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(54) Title: "NON-GMO WHEAT GLUTEN DERIVED GLUTAMINE RICH PEPTIDE BASED BIOSTIMULATING ORGANIC FERTILIZER AND PROCESS FOR PREPARATION THEREOF"

(57) Abstract: The present invention discloses herein non-GMO wheat gluten derived Glutamine rich peptide based biostimulating Organic Fertilizer that enhances photosynthesis and increases chlorophyll content in the plant and to the process for preparation thereof.

"Non-GMO Wheat Gluten Derived Glutamine Rich Peptide Based Biostimulating Organic Fertilizer and Process For Preparation Thereof"

Technical filed of the Invention:

The present invention relates to non-GMO wheat gluten derived Glutamine rich peptide based biostimulating Organic Fertilizer that enhances photosynthesis and increases chlorophyll content in the plant and to the process for preparation thereof.

Background and Prior art:

Every Farmer's dream is to achieve better seed germination for greater production of crops with minimum damages due to plant diseases which greatly impacts the farmer's life socially and economically. The use of synthetic fertilizers and pesticides although has improved the yields, but has also proved detrimental to human life and environment. Hence, the researchers felt a need to study the Bioenergetics and Biochemical aspects of plants, to improve the production of plants in terms of quality and yield which is environmental friendly.

For a plant to grow and thrive, it requires the following different nutrient elements: (i) Carbon (C), hydrogen (H) and oxygen (O) which are available plentiful in air and water; (ii) inorganic elements such as Nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg) as macronutrients in high concentrations; (iii) Micronutrients such as boron (B), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn).

Among these macro and micro nutrients, nitrogen is critical element not only for plants but for other living organisms also. It is required in the synthesis of many cellular components of plants including DNA, chlorophyll, enzymes and proteins. Atmospheric air is the main reservoir of nitrogen. Plant can absorb nitrogen in the form of some fixed and soluble ionic components like nitrate, nitrite and ammonium ions.

However, nutrient input in crop production systems have come under increased scrutiny in recent years due to the potential environmental impact from nitrogen and phosphorus containing inputs.

Modern, intensive agriculture employing continuous and extensive use of chemical fertilizers and pesticides results in loss of natural soil humus, damages fertility of top soil and decreases soil porosity. Artificial chemical fertilizers destroy soil microbial flora resulting in poor soil structure, aeration and decreases nutrient availability. Consequently, roots are oxygen deprived and do not absorb salts effectively. Unabsorbed nitrates are carried away by rainwater into rivers and lakes causing a threat to aquatic life. Excessive nitrates in soil elevate nitrate level in edible plants causing threat to people using them. Pests and diseases become more difficult to control as they become resistant to artificial pesticides.

Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects.

The use of organic fertilizers for improving plant growth and overall development is known very well. Various organic fertilizers are used for plant nutrition. Majority of the organic fertilizers used are farm yard manure, industrial oil cakes unsuitable for animal consumption such as castor oil cake, neem oil cake, linseed oil cake, rapeseed cake, also edible oil cakes such as ground nut cake, are used to improve soil fertility due to the presence of crude proteins, fats and complex carbohydrates. These cakes when added into soil get decomposed over a period of time by the action of bacteria present in soil. The bacteria multiply and the cakes are decomposed allowing uptake of nutrients by plants. However, complete extraction of nitrogen from the natural proteinaceous substances, oilseed cakes is not possible by simple soil application; hence they must be processed by intensive chemical procedures for effective nitrogen utilization. The procedures may introduce synthetic chemicals that are detrimental to environment.

To make aware the consumers that agricultural products used by them are actually obtained from organic ingredients without use of any chemicals and pesticides, farms are inspected as per Organic Standards. If they meet these standards they are certified organic and the produce from these farms is labelled as “Certified Organic Produce”. There are Organic standards for ‘Certified Organic Farming’ such as National Organic Program

(NOP) provided by USDA (US Department of Agriculture), National Program for Organic Production (NPOP) introduced in India, EU standards such as EC 834/2007 and 889/2008 from European Union, Japanese Agricultural Standards (JAS), and Australian Input Standards. These voluntary or legislated standards regulate production methods, farm inputs and in some cases final output for organic agriculture.

Several organic nitrogen fertilizers have been developed by various inventors employing microbial sources and plant, animal origin raw materials, however due to intermediate processes and use of synthetic chemicals for extraction these fertilizers have failed to adhere to the organic standards provided by the USDA.

As per the NOP organic standards by USDA, the use of following materials in organic farming fertilizer compositions is permitted, these include;

- Compost derived animal waste or other materials.
- Fresh plants material for incorporating nitrogen fixing into the soil.
- Raw animal manures.
- Animal and Plant materials – Non Synthetic such as fish meal, fish emulsion, blood meal, feather meal, soybean meal and products of the like nature.
- Non synthetic amino acids produced by plants, animals and microorganisms that have not been genetically modified and are extracted or isolated by hydrolysis or by physical or other non- chemical means that are considered non synthetic.

PCT Publication, WO2013/019933 claims organic fertilizer compositions of 1-6% nitrogen and 1-5% phosphorus comprising an effective amount of a non-genetically modified, enzymatically hydrolyzed plant protein as a nitrogen source and phytic acid as a phosphorus source for delivery to plants. However, the essential element concentrations provided by the compositions are low, the source of hydrolyzed plant protein is not mentioned.

US2008/0302151 relates to the manufacture of the soluble fertilizer for organic agriculture using soybean meal. The limitations of US'151 are that parameters of hydrolysis such as the temperature and pH to be maintained are not mentioned. During

the hydrolysis of soybean meal which contains 48% protein and 45-50% carbohydrate, the reaction mass becomes viscous and difficult to process because of the tendency of carbohydrates to swell and entrap water resulting in difficulty in filtration. Further, the process does not utilize a preservative for the liquid organic fertilizer product which being rich in amino acids and proteins is prone to microbial attack. The commercially available soymeal is solvent extracted using hexane and organic standard requires soymeal to be free from any solvent processing. However, there is no mention of richness of any amino acid, Peptide content and does not talk about the molecular weight of the final product in US'151.

