LECTIN DETOXIFICATION METHOD

PRIORITY UNDER 35 U.S.C Section 119(e) & 37 C.F.R. Section 1.78

[001] This nonprovisional application claims priority based upon the following prior United States Provisional Patent Application entitled: Lectin Detoxification, Application No.: 63/079,132 filed September 16, 2020, in the name of Christina Rahm Cook, which is hereby incorporated by reference for all purposes.

FIELD OF THE INVENTION

[002] The present invention relates generally to purification methods, more specifically but not by way of limitation a method focused on detoxification of lectins while ensuring protection of the nutritional and immunity benefits of lectin.

BACKGROUND

[003] Lectins are carbohydrate binding proteins that are characterized by their capability to attach carbohydrates such as but not limited to mannose, galactose, lactose, N-acetyl glucosamine, N-acetyl galactosamine, fructose with significant specificity. Lectins can bind specifically and reversibly to different types of glycoproteins or carbohydrates. Studies have shown that the longest-lived and healthiest groups of people in the world subsist largely on plant based diets which most often include lectin in larger quantities. Exploratory studies on the health benefits of lectin have been initiated as it has been shown that lectins have important physiological roles including but not limited to antibacterial activity, and antifungal effects, antihuman immunodeficiency virus and antitumor activity. Plants are the main source of lectins that are found in different parts of plants such as seeds, leaves, bark, roots, tubers, and fruits.

[004] It is known in the art that lectins are present in many of the foods we eat that are a major food source for both humans wherein these foods consist of groups such as but not limited to grains and legumes. While these food groups are beneficial for humand they do however have an anti-nutritional characteristic due to the overabundance of lectin. The anti-nutritional properties are most likely caused by lectins impairment of the integrity of the intestinal epithelium and thus this inhibits optimum absorption and utilization of the nutrients that are present in the legumes and other food groups high in lectin. Lectin containing foods are frequently cooked or otherwise processed to reduce the level of the anti-nutritional characteristics and improve the utilization efficiency of legumes and other food groups high in lectin. Additionally, lectins that are thought to survive gastric digestion and have been an allergen, especially in seeds or the oils of seeds and plants. As such, in regards to food safety research has been performed to determine the digestibility of lectins, especially regarding the consumption of high levels of unprocessed or undercooked beans, seeds, plants, roots, fruits, or vegetables.

[005] Lectins can be separated from the plant, roots, fruit, vegetables, oils, and seeds by an aqueous magnetic assisted two-phase system, which can be effectively applied for the extraction and purification of proteins and other biomolecules from plants, seeds, fruits, vegetables, roots, and oils. There is a need for a better process of manufacturing and production of products that have lectins. The present invention disclosed herein involves isolation, purification and characterization of lectin from items such as but not limited to seeds, roots, plants, beans and nuts. In order to assist with the characterization of lectin and its activities in foods, detoxification of the negative characteristics of lectin and protection of positive nutritional benefits which include but are not limited to ant-fungal, anti-viral, and anti-bacterial properties of the lectin should be improved. Furthermore, enhancement of the important physiological roles including but not limited to insecticidal action, antibacterial activity, and antifungal effects and analgesic activity of lectin need to be optimized.

[006] Accordingly, there is a need for a detoxification method and process that is capable of extraction and purification of food groups that are high in lectin so as to remove the anti-nutritional characteristics while maintain the health benefits thereof.

SUMMARY OF THE INVENTION

[007] It is the object of the present invention to provide a method for detoxification of lectin while preserving the nutritional benefits thereof wherein the present invention that separates and purifies lectin through a two-step method based on an aqueous magnetic assisted two-phase system.

[008] Another object of the present invention is to provide a separation and purification method for lectin that employs a gel filtration media utilized in gel filtration chromatography that includes adding various heating and cooling parameters.

[009] A further object of the present invention is to provide a method for detoxification of lectin while preserving the nutritional benefits thereof wherein the present invention that will enhance nutritional value and assist with cleaning the toxins out of environmental seeds, and other items containing lectin.

[0010] Still another object of the present invention is to provide a separation and purification method for lectin wherein the method of the present invention includes the step of forming an aqueous magnetic solution by mixing ammonium sulfate, polyethylene glycol 600 and NaCl and further including the addition of adding one milliliter of crude extract solutions.

[0011] An additional object of the present invention is to provide a method for detoxification of lectin while preserving the nutritional benefits thereof wherein the present invention includes the step of collecting and dialyzing lectin and proteins in the top phase against deionized water.

