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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 February 2012 (02.02.2012)

PCT

(10) International Publication Number
WO 2012/014227 A1

(51) International Patent Classification:
C12N 9/00 (2006.01)

(21) International Application Number:
PCT/IN2011/000495

(22) International Filing Date:
26 July 2011 (26.07.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2109/MUM/2010 26 July 2010 (26.07.2010) IN

(71) Applicant *(for all designated States except US)*: **ADVANCED ENZYME TECHNOLOGIES LTD.** [IN/IN]; Sun Magnetica, A Wing, 5th Floor, Accolade Galaxy, L.I.C. Service Rd., Louiswadi, Thane(W) 400 604, Maharashtra (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RATHI, Chandrakant, Laxminarayan** [IN/IN]; c/o Advanced Enzyme Technologies Ltd., Sun Magnetica, A Wing, 5th Floor, Accolade Galaxy, L.I.C. Service RD., Louiswadi, Thane(W) 400 604 Maharashtra (IN). **PRADHAN, Saylee** [IN/IN]; c/o Advanced Enzyme Technologies Ltd., Sun Magnetica, A Wing, 5th Floor, Accolade Galaxy, L.I.C. Service RD., Louiswadi, Thane(W) 400 604 Maharashtra (IN). **WANI, Ahila** [IN/IN]; c/o Advanced Enzyme Technologies Ltd., Sun Magnetica, A Wing, 5th Floor, Accolade Galaxy, L.I.C. Service RD., Louiswadi, Thane(W) 400 604 Maharashtra (IN).

(74) Agents: **BHATTAD, Uma** et al.; Krishna & Saurastri Associates, 74/F, Venus, Worli Sea Face, Mumbai - 400 018, Maharashtra (IN).

(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, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, 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, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2012/014227 A1

(54) **Title**: AN ENZYME PREPARATION AND METHOD FOR PREPARING PROTEIN CONCENTRATE FROM SOY PROTEIN CONTAINING MATERIALS

(57) **Abstract**: An enzyme preparation for processing a material containing at least one source of soy protein comprising at least at least 10% of at least one pectinolytic enzyme having an activity of at least 3,50,000 u/gm; at least 1% of at least one glucosidolytic enzyme having an activity of at least 200 u/gm at least; and at least 20% at least one glucanolytic enzyme having an activity of at least 1,50,000 u/gm in an inert filler.

TITLE : AN ENZYME PREPARATION AND METHOD FOR PREPARING PROTEIN CONCENTRATE FROM SOY PROTEIN CONTAINING MATERIALS

Technical Field

The present invention relates to an enzyme preparation and method for providing protein concentrates from protein containing materials.

Background

Protein concentrate are widely used as functional and nutritional ingredients in a variety of food products. They are prepared from vegetable as well as animal sources. Important vegetable sources include soya bean, sunflower, peanuts, rapeseed, sesame, milk casein etc. Soya bean contain the highest amount of protein among the legume family. It is widely cultivated for high quality soy protein concentrates and isolates. Soy protein concentrate are generally prepared using aqueous or alcohol extraction methods. The alcohol extraction method results in denaturation of some of the protein as well isoflavones while the aqueous extraction method does not provide a good yield neither it is efficient in removing non-digestible oligosaccharides from protein concentrates.

Soya bean endosperm contains oligosaccharides such as raffinose, stachyose, verbascose. Due to this use of soy and soya related products for human consumption has been long associated with production of abdominal bloating, rumbling and flatus experienced by vegetarians and other heavy soyfood eaters. Verbascose, stachyose, raffinose have been known to be a major cause of flatulence in humans and animals. In the absence of alpha-galactosidases in the mammalian intestinal mucosa, these oligosaccharides escape digestion and are not absorbed. As a consequence, the active microflora in the ileum, and colon, of the large intestine ferment them to form excessive levels of rectal gas, primarily carbon dioxide and hydrogen. In some instances, undigested starch and other carbohydrates contribute to the flatulent effect of diets.

It is therefore desirable to obtain protein concentrate having low levels of oligosaccharides using a method that preserves the proteins from denaturation and losing their nutritional

value. Accordingly, many methods have been put forward for production of protein concentrate having low amount of oligosaccharides among which the promising ones are those that use enzymes because of their specificity with regard to the reaction they catalyze and the substrate they act on.