US200803021151 claims the use of soy meal as soluble liquid fertilizer for organic agriculture. The raw material is soy meal with no other source mentioned.

US5290749 discloses the plant protein hydrolysate that is produced from Corn gluten, wheat gluten or soy protein. US'749 claim removal of carbohydrates from corn gluten and using the remaining protein mass for hydrolysis and producing protein hydrolysate. However, the protein hydrolysis of US'749 is used for inhibiting growth of undesirable plants in plot of soil and not as organic nitrogen fertilizer.

US5180597 describe a method to produce hydrolyzed vegetable protein and the use of this hydrolysate is for savory flavouring –food purpose. The product hydrolyzed vegetable protein is produced using corn gluten or wheat gluten using acid or enzyme hydrolysis, however, the end use as organic fertilizer is not mentioned in US'597.

US6264714 discloses the use of corn gluten as fertilizer without involving any processing technique such as hydrolysis and does not talk about wheat gluten or any other kind of gluten. Further, the undigested fertilizer i.e. corn gluten needs to be digested in soil by microorganism which needs long time to have beneficial effect on plants. In addition, US'714 lacks information on the content of key amino acid Glutamic peptide.

US4491464 claims the use of plant protein hydrolysate as effective nutrient uptake enhancer for potassium and phosphorous. Protein hydrolysate is a mixture of polypeptide, dipeptides and naturally occurring amino acids resulting from the hydrolysis of intact

proteins. The intact protein is plant protein. US'646 also claims a method of using Plant Protein Hydrolysate to increase Phosphorous (P) and potassium (K) content of plant tissue by using a ratio of polyphosphate blend to protein hydrolysate between 200: 1 to 20:1. US'464 are about the effective nutrient uptake enhancer for P and K, however, does not give any information on the content of key amino acid Glutamic peptide. The protein hydrolysate of US'464 is used as nutrient uptake enhancer for phosphorous and potassium and not as nitrogen or Glutamic acid supplementing fertilizer.

PCT/AU94/00703 claims fertilizer component comprising altered protein containing vegetable matter having plant growth enhancing amount of at least one carotenoid. The vegetable matter is specifically corn gluten which contains carotenoid. Corn gluten is used as fertilizer, however, use of wheat gluten as fertilizer is not mentioned in PCT'703.

Though, the above mentioned prior art Patents/Patent Publications teaches the production of an organic fertilizer, however, there are certain limitations to the processes adopted that they cannot be listed as organic as per APEDA (Agriculture Produce and Export Development Agency), USDA and EU standards of organic input production.

In light of the above, researchers around the world felt a need in the art to provide an Organic Fertilizer which not only increases the plant/crop production but also meets the standards of organic farming. They observed that Glutamic Acid is one of the fundamental metabolite produced in the process of formation of vegetable tissue and chlorophyll synthesis. Glutamic acid helps to increase chlorophyll concentration in the plant leading to higher degree of photosynthesis. This makes crops lush green.

Glutamic acid reaction with different organic acids enables the formation of all the other amino acids. Glutamic Acid and L-Aspartic Acid, by transamination give rise to the rest of the other 17 amino acids. Thus, Glutamate occupies a central position in amino acid metabolism in plants. Glutamic acid also acts as a cytoplasm osmotic agent of the "Guard Cell" thus favouring opening of the stomas.

US5840656 describe about use of Glutamic acid, casein hydrolysate and GABA for increasing fertilizer efficiency, improved plant growth.

US6331505 describe about use of glutamic acid and glycolic acid for increasing plant productivity.

US 6534446 describe the use of GABA along with glutamic acid for mitigating plant stress.

However, the source of glutamic acid is not natural and it is not in peptide form in the above mentioned Patents.

In addition to the above, the prior art documents viz. US20150037492, WO99/05918 and US6692933 disclose glutamine enriched peptides derived from wheat gluten for incorporation into dietary products, health food and medicines. However, these patents failed to understand the use of glutamine peptide as effective organic fertilizer.

Hence, after studying the unique and beneficial effect of glutamic acid on plants, the inventors have developed Glutamine rich Peptide Organic Fertilizer using widely available natural source such as non-GMO wheat gluten.

Summary of the Invention:

In view of the above, it is the object of the present invention to provide non-GMO wheat gluten derived Glutamine rich peptide based biostimulating organic fertilizer containing nitrogen, phosphorous and potassium which enhances photosynthesis and increases chlorophyll content in the plant.

The other object of the present invention is to provide cost effective, eco-friendly process for preparing Glutamine rich peptide based biostimulating organic fertilizer without using any chemicals during the production thereby adhering to the norms specified as per APEDA, USDA and EU standards.

The other object is to provide Glutamine peptide based biostimulating organic fertilizer from non-GMO wheat gluten containing high percent of non-synthetic amino acid i.e. glutamic acid.

In a preferred aspect, the present invention provides Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed on the basis of total amino acids content along with nitrogen, phosphorous, potassium and carbohydrates for enhancing photosynthesis and increasing chlorophyll content in the plant.

In another aspect, the present invention provides non-GMO wheat gluten derived dried Glutamine rich Peptide based Biostimulating organic fertilizer which enhances photosynthesis and increases chlorophyll content in the plant comprising total amino acid content of 65-81%, Glutamic acid 36-42% when expressed on the basis of total amino acids content, organic nitrogen 11-13%, total Phosphorous (P_2O_5) 0.24-0.5%, total Potassium (K_2O) 1-2% and carbohydrate 6-15%.

In another aspect, the present invention provides non-GMO wheat gluten derived liquid Glutamine rich Peptide based Biostimulating organic fertilizer which enhances photosynthesis and increases chlorophyll content in the plant comprising total amino acid content of 30-48%, Glutamic acid 36-42% when expressed on the basis of total amino acids content, Organic Nitrogen of 5-8%, Total Phosphorous (P_2O_5) 0.1-0.3%, Total Potassium (K_2O) 0.8-1.2% and carbohydrate of 3-7%.

