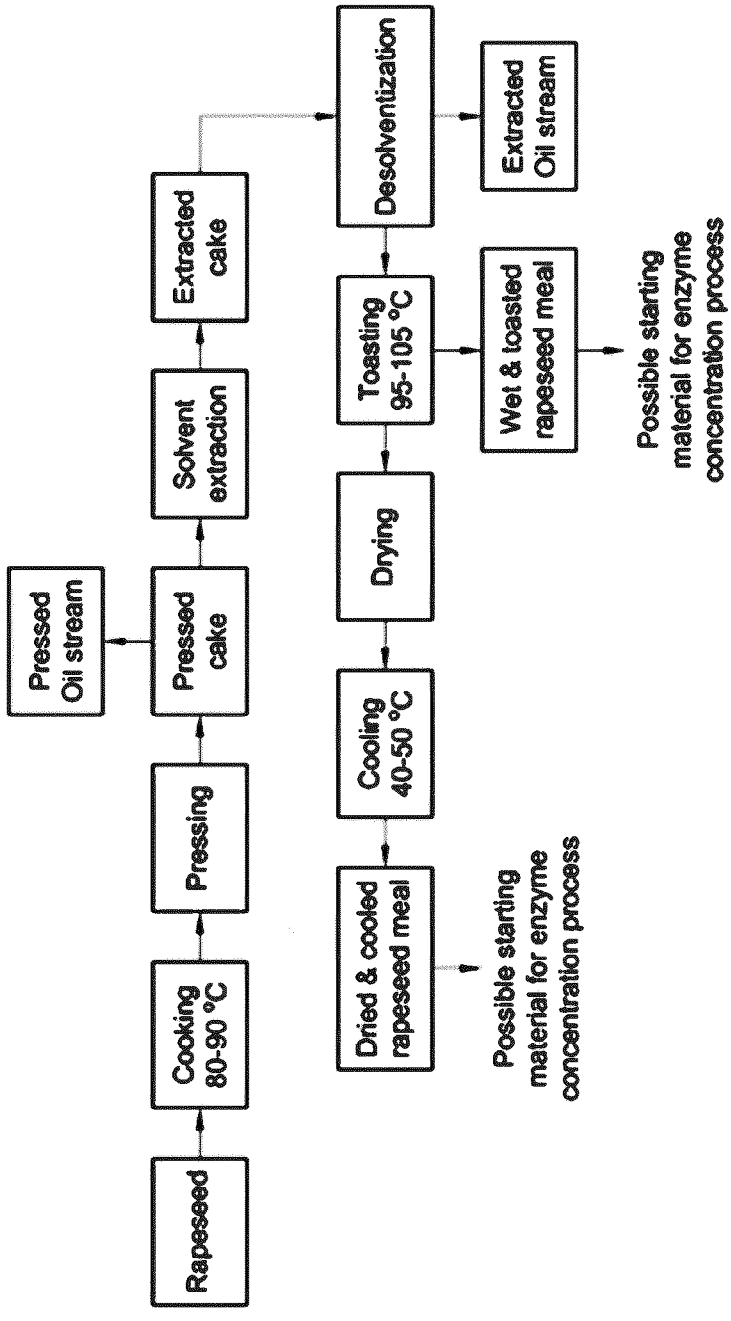
13. A process for the production of a food- or feed product, comprising contacting a plant protein meal according to claim 10 with a food- or feed product.

14. An enzyme composition comprising a pectinase and a cellulase, wherein:

a) the ratio of the pectinase activity / cellulase activity is between: 50 AVJP units - 500 AVJP units pectinase / 1CXU units - 10CXU units cellulase; and wherein optionally,

b) the ratio of arabinofuranosidase activity / cellulase activity is between: 0.7 ARF units - 7 ARF units arabinofuranosidase / 1CXU units - 10CXU units cellulase.