Methods involving use of carbohydrate degrading enzyme such as cellulase, xylanase, mannanase etc have been described in EP 942 922 wherein enzymatic degradation of oligosaccharide is carried out during protein isolation to obtain a mixture of proteins, with hydrolysed carbohydrates and simple sugars. Similarly EP 1309 249 describes a method whereby soy protein product is obtained having a modified sugar profile. This is achieved using enzymatic degradation of oligosaccharides present in the extraction mixture.

Proteins are highly hygroscopic and in general require large quantities of water during extraction. Conventional aqueous extraction methods, even the ones using enzymes employ anywhere between 400% to 600% water by weight of soya flour for extraction purpose. Removing such large quantity of water creates operational problems during the extraction method. The water is ultimately released as effluent having high BOD/COD levels. This posses an environmental hazard as well as drives up the cost of the final product.

There is therefore a need for an efficient and environment friendly method which can provide protein concentrate with better quality at affordable prices.

Summary

According to an aspect, the present invention is directed to an enzyme preparation for processing a material containing at least one source of soy protein comprising at least one pectinolytic enzyme having an activity of at least 3,50,000 u/gm; at least one glucosidolytic enzyme having an activity of at least 200 u/gm; and at least one glucanolytic enzyme having an activity of at least 1,50,000 u/g in an inert filler.

According to another aspect, the present invention relates to use of the enzyme preparation for obtaining a soy protein concentrate.

The invention of the present invention further relates to a method for providing soy protein concentrate comprising the steps of contacting a material comprising at least one source of soy protein with the enzyme preparation in a range of 0.025-1% at a moisture level of 30-70%; incubating the material with the enzyme preparation for 1 to 4 hours; and, processing the material to obtain a soy protein concentrate.

Detailed Description of the Invention

The present invention provides an enzyme preparation and a method for providing protein concentrate from protein containing materials. The enzyme preparation and the accompanied method of the present invention can be used on protein containing materials of vegetable origin, more preferably on cereals and legumes as they contain considerable amount of proteins. Among legumes, soya bean is preferred as it has the highest protein content of all cereals and legumes with inherent protein content ranging from 40% to 50%.

It was surprisingly found that the enzyme preparation and the accompanied method when used on a material containing soya protein are capable of reducing the raffinose and stachyose content of the soy containing material to about 90-100%, preferably between 95-100 %. Further the enzyme preparation and method are capable of reducing the trypsin inhibitor content and substantially increasing the protein content and the PDI (protein dispersibility index). The soy protein concentrate further has better organoleptic properties as compared to the protein concentrate obtained by conventional methods.

According to an embodiment, the protein concentrate obtained by the present invention contains more than 50% protein. Preferably, the protein concentrate obtained contains more than 55% protein. More preferably, the protein concentrate contains more than 60% protein.

In accordance with the present invention, the PDI is increased to about 75-90%, preferably, the PDI is increased to above 85%. Further, the amount of water that is used for the method is substantially reduced.

The enzyme preparation of the present invention comprises enzymes in an effective amount so as to reduce raffinose and stachyose content with improved efficiency and/or increased yield.

According to one embodiment of the present invention, an enzyme preparation for processing a material containing at least one source of soy protein comprising at least at least 10% of at least one pectinolytic enzyme having an activity of at least 3,50,000 u/gm; at least 1% of at least one glucosidolytic enzyme having an activity of at least 200 u/gm at least ; and at least 20% at least one glucanolytic enzyme having an activity of at least 1,50,000 u/gm in an inert filler.

According to an embodiment of the invention, the pectinolytic enzymes that are incorporated in the enzyme preparation have an activity of at least 3,50,000 u/gm. Preferably, the pectinolytic enzymes of the invention have an activity between 3,50,000 – 6,50,000 u/gm. Non-limiting example of pectinolytic enzymes include pectinase.

According to another embodiment of the invention, the glucosidolytic enzymes that are incorporated in the enzyme preparation have an activity of at least 200 u/gm. Preferably, the glucosidolytic enzymes of the invention have an activity between 200 – 1000 u/gm. Without

limitation, glucosidolytic enzymes incorporated in the enzyme preparation of the present invention include beta glucosidase and amyloglucosidase.

According to yet another embodiment of the invention, the glucanolytic enzymes that are incorporated in the enzyme preparation have an activity of at least 1,50,000 u/gm. Preferably, the glucanolytic enzymes of the invention have an activity between 1, 50,000 – 3, 50,000 u/gm. Non-limiting examples of glucanolytic enzymes includes beta glucanase.