[0012] Yet a further object of the present invention is to provide a separation and purification method for lectin wherein the step of determination of the hemagglutinating activity is performed in microtiter plates.

[0013] Another object of the present invention is to provide a method for detoxification of lectin while preserving the nutritional benefits thereof wherein the present invention additionally includes the step of analyzing the sugar specificity of the lectin.

[0014] Still an additional object of the present invention is to provide a separation and purification method for lectin that further includes the step of dialyzing the lectin solution in a phosphate buffer solution containing ethylene diaminete tracetic acid until no hemagglutinating activity is detected.

[0015] Yet another object of the present invention is to provide a method for detoxification of lectin while preserving the nutritional benefits thereof wherein the present invention includes the step of preparing a simulated gastric fluid and mixing the therewith in a water bath to initiate a digestion reaction.

[0016] To the accomplishment of the above and related objects the present invention may be embodied in the form illustrated in the accompanying drawings. Attention is called to the fact that the drawings are illustrative only. Variations are contemplated as being a part of the present invention, limited only by the scope of the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] A more complete understanding of the present invention may be had by reference to the following Detailed Description and appended claims when taken in conjunction with the accompanying Drawings wherein:

[0018] Figure 1 is a diagram of the steps of a preferred embodiment of the method.

DETAILED DESCRIPTION

[0019] Referring now to the drawings submitted herewith, wherein various elements depicted therein are not necessarily drawn to scale and wherein through the views and figures like elements are referenced with identical reference numerals, there is illustrated a lectin detoxification method 100 constructed according to the principles of the present invention.

[0020] An embodiment of the present invention is discussed herein with reference to the figures submitted herewith. Those skilled in the art will understand that the detailed description herein with respect to these figures is for explanatory purposes and that it is contemplated within the scope of the present invention that alternative embodiments are plausible. By way of example but not by way of limitation, those having skill in the art in light of the present teachings of the present invention will recognize a plurality of alternate and suitable approaches dependent upon the needs of the particular application to implement the functionality of any given detail described herein, beyond that of the particular implementation choices in the embodiment described herein. Various modifications and embodiments are within the scope of the present invention.

[0021] It is to be further understood that the present invention is not limited to the particular methodology, materials, uses and applications described herein, as these may vary. Furthermore, it is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. It must be noted that as used herein and in the claims, the singular forms "a", "an" and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an element" is a reference to one or more elements and includes equivalents thereof known to those skilled in the art. All conjunctions used are to be understood in the most inclusive sense possible. Thus, the word "or" should be understood as having the definition of a logical "or" rather than that of a logical "exclusive

or" unless the context clearly necessitates otherwise. Structures described herein are to be understood also to refer to functional equivalents of such structures. Language that may be construed to express approximation should be so understood unless the context clearly dictates otherwise.

[0022] References to "one embodiment", "an embodiment", "exemplary embodiments", and the like may indicate that the embodiment(s) of the invention so described may include a particular feature, structure or characteristic, but not every embodiment necessarily includes the particular feature, structure or characteristic.

[0023] Referring in particular to the Figures submitted as a part hereof, in step 101 Dried seeds, plants, roots, fruits, vet were ground to a fine powder passed through a 50-mesh sieve. Step 103 Then, the powder is mixed with phosphate-buffered saline by agitation for a period of ten to twelve hours. During the mixing process it is contemplated within the scope of the present invention that the mixture can be subjected to alternate temperatures that are either warmer or colder than the environment in which the mixture is located. Step 105 the mixture is filtered and centrifuged to produce a supernatant as the crude extract. Step 107, the crude extract is purified using the aqueous magnetic two-phase solution as discussed herein. The aqueous magnetic two-phase solution is formed by mixing an amount of ammonium sulfate, polyethylene glycol 600 and NaCl and further adding the crude extract solutions. It should be understood within the scope of the present invention that the amounts for the immediately aforementioned can vary depending on the total end volume production that is desired. Furthermore, alternate amounts will vary based on the type of material such as but not limited to plant, seed or root. The total weight of the mixture can range between two and twenty grams. Additionally, the pH there is maintained between a range of five to ten.