The organic fertilizer of the present invention enhances photosynthesis and increases chlorophyll content in the plant, offers better seed germination, helps in production of other amino acids, activates defence mechanism of plants against diseases.

In another preferred aspect, the present invention provides a process for production of Glutamine rich peptide based biostimulating organic fertilizer from non-GMO wheat gluten with high protein content of 65-81%. The process step comprises;

i. Hydrolyzing wheat gluten slurry with phosphatase enzyme such as phytase at 0.5% of wheat gluten mass at pH 2.5-7.2 and at temperature 30-85°C to obtain phosphatase digested wheat gluten slurry with high content of bioavailable plant origin phosphorous;

ii. Hydrolyzing phosphatase digested wheat gluten slurry with carbohydratase enzyme at pH 2.0- 6.0 and at temperature 40-95°C to hydrolyse carbohydrates into sugars and to lower the viscosity of the reaction mass;
iii. Conducting proteolysis using proteolytic enzymes and wet milling simultaneously the phytate and carbohydrate digested wheat gluten hydrolysate in a wet milling machine at pH 6- 9.5 and at temperature 45-75°C to obtain a reaction mass with high degree of hydrolysis and reduced viscosity;
iv. Filtering the reaction mass of step (iii) through ultra-filtration membranes of 10-50 KD to obtain low molecular weight peptides in the permeate and recovering the unutilized enzymes from the retentate using 30 KD membrane for recycling;
v. Subjecting the low molecular weight peptides of the permeate of step (iv) to high pressure nano filtration using filters of 400-800 daltons to obtain liquid Glutamine rich peptide; and
vi. Heating the liquid Glutamine rich peptide at a temperature in the range of 95-125°C, adjusting the pH if required, concentrating and drying to obtain dried Glutamine rich peptide with high content of glutamic acid along with organic nitrogen, phosphorous and potassium.

Wet milling was conducted during the proteolysis process which crushes the wheat gluten particles to very fine size preferably 50-100 micron that increases the surface area of gluten protein molecule and improves access of enzyme to protein molecule for better digestion and yields a reaction mass with high degree of hydrolysis and Glutamine rich peptide with high content of glutamic acid.

Further, the wet milling conducted during proteolysis helps in better extraction and release of Phosphorous and potassium from wheat gluten.

The unutilized enzymes obtained after filtration are reused in the process thereby avoiding excess use of phytase, carbohydrate and protein digesting enzymes making the process cost effective.

In another aspect, for making liquid form of Glutamine rich peptide based biostimulating organic fertilizer, the pH of the liquid in step (v) was adjusted to 2.0- 7.0 using any acid

including non-synthetic acetic acid derived from sugar industry or citric acid from fermentation industry and/or combinations thereof, or any alkaline substances including natural sodium carbonate to improve storage stability of the liquid organic fertilizer and reduce susceptibility of the fertilizer to microbial attack.

In yet another aspect, the Glutamine rich peptide based biostimulating organic fertilizer obtained by the present process comprises 36-42% when expressed on the basis of total amino acids.

Detailed description of the Invention:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and appreciated.

Source of Wheat Gluten: Vital Wheat gluten used in present invention is procured from local market in India. The Wheat gluten used was manufacture by Roquette France.

The term 'Glutamine rich peptide' as referred in the specification means and include high percentage of non-synthetic key amino acid i.e. Glutamic acid that is bound to other amino acids and which is released on acid hydrolysis.

The present invention discloses Glutamine rich peptide based biostimulating Organic Fertilizer derived from non-GMO plant protein products selected from wheat gluten, corn gluten meal, rice gluten ,pea protein, mustard and/or combinations thereof; preferably wheat gluten. The Glutamine rich Peptide based organic fertilizer enhances photosynthesis and increases chlorophyll content in the plant, offers better seed germination, helps in production of other amino acids, activates defence mechanism of plants against diseases.

Accordingly, Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed on the basis of total amino acids along with nitrogen, phosphorous, potassium and carbohydrates for enhancing photosynthesis and increasing chlorophyll content in the plant.

In an embodiment, the present invention provides Non-GMO wheat gluten derived dried glutamine rich peptide based biostimulating organic fertilizer comprising total amino acid content of 65-81%, Glutamic acid 36-42% when expressed on the basis of total amino acids, organic nitrogen of 11-13%, total phosphorous (P_2O_5) 0.24-0.5%, total potassium (K_2O) 1-2% and carbohydrate 8-15%.

In another embodiment, the present invention provides wheat gluten derived liquid glutamine rich peptide based biostimulating organic fertilizer comprises total amino acid content of 30-48%, Glutamic acid 36-42% when expressed on the basis of total amino acids, organic nitrogen of 5-8%, total phosphorous (P_2O_5) 0.1-0.3%, total potassium (K_2O) 0.8-1.2% and carbohydrate of 3-7%.

In yet another embodiment, the present invention provides a process for preparation of Glutamine rich peptide based biostimulating organic Fertilizer from non-GMO wheat gluten which is described elaborately herein below:

The protein rich non-GMO wheat gluten was added to demineralized water (DM) to form slurry having solid content of 6-15%. The slurry was heated to 50-80°C. This process was performed in a stainless steel (SS) jacketed reactor installed internally with a cooling coil. The reaction mass was allowed to go into homogenous slurry form.

Phytates and Phytic acid are the principal storage form of phosphorus in plant origin products. Phytates however hinders the proteolysis to certain extent and thus impacts the extraction of amino acids from the plant source. To overcome the problem, the process involves a step of hydrolysing the phytates and phytic acid using phosphatase enzyme such as phytase. Phytase not only helps in improving digestibility of proteins during proteolysis process by hydrolysing the phytates present as phytates but also helps in producing bioavailable phosphorous which is one of the essential element in the plant growth.

Accordingly, wheat gluten slurry obtained is subjected to hydrolysis using phosphatase enzyme such as phytase at 0.5% of wheat gluten mass at pH 2.5-7.2 and at temperature

30-85°C to obtain phosphatase digested wheat gluten slurry with high content of bioavailable phosphorous.