According to a still another embodiment of the invention, the enzyme preparation optionally further comprises one or more enzymes selected from enzymes having cellulolytic activity, rnananolytic activity, alpha galactosidolytic activity, Xylanolytic activity or amylolytic activity. Enzymes having such activity which can be employed in the enzyme preparation of the present invention include but are not limited to mannanase, amylase, alpha galactosidase, xylanase etc.

According to a further embodiment of the invention, the enzyme preparation is optionally augmented with additional proteolytic, and cell wall degrading enzymes depending on the type of protein material used. Examples of such enzyme include but not limited to arabinase, protease, and /or beta glucanase, pentosanase, polygalactrunase, pectin methyl esterase, phytase, endocellulase, hemicellulase used either alone or in combination thereof.

According to a preferred embodiment of the invention, the enzyme preparation optionally further comprises at least one enzyme selected from enzymes having xylanase activity, mannanase activity, esterase activity, protease activity, phytase activity or amylase activity.

According to an embodiment of the invention, the enzymes having xylanase activity of at least 5000 u/gm are incorporated. According to a preferred embodiment, xylanases having an activity between 5,000–30,000 u/gm are incorporated. Non-limiting example of xylanase incorporated in the invention is pentosanase.

According to an embodiment of the invention, the enzymes having mannanase activity of at least 10,000 u/gm are incorporated. According to a preferred embodiment, mannanases having an activity between 10,000–50,000 u/gm are incorporated.

According to an embodiment of the invention, the enzymes having esterase activity of at least 200 u/gm are incorporated. According to a preferred embodiment, esterases having an activity between 200- 1000 u/gm are incorporated. Non-limiting example of the enzyme having esterase activity is pectin methyl esterase.

According to an embodiment of the invention, the enzymes having protease activity of at least 10,000 u/ gm are incorporated. According to a preferred embodiment, proteases having an activity between 10,000 -50,000 u/gm are incorporated. According to an embodiment of the invention, proteases are selected from at least one of basic, neutral or acid protease. According to a preferred embodiment, the enzyme having protease activity is acid protease. According to yet another preferred embodiment, the enzyme having protease activity is fungal acid protease.

According to an embodiment of the invention, enzymes having phytase activity of at least 2,000 u/ gm are incorporated. According to a preferred embodiment, amylases having an activity between 2,000 – 8,000 u/ gm are incorporated.

According to an embodiment of the invention, the enzymes having amylase activity of at least 3,500 u/ gm are incorporated. According to a preferred embodiment, amylases having an activity between 3,500 – 6,000 u/ gm are incorporated. According to an embodiment of the invention, amylases are selected from at least one of alpha amylase or beta amylase. According to a preferred embodiment, the enzyme having amylase activity is alpha amylase. According to yet another preferred embodiment, the enzyme having amylase activity is fungal alpha amylase.

The enzyme preparation further comprises at least one inert filler selected from but not limited to lactose, sucrose, starch derivatives, microcrystalline cellulose, malto dextrin or the like.

According to an embodiment of the invention, the source of soy protein is selected from but not limited to soya grits, defatted soy flour or soya meal.

In accordance with another embodiment, the invention relates to use of the enzyme preparation of to provide a soy protein concentrate from a material containing at least one source of soy protein.

Further, the invention relates to a method for providing soy protein concentrate comprising the steps of contacting a material comprising at least one source of soy protein with the enzyme preparation of the present invention in a range of 0.025-1% at a moisture level of 30-70%; incubating the material with the enzyme preparation for 1 to 4 hours; and, processing the material to obtain a soy protein concentrate.

According to an embodiment, the method comprises steps of spraying water onto a material containing at least one source of soy protein to moisten it and treating the said material with the enzyme preparation of the present invention in a range of 0.025-1% at a moisture level of 30-70%, incubating the material with the enzyme preparation for 1 to 4 hours. The enzyme preparation acts on oligosaccharides, cellulosic material and other carbohydrates present in the said material; solubilises them and increases the availability of the inherent protein and quality of the final product. The material is then processed to obtain a protein concentrate having little or no oligosaccharide content.

According to a preferred embodiment, the method comprises spraying an enzyme preparation of the present invention in a range of 0.025-1% at a moisture level of 30-70% on to a material containing at least one source of soy protein and incubating the material with the enzyme preparation for 1 to 4 hours. The enzyme preparation acts on oligosaccharides, cellulosic material and other carbohydrates present in the said material; solubilises them and increases the availability of the inherent protein and quality of the final product. The material is then processed to obtain a protein concentrate having little or no oligosaccharide content.