Fig. 1


Rapeseed
Cooking 80-90 °C
Pressing
Pressed cake
Pressed Oil stream
Solvent extraction
Extracted cake
Desolventization
Extracted Oil stream
Toasting 95-105 °C
Wet & toasted rapeseed meal
Possible starting material for enzyme concentration process
Drying
Cooling 40-50 °C
Dried & cooled rapeseed meal
Possible starting material for enzyme concentration process

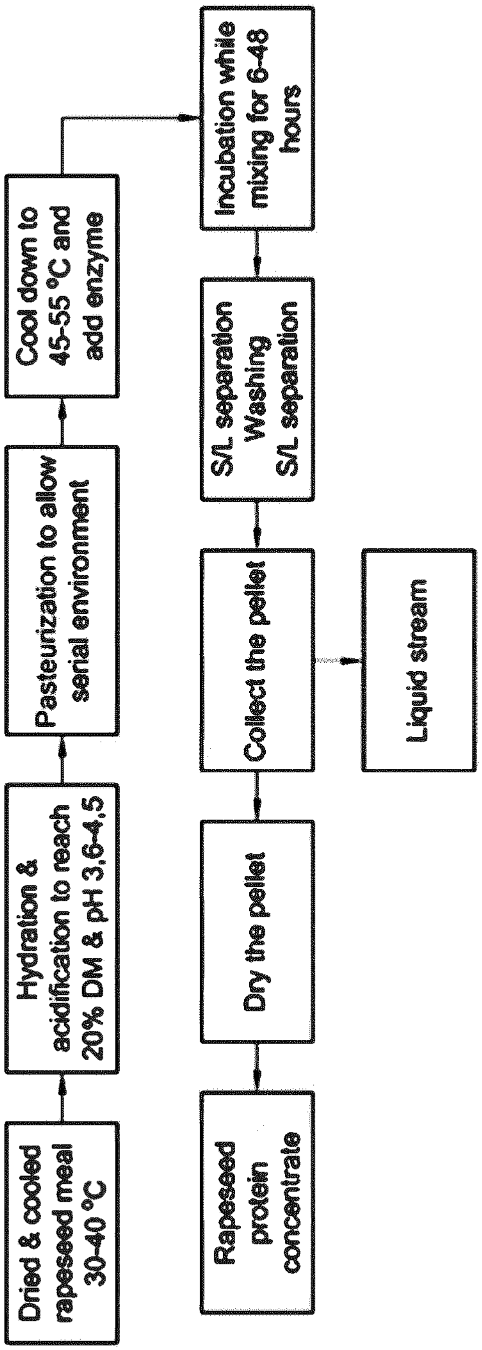

Dried & cooled rapeseed meal 30-40 °C
Hydration & acidification to reach 20% DM & pH 3,6-4,5
Pasteurization to allow serial environment
Cool down to 45-55 °C and add enzyme
Incubation while mixing for 6-48 hours
S/L separation Washing S/L separation
Collect the pellet
Liquid stream
Dry the pellet
Rapeseed protein concentrate