The Phosphatase enzyme Phytase is derived from various fungi or bacteria including *Aspergillus niger* and *Escherichia coli*.

It was further observed that during hydrolysis of the carbohydrate containing protein rich substances, carbohydrates tend to swell and increase the viscosity of the whole reaction mass making it difficult for filtration for further processing after hydrolysis. The swollen carbohydrate particle also entraps the hydrolysed protein liquid into them influencing the yield of Glutamine peptide rich organic fertilizer. In order to avoid/reduce the viscosity build-up of the slurry mixture and to improve the yield of hydrolysed proteins, it is necessary to breakdown the complex carbohydrates present in the slurry using carbohydrate digesting enzymes.

Accordingly, the pH of the slurry was adjusted at 2.0-6.0 using organic or inorganic acid for optimum functioning of carbohydrate digesting enzymes selected from the group consisting of pectinase from *Aspergillus* species, β-mannanase from *Aspergillus* species, *Bacillus* species, cellulase from *Aspergillus* species, *Trichoderma* species, xylanase from *Aspergillus* species and amylase from *Bacillus* species and glucoamylase from *Aspergillus* species. The carbohydrate enzyme of concentration in the range of 0.1 to 1.5% was added to the slurry at least 4-7 hours prior to the proteolysis. The carbohydrate digestion/hydrolysis was conducted under stirring for 3-6 hours at 40°- 95°C and at pH 4-6. Alternately, the digestion/hydrolysis can proceed without stirring just by circulation of liquid for 8-10 hrs within the reaction kettle by pump.

During proteolysis, phytate and carbohydrate digested liquid hydrolysate containing high molecular weight crude proteins from non-GMO wheat gluten are hydrolysed into smaller peptides and amino acids using proteolytic enzymes. The proteolytic enzymes include any GMO or non GMO protease enzyme including any one or combination of natural plant based proteases such as papain or bromelain; animal origin protease such as pancreatin, pepsin, trypsin; non-GMO fungal proteases and bacterial proteases, neutral and alkaline proteases; exo peptidase and endo peptidase proteases and/or combinations thereof;

preferably the proteolytic enzyme used is papain being a plant origin Non- GMO enzyme, though other enzymes like pancreatin, exo peptidase and endopeptidase enzyme also offers equal outputs.

To the carbohydrate digested liquid hydrolysate, alkali was added which was selected from synthetic and natural (non-synthetic alkali) sources to adjust the pH to 6.0 - 9.5 followed by addition of suitable proteolytic enzyme at 1-5% of protein present. The proteolysis was carried out at 45-75°C for 6-24 hours. The pH was adjusted during process, if required and the temperature of the slurry was regulated by heating or cooling processes to achieve optimum temperature of 55-60°C required for efficient activity of proteolytic enzyme.

The reaction mixture during the proteolysis was circulated and processed through wet milling machine in order to crush the wheat gluten particles to very fine size preferably 50-100 micron that increases the surface area of gluten protein molecule and improves access of enzyme to protein molecule for better digestion and yields a reaction mass with high degree of hydrolysis and Glutamine rich peptide with high content of glutamic acid.

The wet milling during proteolysis helps better extraction of proteins and release of Phosphorous (P) and Potassium (K) from wheat gluten.

The proteolysis was continued until the mixture attained the degree of hydrolysis of 20-30% determined using Sorenson's method and substantial reduction in viscosity of hydrolysate is noted. The higher degree of hydrolysis was also achieved by increasing the proteolysis time and increasing the dosage of protease enzyme.

The Glutamine Peptide rich hydrolysate liquid was filtered with press filter or decanter at the temperature of about 50-60°C followed by micron filtration to remove suspended, unreacted or undissolved particles. The liquid Glutamine Peptide based Organic Fertilizer was then fed to ultra-filtration employing MWCO of 10-50 KD, preferably 15-30 KD for separating high molecular peptides, carbohydrates and unutilized enzymes. The permeate containing low molecular peptides was used for further processing to nano-filtration.

The unutilized enzymes present in the retentate of the ultrafiltration step was recovered by subjecting the retentate to 20-30 KD ultra-filtration and reused in next cycle of extractions. This recovery and recycling of the unutilized enzyme helped in reducing the additional enzyme requirement in next processing. Alternately, if the quantity of freshly added enzyme was not reduced during processing for next batches it was inferred that the recycled enzyme was utilized for extraction of Glutamine peptide from gluten.

The low molecular weight peptides obtained in the permeate after first ultrafiltration was fed to high pressure nano-filtration at MWCO of 400-800 daltons to obtain liquid Glutamine rich peptide based biostimulating organic Fertilizer.

The nano filtered liquid was then heated at 95-125°C for 1-2 hrs to inactivate the traces of enzymes, if present. The liquid was then subjected to concentration process such as vacuum evaporator to obtain concentrated Glutamine rich peptide based biostimulating organic fertilizer which was further dried using known drying methods.

The liquid Glutamine rich peptide was further dried using suitable dryer to keep all its vital peptides intact at low temperature. The dried powder can then be converted into various forms such as granule, tablet, pellet, small ball in order to suit the end use, application and handling.

For making storage stable liquid form of Glutamine rich peptide based biostimulating organic fertilizer, the pH of the liquid was adjusted to 2.0-6.0 using organic or inorganic acidic substances including non-synthetic acetic acid derived from sugar industry or citric acid from fermentation industry and/or combinations thereof, or any alkaline substances including natural sodium carbonate to improve storage stability of the liquid organic fertilizer and reduce susceptibility of the fertilizer to microbial attack.