According to an embodiment, the pH of the method pH is maintained in the acidic range more preferably between pH 4 to 6. More preferably, the pH is maintained at 4.7. The pH of the method is regulated by spraying acid such as Hydrochloric acid or formic acid, acetic acid, citric acid and the like.

According to another embodiment, the method is carried out at an optimum temperature for the functioning of the enzyme preparation. According to a preferred embodiment, the temperature is maintained at 40^{o}-60^{o} C. More preferably, the temperature is maintained at 55^{o} C.

According to a further embodiment, the material is incubated for 1-4 hours, preferably for 2 hours. The method optionally further comprises agitation of the material during incubation.

According to an embodiment of the invention, the processing step involves drying the material at a temperature of 50°-60° C and pulverization to obtain a powdered concentrate. Preferably, drying of the material is carried out at a temperature of 55° C. However, any suitable conditions and method may be used to obtain a protein concentrate in a desired form.

The present invention is described in further detail by the following non-limiting examples:

EXAMPLES

Example 1

Enzyme Preparation 1

The enzyme preparation of the present invention comprises pectinase having activity 350000 – 550000 u/gm with the percent ranging from 10% -18%, beta glucanase enzyme having activity range from 150000 -300000 u/gm with percent ranging from 20-35%, beta glucosidase enzyme having activity range from 200 -700 u/gm with percent ranging from 1-4%, with inert filler like lactose, maltodextrin, starch derivatives, microcrystalline cellulose Q.S.

The following results were obtained with different concentrations of the enzyme preparation:

Sample	Moisture content by infrared moisturometer	Protein content %	PDI %	Raffinose Hydrolysis %	Trypsin inhibitor units mg/gm	Starch by enzymatic method %

Control (Soya material without enzyme)	4.92	52.0	80.75	-	124.21	18.4
Preparation 1 0.025%	4.98	54.2	80.89	75.8%	115.1	17.4
Preparation 1 0.05%	4.88	54.8	80.9	75.9%	110.2	15.6
Preparation 1 0.075%	4.78	54.9	81.2	76.12%	110.2	14.8
Preparation 1 0.1%	4.82	55	81.3	76.2%	105.8	14.7
Preparation 1%	4.81	58	81.4	76.9%	101.2	14.5

Example 2

Enzyme Preparation 2

The enzyme preparation of the present invention comprises pectinase enzyme having activity 550000 – 650000 u/gm with the percent ranging from 40% -50%, beta glucanase enzyme having activity range from 250000 -350000 u/gm with percent ranging from 25-35%, amylo glucosidase enzyme having activity range from 500 -1000 u/gm with percent ranging from 2-5%, with inert filler like lactose, maltodextrin, starch derivatives, microcrystalline cellulose Q.S

The following results were obtained for the soy protein concentrate with different concentrations of the enzyme preparation:

Sample	**Moisture content by infrared moisturometer**	**Protein content %**	**PDI %**	**Raffinose Hydrolysis %**	**Trypsin inhibitor units mg/gm**	**Starch by enzymatic method %**
Control (Soya material without enzyme)	4.92	52.0	80.75	-	124.21	18.4
Preparation 2 0.025%	4.89	54.8	80.89	76.8%	120.4	17.6
Preparation 2 0.05%	4.87	55.1	80.98	77%	118.2	16.8

Preparation 2 0.075%	4.78	55.4	81.5	77.2%	115.2	16.7
Preparation 2 0.1%	4.82	55.9	81.6	77.28%	104.8	16.4
Preparation 2 1%	4.88	58.3	81.9	78.9%	102.2	15.2

Example 3

Enzyme Preparation 3

The enzyme preparation of the present invention comprises pectinase enzyme having activity 350000 – 550000 u/gm with the percent ranging from 10% -18%, beta glucanase enzyme having activity range from 150000 -350000 u/gm with percent ranging from 20-35%,beta glucosidase enzyme having activity range from 200 -700 u/gm with percent ranging from 1-4%,, pentosanase enzyme having activity 5000-30000 u/gm with percent ranging from 1- 3.5%, esterase enzyme ranging from 200 -1000u/gm with percent range from 2-5%,mannanase ranging from 10000-50000 u/gm with the percentage range of 2-6%,fungal acid protease having activity 10000 to 50000 u/gm with the percentage range of 1% to 6%,phytase enzyme having activity between 2000-8000 u/gm with percent range from 2-8%, Fungal alpha amylase ranging from 3500 to 6000 u/gm with the percentage of 1% to 6% with inert filler like lactose, maltodextrin, starch derivatives, microcrystalline cellulose Q.S