Fig. 2

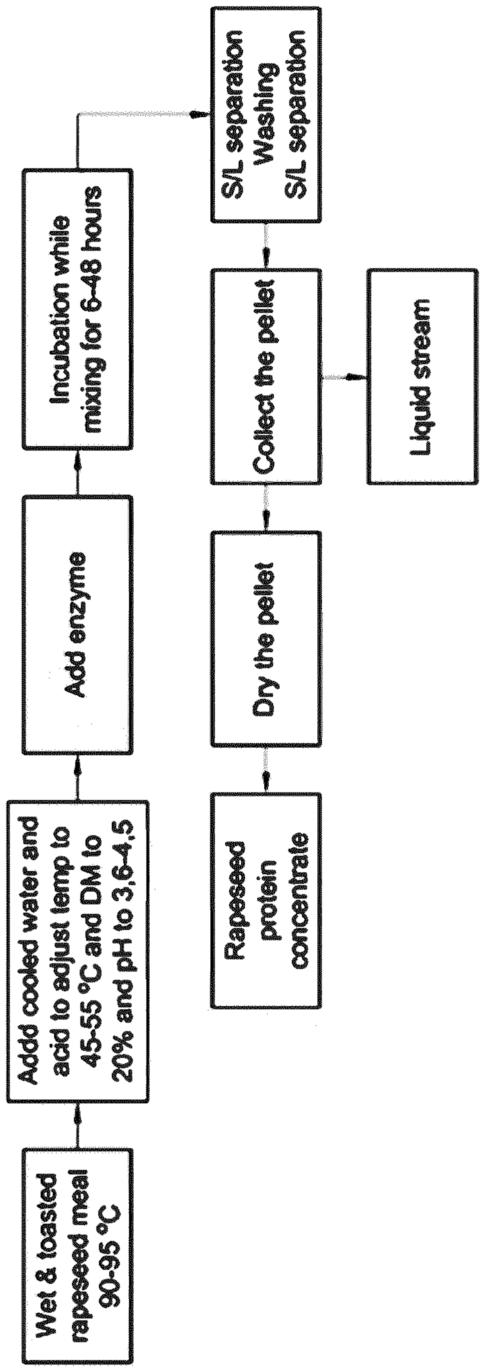

Wet & toasted rapeseed meal 90-95 °C
Addd cooled water and acid to adjust temp to 45-55 °C and DM to 20% and pH to 3,6-4,5
Add enzyme
Incubation while mixing for 6-48 hours
S/L separation
Washing
S/L separation
Collect the pellet
Liquid stream
Dry the pellet
Rapeseed protein concentrate

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/068892

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23J1/14 A23J3/34 A23K10/14 A23K10/30 A23K20/147
A23L33/185 C12N9/42

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23J A23L A23K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 105 779 543 A (UNIV WUHAN POLYTECHNIC) 20 July 2016 (2016-07-20) abstract; claims 1-7; examples 1-3	1-13

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2024	09/10/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bender, Pia

1

International application No
PCT/EP2024/068892

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Rodrigues Ivo M. ET AL: "bioresources.com", , 18 February 2014 (2014-02-18), pages 1-16, XP093208422, Retrieved from the Internet: URL:https://bioresources.cnr.ncsu.edu/wp-content/uploads/2016/06/BioRes_09_2_2010_Rodrigues_CR_Enzy_Hydrol_Rapeseed_Meal_Carbohydrates_4812.pdf [retrieved on 2024-09-26] Introduction Rapeseed Meal Screening and Chemical Composition Enzymatic Hydrolysis of Treated Rapeseed Meal; figures 2, 7; tables 1, 3-5 -----	1,2,5,6, 8-14
X	WO 2012/014227 A1 (ADVANCED ENZYME TECHNOLOGIES LTD [IN] ET AL.) 2 February 2012 (2012-02-02) examples 1, 3 -----	14
X	WO 2013/170017 A2 (UNIV AKRON [US]) 14 November 2013 (2013-11-14) table 1 -----	14
A	CN 107 373 011 A (HEFEI JINGGU RICE PROC CO LTD) 24 November 2017 (2017-11-24) the whole document -----	1-14
A	WO 2008/144939 A1 (CA MINISTER AGRICULTURE & FOOD [CA] ET AL.) 4 December 2008 (2008-12-04) page 45, paragraph 00163 - page 46, paragraph 00164; table 7 -----	1-14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/068892

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 105779543 A	20-07-2016	NONE	
WO 2012014227 A1	02-02-2012	NONE	
WO 2013170017 A2	14-11-2013	CA 2872907 A1	14-11-2013
		US 2015118730 A1	30-04-2015
		US 2018118794 A1	03-05-2018
		WO 2013170017 A2	14-11-2013
CN 107373011 A	24-11-2017	NONE	
WO 2008144939 A1	04-12-2008	CA 2688464 A1	04-12-2008
		DK 2155779 T3	18-02-2013
		EP 2155779 A1	24-02-2010
		US 2010249378 A1	30-09-2010
		WO 2008144939 A1	04-12-2008