In an embodiment, the liquid and dried glutamine rich peptide composition is given below in **Table A:**

Parameter	Dry Basis	Liquid Conc. form
Organic Nitrogen	11-13%	5 - 8%
Organic Phosphorous P2O5	0.24-0.5%	0.1-0.3%
Organic Potassium K2O	1-2%	0.8-1.2%
Carbohydrate	6-15%	3-7%
Total amino acids (AA)	65-81%	30-48%
Glutamic acid (gm/100 gm Amino acids)	36-42 gm /100 gm AA	36-42 gm/100 gm AA

In another embodiment, the present invention provides a liquid glutamine rich peptide composition comprising total amino acid content of 30-48%, glutamic acid 36-42% when expressed on the basis of total amino acids, organic nitrogen of 5-8%, total phosphorous (P_2O_5) 0.1-0.3%, total potassium (K_2O) 0.8-1.2% and carbohydrate of 3-7% along with acceptable excipients for photosynthesis and chlorophyll enhancement, enhanced seed germination, help in production of other amino acids, and to activate the defense mechanism of the plant against diseases.

In yet another embodiment, the present invention provide dried Glutamine rich peptide based organic fertilizer composition comprising total amino acid content of 65-81%, Glutamic acid 36-42% based on total amino acids , organic nitrogen 11-13%, total phosphorous (P_2O_5) 0.24-0.5%, total potassium (K_2O) 1-2% and carbohydrate of 6-15% along with acceptable excipients for photosynthesis and chlorophyll enhancement, enhanced seed germination, help in production of other amino acids, and to activate the defense mechanism of the plant against diseases.

The Glutamine rich Peptide based organic fertilizer composition can be prepared in liquid, gel, paste, pulp as well different solid forms like powder, granule, pellet, tablet, round ball and the like.

The Liquid and Dry Glutamine rich peptide based organic fertilizer has excellent storage stability of 36 months when kept in cool and dry place away from sunlight and is endo toxin free.

In another embodiment, the present invention provides a method for enhancing the photosynthesis and chlorophyll content in the plant, better seed germination, assisting in production of other amino acids in plants and to activate defence mechanism of plants against diseases comprising contacting the plant with Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed/translated on the basis of total amino acids along with nitrogen, phosphorous, potassium and carbohydrates by means of spraying on crops, injecting, through irrigation, drenching, applying in soil or mixing with fertilizers.

In yet another embodiment, the present invention provides Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed/translated on the basis of total amino acids along with nitrogen, phosphorous, potassium and carbohydrates for use in photosynthesis and chlorophyll enhancement, to enhance seed germination, assist production of other amino acids in plants and to activate the defence mechanism of the plant against diseases.

While the present invention has been described in terms of its specific embodiments, certain modifications and equivalents will be apparent to those skilled in the art and are included within the scope of the invention. The examples included herein are provided to illustrate particular aspects of the disclosure and do not limit the scope of the present invention.

Example 1:

500 Kgs Vital wheat gluten of Roquette France make was ground to 100 mesh in pulveriser and then added in 4000 Ltr of demineralized UV treated water to obtain gluten to water ratio of 1: 8. The reaction was carried out in stainless steel SS316 reactor. The reaction mass was slurried at a temperature of 65°C to get homogenous slurry.

The pH of the homogeneous slurry was adjusted with organic acid citric acid at 5.5 for optimum functioning of phytase enzyme. The phytase enzyme was added each at the rate of 0.5% of wheat gluten mass and digestion was continued for 3 hours at 50-55°C.

Further in next step, the pH of the homogeneous slurry was adjusted with organic acid citric acid at 5.1 for optimum functioning of carbohydrate enzyme. The carbohydrate enzymes pectinase and amylase were added each at the rate of 0.5% of carbohydrate present in wheat gluten and carbohydrate digestion was continued for 3 hours at 55°C and then at 85°C for additional 2 hours until the viscosity of liquid hydrolysate was reduced and flowable. Further, to the liquid hydrolysate was added proteolytic natural enzyme papain at 1.0% of substrate (wheat gluten) used. The pH of the reaction mass was adjusted to 7.0 using alkali such as natural soda or sodium hydroxide. During the proteolysis the reaction mass was subjected to wet milling machine wherein the reaction mass was crushed continuously by passing the reaction slurry through wet milling to reduce the surface area of large protein molecule and improve access of enzyme to protein molecule for better digestion. The proteolysis was conducted at 60°C for 15 hours until degree of hydrolysis was in the range of 22-24% as calculated using Sorenson's method.

The hydrolysate liquid after proteolysis was subjected to filtration through decanter followed by micron filtration. The filtration yielded 3800 Ltr liquid containing 9.12% dissolved solids. The filtered liquid was then subjected to ultra-filtration of 15 KD membrane and the retentate from this ultrafiltration was subjected to 30 KD ultrafiltration to recover the unutilized enzymes for reycle. The permeate obtained after 1st ultrafiltration was fed for further nano filtration at 800 daltons which yielded 3240 Ltr of permeate with 8.5% dissolved solids. This permeate was further heated at 95-125°C for 2 hour to inactivate any traces of enzymes and concentrated under vacuum at 80°C to desired solid content suitable for drying. The concentrated liquid was then fed to spray dryer tower with nozzle arrangement to dry the product at low temperature to yield 275 kg of the dry powder with: - Total nitrogen of 12.01%, Total Phosphorous 0.29%, Total Potassium 1.44%, carbohydrate of 6.5 %, Total peptides and amino acids of 77.846 % and Glutamic acid content of 32.1% which translates to 41.23 gm Glutamic /100 gm Total amino acid.

The amino acid present in the Glutamine rich Peptide based organic fertilizer obtained by the process of Example 1 is given in **Table 1**, wherein about 1/3rd of the product is Glutamic acid and 41.23% (32.1 /77.846 = 41.23%) if expressed based on total amino acid content.