The following results were obtained for the soy protein concentrate with different concentrations of the enzyme preparation:

Sample	Moisture content by infrared moisturometer	Protein content %	PDI %	Raffinose Hydrolysis %	Trypsin inhibitor units mg/gm	Starch by enzymatic method %
Control (Soya material without enzyme)	4.92	52.0	80.75	-	124.21	18.4
Preparation 3 0.025%	4.72	61.28	86.54	99.89%	3.58	8.12
Preparation 3 0.05%	4.98	61.99	86.99	99.98%	2.01	7.58
Preparation 3 0.075%	4.73	61.99	86.99	100%	2.01	7.58
Preparation 3 0.1%	4.81	61.99	86.99	100%	2.01	7.58
Preparation 3 1%	4.72	61.99	86.99	100%	2.01	7.58

Example 4

Enzyme Preparation 4

The enzyme preparation of the present invention comprises pectinase enzyme having activity 350000 – 550000 u/gm with the percent ranging from 10% -18%, beta glucanase enzyme having activity range from 150000 -350000 u/gm with percent ranging from 20-35%, beta glucosidase enzyme having activity range from 200 -700 u/gm with percent ranging from 1-4%,, pentosanase enzyme having activity 5000-30000 u/gm with percent ranging from 1- 3.5%, esterase enzyme ranging from 200 -1000u/gm with percent range from 2-5%, mannanase ranging from 20000-50000 u/gm with the percentage range of 5-10%, fungal acid protease having activity 30000 to 50000 u/gm with the percentage range of 2% to 8% phytase enzyme having 5000-8000 u/gm with percent range from 5-10%, Fungal alpha amylase ranging from

3500 to 6000 u/gm with the percentage of 5% to 6% with inert filler like lactose, maltodextrin, starch derivatives, microcrystalline cellulose Q.S

The following results were obtained for the soy protein concentrate with different concentrations of the enzyme preparation 3 (example 3):

Sample	**Moisture content by infrared moisturometer**	**Protein content %**	**PDI %**	**Raffinose Hydrolysis %**	**Trypsin inhibitor units mg/gm**	**Starch by enzymatic method %**
Control (Soya material without enzyme)	4.92	52.0	80.75	-	124.21	18.4
Preparation 4 0.025%	4.73	61.28	86.54	99.89%	3.58	8.12
Preparation 4 0.05%	4.81	61.99	86.99	99.98%	2.01	7.58
Preparation 4 0.075%	4.77	61.99	86.99	100%	2.01	7.58
Preparation 4 0.1%	4.8	61.99	86.99	100%	2.01	7.58
Preparation 4 1%	4.76	61.99	86.99	100%	2.01	7.58

Example 5

EFFECT OF BOUND AND UNBOUND MOISTURE STUDY AT DIFFERENT MOISTURE LEVELS

Process:

The Initial moisture was checked immediately (after spraying of solution). The final moisture after 2 hours of enzyme reaction at 55 degrees was checked.

The moisture content was checked by oven method

From the table below, it was observed that the enzymatic method of the present invention acts on polysaccharides present in the protein containing material, solubilises them and increases the final moisture of the product which in turn increases the protein content of the final product.

Sample	%initial moisture after spraying the solution immediately	% final moisture after enzyme reaction at 55 degrees 2 hours	**% bound moisture release(final-initial/initial)**
Control (With 40% moisture without enzyme)	40.09	40.1	**Nil**
Enzyme preparation 3 (0.05%) with 40% moisture	40.1	44.74	**11.57%**
Control With 50% moisture without enzyme	50.92	50.98	**Nil**
Enzyme preparation 3 (0.05%) With 50% moisture	50.1	56.1	**11.97%**
Control With 60% moisture without enzyme	60.15	60.12	**Nil**
Enzyme preparation 3 (0.05%) With 60% moisture	60.2	67.7	**12.45%**
Control With 70% moisture without enzyme	70.55	70.52	**Nil**
Enzyme preparation 0.05% With 70% moisture	70.01	78.89	**12.68%**

Example 6

Experiment done on soya bean meal with enzyme preparation 3 of the invention:

Soyabean meal specifications

Moisture content	8.5 %
Protein	46%
Sucrose	4.44%
Glucose	0.255%
Raffinose containing oligosaccharides	5.43%

Process

SUBSTRATE: SOYABEAN MEAL AT 55 DEGREES AT PH 4.7 WITH 25% -50% MOISTURE

100 gm of soybean meal was taken in a bottle and initial moisture content was checked (8.5% moisture). The enzyme preparation 3 (Example 3) of the invention was sprayed by laboratory sprayer. The bottle was closed with the lid. The samples are mixed in the laboratory blender for 10 seconds and kept in a water bath at 50 degrees for 2 hrs (under closed condition). After 2 hours, the sample was removed from the water bath and dried by laboratory drying process

Various parameters of the soy protein concentrate were studied e.g. moisture content, protein content, soluble sugars, fats, hydrolyzed raffinose and stachyose content using methods known in the art. The following is the analysis of the soya protein concentrate obtained on treatment:

Sample	Temperature degree celcuis	Moisture in %	Dosage	Protein content	Glucose gm/100gm	Sucrose gm/100gm	Rafiinose gm/100gm
Control	50 degrees	25%	-	46%	0.255	4.44	5.43
Enzyme preparation 3	50 degrees	25%	0.05%	50.2	8.674	3.49	0.3(94.47%) reduction
	50 degrees	30%	0.05%	51.75	10.84	3.4	0.05(99.07 %) reduction
	50 degrees	40%	0.05%	52.8	17.3	3.08	0.001 (99.98) % reduction
	50 degrees	50%	0.05%	53.3	18.6	2.42	0.0001 (100%) Reduction.

Example 7

SUBSTRATE: SOYA GRITS, 50% MOISTURE AND DOSAGE OF ENZYME PREPARATION 3 (0.05%), PH 4.7, Temp: 50c to 55c.

Process

5 KG of soya grits were taken and dry tempered for 30 minutes. Soya grits were treated with 2.5gm of enzyme ENZYME PREPARATION+15gm citric acid and 2 kg of water. PH was maintained at 4.7. The sample was added in the blender and after 1 hour of incubation temperature attaining 55 degrees equilibrium (PH 5.5). The sample was agitated every 20min for 15-20 seconds. After 1 more hour of incubation, temperature was checked again and maintained at 50-55 degrees (ph=6). 0.5 liters water +5 gm citric acid was added to the batch. The sample was kept for 2 hours of incubation, pH was checked and maintained accordingly. After 1 more hour of incubation, the liquid and solid was separated and collected. The sample was dried at 55 degrees for 20 hours to get soy protein concentrate with upto 5% moisture.

Various parameters of the soy protein concentrate were studied e.g. moisture content, protein content, soluble sugars, fats, hydrolyzed raffinose and stachyose content using methods known in the art. The following is the analysis of the soya protein concentrate obtained on treatment:

PARAMETERS	CONTROL SOYA GRIT	TRETAED SOYA GRITS with enzyme preparation 3
MOISTURE %	9.24%	5.89%
PROTEIN %	50.72%	61.81%
SOLUBLE SUGAR%	0.892%	2.170%
FAT %	18.16%	20.91%
RAFFINOSE CONTAINING OLIGOSACCHARIDES		
GLUCOSE (%)	0.258	1.789
SUCROSE (%)	4.21	5.259
RESIDUAL RAFFINOSE (%)	6.560	0.030
RESIDUAL STACHOSE (%)	7.87	0.0481
% RAFFINOSE HYDROLYSED	-	99.54%
%STACHYOSE HYDROLYSED	-	99.38%
TRYSPIN INHIBITOR Mg/gm	111.28 Mg/gm	0.102 Mg/gm
Total phosphorus by AOCS Method %	1.1	1.31
Phytin phosphorus % Titration method	0.231	0.168
Non phytin phosphorus % (subtraction of phytin phosphorus from total phosphorus)	0.869	1.142

Example 8

SUBSTRATE: SOYA GRITS, 70% MOISTURE AND DOSAGE OF ENZYME PREPARATION 3 (0.05%), PH 4.7, Temp: 50c to 55c.

Process

Soya grits were put in a blender and ground and sieved to get different fraction which were then separated. Taken the fraction as 3 kg of higher particle size (6 to 8 mm), 1 kg of lower particle fraction(3 to 5 mm) and 1 kg of powder(0.5 to 1 mm). 2.5 kg of water were added with

enzyme at dosage of 500gms/T, 5 gm of citric acid. Enzyme reaction was carried out at 55 degrees for 2 hours in blender. The samples were air dried over night.

Various parameters of the soy protein concentrate were studied e.g. moisture content, protein content, soluble sugars, fats, hydrolyzed raffinose and stachyose content using methods known in the art. The following is the analysis of the soya protein concentrate obtained on treatment:

PARAMETERS	CONTROL SOYA GRIT	TREATED SOYA GRIT with enzyme preparation 3
MOISTURE %	9.46%	7.43%
PROTEIN %	50.52%	61.06%
SOLUBLE SUGAR%	0.892%	1.28%
FAT %	18.69%	20.91%
RAFFINOSE CONTAINING OLIGOSACCHARIDES		
GLUCOSE (%)	0.258	0.926
SUCROSE (%)	4.146	5.168
RESIDUAL RAFFINOSE (%)	6.581	0.1297
RESIDUAL STACHOSE (%)	7.1772	0.1556
% RAFFINOSE HYDROLYSED	-	98.13%
%STACHYOSE HYDROLYSED	-	97.83%
TRYSPIN INHIBITOR Mg/gm	101.28 Mg/gm	1.95 Mg/gm
Total phosphorus by AOCS Method %	1.1	1.36
Phytin phosphorus % Titration method	0.227	0.163
Non phytin phosphorus % (subtraction of phytin phosphorus from total phosphorus)	0.873	1.197

Example 9

SUBSTRATE: Defatted soya flour, 70% MOISTURE AND DOSAGE OF ENZYME PREPARATION 3 (0.05%), PH 4.7, Temp: 50c to 55c.

Process

5 KG of defatted soya flour were taken and 3.5 kg of water were added. The material was treated is with 500GM/T of enzyme +5gm citric acid to adjust ph to 4.7. The sample was added in the blender after attaining 55 degrees. Enzyme reaction was carried out at 55 degrees for 2 hours. The samples were dried.

Various parameters of the soy protein concentrate were studied e.g. moisture content, protein content, soluble sugars, fats, hydrolyzed raffinose and stachyose content using methods known in the art. The following is the analysis of the soya protein concentrate obtained on treatment:

PARAMETERS	CONTROL DEFATTED SOYA	TRETAED Defatted SOYA FLOUR with enzyme preparation 3
MOISTURE %	9.46%	9.50%
PROTEIN %	50.52%	62.98%
SOLUBLE SUGAR%	0.892%	3.670%
FAT %	18.69%	20.66%
RAFFINOSE CONTAINING OLIGOSACCHARIDES		
GLUCOSE (%)	0.258	0.8780
SUCROSE (%)	4.146	5.289
RESIDUAL RAFFINOSE (%)	6.581	0.031
RESIDUAL STACHOSE (%)	7.1772	0.048
% RAFFINOSE HYDROLYSED	-	99.48%
%STACHYOSE HYDROLYSED	-	99.40%
TRYSPIN INHIBITOR Mg/gm	101.28 Mg/gm	0.12 Mg/gm
Total phosphorus by AOCS Method %	1.1	1.26
Phytin phosphorus %(Titration method)	0.227	0.175
Non phytin phosphorus %(subtraction of phytin phosphorus from total phosphorus)	0.873	1.085

From the above examples, it can be seen that Soy Protein concentrates obtained using the enzymatic preparation and method of the present invention contains more than 60 % of protein by weight of dry solids. Raffinose and stachyose reduction (hydrolysis) is observed to be more than 90% and upto 100 %.

The improved proteins are obtained even when water is used in the range of 40% to 70%. This can be attributed to the catalytic activity of the enzyme preparation in accordance with teachings of the present invention. Inventors of the present invention have been able to break down the moisture holding capacity of the protein containing endosperms using enzymes thus making more moisture available for the enzyme reaction. This in turn enables the inventors to use low amount of water during the method and still obtain a protein concentrate with reduced amount of oligosaccharide. In addition to higher yield of protein concentrate obtained w/w of dry solid and reduction of oligosaccharides , cellulosic, and other carbohydrates content, the concentrate obtained also has a higher PDI, lesser tripsin inhibitor units and better organoleptic properties than the protein concentrate obtained by conventional method.

Various modifications may be made to the various embodiments of the present invention in part or whole without departing from the spirit and scope of the appended claims.