Table 1:

Amino acids (acid hydrolysis)	
Alanine	1.67±0.09 g/100g
Arginine	0.844±0.051 g/100g
Aspartic acid	2.27±0.12 g/100g
Glutamic acid	32.1±1.5 g/100g
Glycine	3.08±0.20 g/100g
Histidine	1.28±0.18 g/100g
Hydroxyproline	<0.05 (LOQ) g/100g
Isoleucine	2.45±0.20 g/100g
Leucine	4.69±0.33 g/100g
Lysine	0.986±0.056 g/100g
Ornithine	0.806±0.161 g/100g
Phenylalanine	3.85±0.21 g/100g
Proline	11.0±0.9 g/100g
Serine	2.82±0.16 g/100g
Threonine	1.79±0.10 g/100g
Tyrosine	2.67±0.29 g/100g
Valine	2.80±0.18 g/100g
DJ011 DJ Cystine, methionine (oxidative) Method : ISO 13903:2005; EU 152/2009(F)	
Cystein+ Cystine	1.20±0.11 g/100g
Methionine	0.968±0.074 g/100g
DJ009 DJ Tryptophane Method : EU 152/2009	
Tryptophan (Total)	0.572±0.045 g/100g

Example 2:

The non-GMO wheat gluten was processed as per Example 1 except the enzyme used for carbohydrate digestion was pectinase and glucoamylase at 0.5% each based on carbohydrate content and hydrolysis was conducted for 4 hours for pectinase and 4 hour for glucoamylase. In proteolysis papain enzyme was used at 1.5% and at same temperature and duration of 18 hrs at 60°C.

The product obtained is 258kg of the dry powder with Total nitrogen of 12.2%, carbohydrate of 6.5%, Total peptides and amino acids of 12.2x6.25 = 76.25% and

Glutamic acid content of 32.4% which translates to 42.49 gm Glutamic acid /100gm amino acid.

Example 3: Use of the Glutamine Rich peptide based Organic Fertilizer on seed germination

Cotton seeds were treated with Glutamine Rich peptide based Organic Fertilizer produced as per Example 1 to observe its effect on germination.

Soluble Glutamine Rich peptide based organic fertilizer produced using present invention having specifications: - total amino acid content of 76.8%, Glutamic acid 32.1%, Organic Nitrogen of 12.01%, Total Phosphorous (P_2O_5) - 0.29%, Total Potassium (K_2O) - 1.44% and carbohydrate of 6.5% was used for seed treatment.

1 Kg of Cotton seeds were coated with 10 gms Soluble Glutamine Rich peptide based organic fertilizer diluted 50 ml water and allowed to dry. The seeds were planted next day morning along with untreated seeds coated with in 50 ml of plain water only for 20 min and dried and the results are given in **Table 2** below:

Table 2:

Day	Untreated cotton seeds % Germination %	Treated cotton seeds at 1% Glutamine Rich peptide Germination %
3	0	60
4	0	75
5	0	85
6	10	95
7	60	96

Example 4: Use of the Glutamine Rich peptide based Organic Fertilizer on Fresh weight

Coriander was planted in 10 are (1 are = 1000 sq ft) (10,000 sq ft) with a distance of 5 inch x 1 ft in row.

7 days after growth of coriander following treatment was made.

Soluble Glutamine Rich peptide (BioLifeS75) based organic fertilizer produced using this invention having specifications: - total amino acid content of 76.8%, Glutamic acid 32.1%, Organic Nitrogen of 12.01%, Total Phosphorous (P_2O_5) - 0.29%, Total Potassium (K_2O) -1.44% and carbohydrate of 6.5% was used for foliar and drench treatment.

Treatment: - Foliar application at 2 gm (BioLifeS75) per Ltr for water using knapsack sprayer and Drench at the rate of 1 gm (BioLifeS75) per Ltr of water.

Qty of water required for 10,000 sq ft foliar spray:-20 Ltr

Qty of water used for 10,000 sq ft root drench = 50 Ltr

The fresh weight was noted at the end of 15 days.

The results are given in **Table 3** below.

Table 3:

Days	Average Saleable Fresh Weight of coriander plant Untreated	Average Saleable Fresh Weight of coriander plant Treated
2 weeks after treatment	22 gm	36 gm
Increase in %	-	63%
Color of leaves	Green	Lush Green

From the treatment it is evident that the spray and drench of the produce of invention offers increase in weight of plants and increases greenness in plant. The increased greenness is due to the increased chlorophyll content in the plants.

Example 5: Use of the Glutamine Rich peptide based Organic Fertilizer on cucumber for yield increase

Cucumber was planted in $1/4^{th}$ acre.

Spraying treatments were started after 25 days of transplanting and every 7 days for 3 times through the growing season made.

Soluble Glutamine Rich peptide (BioLifeS75) based organic fertilizer produced using this invention having specifications: - total amino acid content of 76.8%, Glutamic acid 32.1%, Organic Nitrogen of 12.01%, Total Phosphorous (P_2O_5) - 0.29%, Total Potassium (K_2O) -1.44% and carbohydrate of 6.5% was used for foliar and drench treatment.

Treatment: - Foliar application at 2 gm per Ltr for water using knapsack sprayer and Drench at the rate of 1 gm per Ltr of water. The results are given in **Table 4** below:

Table 4:

Treatment	**Fruit length cm**	**Fruit diameter cm**	**Fruit weight gms**	**Total yield Mt./acre**	**Increase over control**
Control	**17**	**2.93**	**102**	**3.06**	**-**
2 gm /Ltr of the product from Example 2	**21**	**3.2**	**158**	**4.86**	**58.82%**

The cucumber was very fresh and the appearance was much better than untreated.

Example 6: Use of the Glutamine Rich peptide based Organic Fertilizer on chlorophyll content in cow pea

Cow pea (blackeyed pea) was planted in 1 acre soil. The cow pea seeds were planted in the mid July during monsoon season under atmospheric conditions. The minimum and maximum atmospheric temperature was 2°C and 20°C respectively.

The Agricultural University recommended fertilizer dosage of NPK fertilizer was added in soil.

One spray of preparation of Example 1 was sprayed at 250 gms per acre in 100 Ltr of water before flowering and 1 drench of 250gms per acre in 250 ltr water was given at root zone using knapsack pump.

After flowering the cowpea plants were harvested and washed with distilled water.

The total chlorophyll content was analyzed and the results are given in **Table 5** below.

Table 5:

Treatment	Chlorophyll "a" mg/gm	Chlorophyll "b" mg/gm	Total Chlorophyll mg/gm
Control Untreated	**0.761**	**0.146**	**1.46**
2 gm /Ltr of the product from example no 2	**1.455**	**0.312**	**2.13**

Example 7:

The non-GMO wheat gluten was processed as per Example 1 except the enzyme used for carbohydrate digestion was pectinase and amylase at 0.5% each based on carbohydrate content and hydrolysis was conducted for 4 hours for pectinase and 4 hour for amylase. In proteolysis pacreatin 4NF enzyme was used at 1.5% and at same temperature and duration of 18 hrs at 55-58°C.The pH was adjusted at 7.8 to 8.0 during the reaction.

The product obtained is 212kg of the dry powder with Total nitrogen of 12.1%, Total phosphorous -0.4%, Total Potassium – 1.5%, carbohydrate of 7.5 %, Total peptides and amino acids of 12.1x 6.25 = 75.62% and Glutamic acid content of 31.0 % which translates to 41 gm Glutamic acid /100gm amino acid.

Example 8:

The non-GMO wheat gluten was processed as per Example 1 except the enzyme used for carbohydrate digestion was pectinase and amylase at 0.5% each based on carbohydrate content and hydrolysis was conducted for 4 hours for pectinase and 4 hour for amylase. In proteolysis Protease enzyme Alcalase 2.4 L (Manufacturer Novozyme) was used at 1.5% and at same temperature and duration of 18 hrs at 55-60°C.The pH was adjusted in 7.8 to 8.2 during the reaction.

The product obtained is 220kg of the dry powder with Total nitrogen of 12.0%, Total phosphorous -0.41%, Total Potassium – 1.65%, carbohydrate of 6.5 %, Total peptides and

amino acids of 12.0x 6.25 = 75 % and Glutamic acid content of 30.5% which translates to 40.67 gm Glutamic acid /100gm amino acid.

Example 9: Liquid Glutamine Rich peptide based organic fertilizer
The non-GMO wheat gluten was processed as per Example 1 .The phosphatise enzyme phytase is used at 0.5% of wheat gluten in similar manner as described in example 1 as except the enzyme used for carbohydrate digestion was pectinase and amylase at 0.5% each based on carbohydrate content and hydrolysis was conducted for 4 hours for pectinase and 4 hour for amylase.

In proteolysis papain enzyme was used at 1.7% and at same temperature and duration of 18 hrs at 60°C. The wet milling was carried out as per Example No. 1.

The product after filtration steps is concentrated in vacuum evaporator to have the Liquid Glutamine Rich peptide based organic fertilizer comprising total amino acid content of 39%, glutamic acid of 15.6% which equals 15.6/39 = 40 % when expressed on the basis of total amino acids , organic nitrogen of 6.3 %, total phosphorous (P_2O_5) 0.15 %, total potassium (K_2O) 0.9% and carbohydrate of 3.5 % The yield of the Liquid Glutamine Rich peptide based organic fertilizer is 490 kg.

Industrial Advantages:

- The invention provides production of liquid and solid Glutamine rich peptide based organic fertilizer from non-GMO wheat gluten by employing enzymatic sources and non-synthetic chemicals that adhere to organic farming standards.

- Glutamic acid is single most abundant amino acids present in the plant. Glutamic acid reaction with different organic acids obtains all the other amino acids which are essential for overall plant development.

- Glutamine rich peptide based organic fertilizer contains very high % of key amino acid - Glutamic acid, approx. 36% to 42% when translated on the basis of total amino acid content.

- Glutamine rich peptide based organic application is specifically of great use when plants are under stress conditions which make the synthesis of amino acids more difficult.

- Glutamine rich peptide based organic fertilizer improves seed germination and plant yield by increasing the chlorophyll content during photosynthesis.

- Glutamine rich peptide based organic fertilizer offers three essential nutrients viz. Nitrogen, Potassium and Phosphorous.

We Claim,

1. Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed /translated on the basis of total amino acids along with bioavailable nitrogen, phosphorous, potassium and carbohydrates for enhancing photosynthesis and increasing chlorophyll content in the plant.

2. The glutamine rich peptide based biostimulating organic fertilizer according to claim 1, wherein said fertilizer in dried form comprises total amino acid content of 65-81%, Glutamic acid 36-42% when expressed /translated on the basis of total amino acids, organic nitrogen of 11-13%, total phosphorous (P_2O_5) 0.24-0.5%, total potassium (K_2O) 1-2% and carbohydrate 6-15%.

3. The glutamine rich peptide based biostimulating organic fertilizer according to claim 1, wherein said fertilizer in liquid form comprises total amino acid content of 30-48%, Glutamic acid 36-42% based on total amino acids, organic nitrogen of 5-8%, total phosphorous (P_2O_5) 0.1-0.3%, total potassium (K_2O) 0.8-1.2% and carbohydrate of 3-7%.

4. An eco-friendly and cost effective process for preparing Glutamine rich peptide based biostimulating organic fertilizer from non-GMO Wheat Gluten, comprising;
    i. Hydrolyzing wheat gluten slurry with phosphatase enzyme such as phytase at 0.5% of wheat gluten mass at pH 2.5-7.2 and at temperature 30-85°C to obtain phosphatase digested wheat gluten slurry with high content of bioavailable plant origin phosphorous;
    ii. Hydrolyzing phosphatase digested wheat gluten slurry with carbohydratase enzyme either alone or in combination at pH 2.0- 6.0 and at temperature 40-95°C to hydrolyse carbohydrates into sugars and to lower the viscosity of the reaction mass;

iii. Conducting proteolysis using proteolytic enzymes and wet milling simultaneously the phytate and carbohydrate digested wheat gluten hydrolysate in a wet milling machine at pH 6- 9.5 and at temperature 45-75°C to obtain a reaction mass with high degree of hydrolysis and reduced viscosity;

iv. Filtering the reaction mass of step (iii) through ultra-filtration membranes of 10-50 KD to obtain low molecular weight peptides in the permeate and recovering the unutilized enzymes from the retentate using 30 KD membrane for recycling;

v. Subjecting the low molecular weight peptides of the permeate of step (iv) to high pressure nano filtration using filters of 400-800 daltons to obtain liquid Glutamine rich peptide; and

vi. Heating the liquid Glutamine rich peptide at a temperature in the range of 95-125°C, adjusting the pH if required, concentrating and drying to obtain dried Glutamine rich peptide with high content of glutamic acid.