We claim:

1. An enzyme preparation for processing a material containing at least one source of soy protein comprising:
   (a) at least 10% of at least one pectinolytic enzyme having an activity of at least 3,50,000 u/gm;
   (b) at least 1% of at least one glucosidolytic enzyme having an activity of at least 200 u/gm at least ; and
   (c) at least 20% at least one glucanolytic enzyme having an activity of at least 1,50,000 u/gm in an inert filler.

2. The enzyme preparation as claimed in claim 1, wherein the pectinolytic enzyme is pectinase having an activity between 3,50,000 to 6,50,000 u/gm.

3. The enzyme preparation as claimed in claim 1, wherein the glucosidolytic enzyme is selected from at least one of beta glucosidase and amyloglucosidase having an activity between 200 to 1000 u/gm.

4. The enzyme preparation as claimed in claim 1, wherein the glucanolytic enzyme Is selected beta glucanase having an activity between 1,50,000 to 3,50,000 u/gm.

5. The enzyme preparation as claimed in claim 1, wherein the enzyme preparation optionally further comprises at least one enzyme selected from enzymes having xylanase activity, mannanase activity, esterase activity, protease activity, phytase activity or amylase activity.

6. The enzyme preparation as claimed in claim 5, wherein the enzyme having xylanase activity is pentosanase.

7. The enzyme preparation as claimed in claim 5, wherein the enzyme having esterase activity is pectin methyl esterase.

8. The enzyme preparation as claimed in claim 5, wherein the enzyme having protease activity is selected from proteases having basic, neutral or acid protease.

9. The enzyme preparation as claimed in claim 5, wherein the enzyme having amylase activity is selected from alpha amylase or beta amylase.

10. The enzyme preparation as claimed in claim 1, wherein the filler is selected form at least one of lactose, sucrose, microcrystalline cellulose or malto dextrin.

11. The enzyme preparation as claimed in claim 1, wherein the source of soy protein is selected from soya grits, defatted soy flour or soya meal.

12. Use of the enzyme preparation of claim 1 to provide a soy protein concentrate from a material containing at least one source of soy protein.

13. A method for providing soy protein concentrate comprising the steps of:
    (a) contacting a material comprising at least one source of soy protein with an enzyme preparation of claim 1 in a range of 0.025-1% at a moisture level of 30-70%;
    (b) incubating the material with the enzyme preparation for 1 to 4 hours; and,
    (c) processing the material to obtain a soy protein concentrate.

14. The method as claimed in claim 13, optionally further comprises agitation of the material during incubation.

15. The method as claimed in claim 13, wherein the processing comprises drying and pulverizing the material to obtain powdered soy protein concentrate.

17. The method as claimed in claim 13, wherein the treatment of the soybean material is carried out at a ph of 4-6.

18. The method as claimed in claim 17, wherein the treatment of the soybean material is carried out at a ph of 4.7.

19. The method as claimed in claim 13, wherein the treatment of the soybean material is carried out at a temperature of 40^{0}-60^{0} C.

20. The method as claimed in claim 19, wherein the treatment of the soybean material is carried out at a temperature of 55 ^{0}C.

21. The method as claimed in claim 13, wherein the source of soy protein is selected from soya grits, defatted soy flour or soya meal.

INTERNATIONAL SEARCH REPORT PCT/IN2011/000495 06.01.2012

International application No.
PCT/IN 11/00495

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/00 (2011.01)
USPC - 435/183
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)
USPC: 435/183
IPC: C12N 9/00 (2011.01)

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)
PubWEST(USPT,PGPB,EPAB,JPAB); Google; Google Patents
Search Terms: Activity, pectinase, glucosidase, glucanase, soy protein, amyloglucosidase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,958,015 A (Gay) 18 March 1976 (18.05.1976) col 3, ln 20-35; col 4, ln 1-50; col 6, ln 32-33; claim 5	1-15, 17-21
Y	US 3,640,723 A (Uhlig et al.) 08 February 1972 (08.02.1972) col 1, ln 41-42; col 2, ln 20-24	1-15, 17-21
Y	US 2009/0155238 A1 (Weiner et al.) 18 June 2009 (18.06.2009) para [0054]; [0668]	1-15, 17-21
Y	US 2010/0074996 A1 (Marcussen et al.) 25 March 2010 (25.03.2010) para [0024]; [0042]; [0044]; [0156]-[0157]	1-15, 17-21

☐ Further documents are listed in the continuation of Box C. ☐

* 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
21 December 2011 (21.12.2011)	06 JAN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774