5. The process according to claim 4, wherein the Phosphatase enzyme Phytase is derived from various fungi or bacteria including *Aspergillus niger* and *Escherichia coli.*

6. The process according to claim 4, wherein the carbohydratase enzymes are selected from pectinase from *Aspergillus* species, β-mannanase from *Aspergillus* species, *Bacillus* species, cellulase from *Aspergillus* species, *Trichoderma* species, xylanase from *Aspergillus* species and amylase from *Bacillus* species and glucoamylase from *Aspergillus* species either alone or in combination thereof.

7. The process according to claim 4, wherein the proteolysis is carried using proteolytic enzymes at 1.0-1.5% of the substrate used either alone or combinations thereof.

8. The process according to claim 7, wherein the proteolytic enzymes are selected from GMO or non GMO protease enzyme selected from natural plant based proteases such as papain or bromelin; animal origin protease such as pancreatin,

pepsin, trypsin; non-GMO fungal proteases and bacterial proteases, neutral protease, and alkaline proteases; exo peptidase and endo peptidase proteases alone or in combinations thereof.

9. The process according to claim 4, wherein the wet milling step (iii) comprises breaking down the wheat gluten particles to very fine size of 50-100 micron.

10. The process according to claim 4, wherein the glutamic acid content in the peptide is 36 to 42% of the total amino acids.

11. The process according to claim 4, wherein, the pH of the liquid Glutamine rich peptide of step (iv) was adjusted to 2.0-6.0 using any acidic substances including non-synthetic acetic acid or citric acid from fermentation industry and/or combinations thereof, or any alkaline substances including natural sodium carbonate.

12. The process according to claim 4, wherein the wet milling during proteolysis enhances extraction and release of phosphorous and potassium from wheat gluten.

13. The fertilizer composition comprising glutamine rich peptide based organic fertilizer according to any of the preceding claims along with acceptable excipients for chlorophyll enhancement, enhanced seed germination, production of other amino acids and to activate the defence mechanism of the plant against diseases.

14. The fertilizer composition according to claim 13, wherein said composition can be formulated in the form of liquid, gel, paste, pulp and in solid forms such as powder, granule, tablet, pellet, round ball and the like.

15. A method for enhancing the photosynthesis and chlorophyll content in the plant, better seed germination, assisting in production of other amino acids in plants and to activate defence mechanism of plants against diseases comprising contacting the plant with Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when

expressed/translated on the basis of total amino acids along with nitrogen, phosphorous, potassium and carbohydrates by means of spraying on crops, injecting through irrigation, drenching, applying in soil or mixing with fertilizers.

16. Non-GMO wheat gluten derived glutamine rich peptide based bio stimulating organic fertilizer comprising Glutamic acid 36-42% when expressed/translated on the basis of total amino acids along with nitrogen, phosphorous, potassium and carbohydrates for use in photosynthesis and chlorophyll enhancement, to enhance seed germination, assist production of other amino acids in plants and to activate the defence mechanism of the plant against diseases.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 15/50207

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C05F 11/00 (2016.01)
CPC - C05F 11/00, C05G 3/0076
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)
IPC (8): C05F 11/00 (2016.01)
CPC: C05F 11/00, C05G 3/0076

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/68.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google patents, Google scholar, Google web, PatBase, Proquest Dialog
wheat Gluten; glutamine /glutamic acid; peptide/amino acid/protein; fertilizer; enhances/increases; photosynthesis/chlorophyll content; plant/crop; seed germination/amino acids/defense; hydrolyze; phosphatase enzyme/phytase; carbohydratase; proteolytic/protease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 5,840,656 A (KINNERSLEY et al.) 24 November 1998 (24.11.1998) Abstract; Col. 2, ln 1-7; Col. 3, ln 10-18; Col. 3, ln 46-58; Col. 6, ln 23-37; Col. 5, ln 53-67; Col. 6, ln 1-22	1-3, 13-14/(1-3), 15-16 --- 4-12, 13-14/(4-12)
Y	US 2013/0192322 A1 (MILLER) 01 August 2013 (01.08.2013) Abstract para [0009]; para [0013]; para [0015]	1-3, 13-14/(1-3), 15-16
Y --- A	US 6,372,282 B1 (EDENS et al.) 16 April 2002 (16.04.2002) Abstract; claim 18; Col. 9, ln 22-41; Table 6; Col. 9, ln 22-30	1-3, 13-14/(1-3), 15-16 --- 4-12, 13-14/(4-12)
Y	COLLA et al. "Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis" Front Plant Sci. 2014. Vol. 5. No. 448. pp 1-6. especially, Abstract; page 2, Col. 1, para 5; page 2, Col. 2, para 1; page 5, Col. 1, para 2; page 5, Col. 2, para 1page 5, Col. 1, para 2; page 5, Col. 2, para 1	1-3, 13-16
Y	US 6,124,241 A (KINNERSLEY) 26 September 2000 (26.09.2000) claim 1; claim 32; Abstract	13-14/(1-3), 15-16

☒ 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
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"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
29 November 2016	28 DEC 2016
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. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 15/50207

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/052723 A1 (CHAUDHRY) 16 April 2015 (16.04.2015) Abstract; claim 8; page 4, para 3-4; claim 1; page 8, para 1-4; page 10, para 2-4; page 13, para 3-4; claim 5-6	4-12, 13-14/(4-12)
A	WO 2010/027846 A1 (DANISCO US INC. et al.) 11 March 2010 (11.03.2010) Abstract; claim 1; page 2, ln 23-26; page 3, ln 8-12	4-12, 13-14/(4-12)
A	US 5,989,600 A (NIELSEN et al.) 23 November 1999 (23.11.1999) Col. 2, ln 30-37	4-12, 13-14/(4-12)