[0024] Step 109, subsequent separation the lectin and proteins in the top phase of the mixture are collected and dialyzed against deionized water. While no particular time is required, good results have been achieved

executing this step for twenty four to forty eight hours. The step employs utilization of a dialysis bag having 500-10000-Da molecular weight cutoffs so as to remove salt and polyethylene glycol. Step 111, The lectin is purified through gel chromatography. The lectin solution is prepared by concentrating the dialyzed lectin solution. Step 113, subsequent concentration of the dialyzed lectin solution the lectin solution is filtered utilizing an appropriate membrane. Step 115. Ensuing filtration five to ten milliliters of the filtered solution is applied to filtration media utilizing gel filtration chromatography. It should be understood within the scope of the present invention that the filtration media is pre-equilibrated with a phosphate buffer. It is contemplated within the scope of the present invention that the filtration media could have alternate pH levels.

[0025] Step 117 a fraction of the solution is prepared for the determination of hemagglutinating activity. The lectin solution is collected and pooled and subsequently dialyzed and lyophilized to obtain a lectin lyophilized powder for use. It is contemplated within the scope of the present invention that the hemaglutinating activity is performed in microtiter plates. Step 119, The lectin lyophilized powder needs to be formulated into a lectin solution. Ensuing formulation, part of the of lectin solution is twofold serially diluted with stroke-physiological saline. Step 121, the sugar specificity of lectin needs is analyzed. The sugar specificity of the lectin is analyzed in a manner analogous to the hemagglutination test. Step 123, the sugar base that is tested is formulated into alternate concentrations of aqueous solutions. Step 125, equal volumes of the sugar solution and the lectin solution are mixed, and stored at room temperature. It is contemplated within the scope of the present invention that the mixture of the lectin solution and the sugar solution are stored at ambient temperature for a period of one to two hours.

[0026] Step 127, the mixture of lectin solution and sugar solution is diluted to detect hemagglutinating activity. Step 128, a simulated gastric fluid needs is prepared. The lectin solution is mixed with the simulated gastric solution having a pH of 1.2 in a water bath at 37 °C to start the digestion reaction. The

pepsin reaction is terminated by adding Na_2CO_3 solution with a pH of 11.0 after 10 min, 20 min, 30 min, 40 min, 50 min, and 60 min, respectively. Simulated intestinal fluid is also prepared similarly with minor modifications. The trypsin reaction should be immediately terminated by heating the fluid to boiling. Step 129, water is utilized as a blank control to observe the inhibition of the lectin hemagglutinating activity by different glycosyl groups. Step 121 through step 127 is performed three to five times so as to determine the characteristics inidicating the best path of detoxification.

[0027] Step 131, the lyophilized powder lectin needs is dissolved in a phosphate buffer to prepare a lectin solution. Step 132, the lectin lyophilized powder needs to be determined for pH stabilization. This can occur and can be conducted by dissolving in buffers of desired pH to formulate a solution. After standing at 4 °C for 12 h-24 h, the acid–base stability of the lectin needs to be studied by a hemagglutinating experiment and fluorescence spectroscopy, respectively. Glycine–HCl buffer solution at pH 2.0, citrate buffer solution at pH 3.0 to 5.0, phosphate buffer at pH 6.0 to 8.0, and glycine–sodium hydroxide buffer solution at pH 9.0 to 11.0 should be used to maintain the pH.

[0028] Step 133, ensuing dissolution of the lectin powder, the hemagglutinating activity of the lectin solution can be determined. Step 135, the lectin solution is dialyzed in a phosphate buffer solution containing ethylene diaminete tracetic acid until no hemagglutinating activity is detected. Step 137 the lectin solution is then dialyzed against phosphate buffer solution to remove the ethylene diaminete tracetic. Step 139 the obtained sample solution is subjected to a hemagglutinating activity analysis to confirm that the hemagglutinating activity is lost. Step 141, the lectin solution displaying no hemagglutinating activity is mixed with different metal salt solutions and then diluted to detect hemagglutinating activity to

determine the effect of metal salt ions on the recovery of hemagglutinating activity of the seed, plant or root.

[0029] Metal ions function to provide a key role in maintaining the stability of the lectin structure and maintaining its specific biological activity. Ethylenediaminetetraacetic acid is a divalent metal ion-chelating agent. When the lectin fully reacts with Ethylenediaminetetraacetic acid, the hemagglutinating activity of lectin is lost, and the reaction is generally reversible. Mn^{2+} and Ca^{2+} ions could completely restore the hemagglutinating activity of the lectin while Mg^{2+} ions could only partially restore the activity. It is concluded in the scope of the present invention that the Mn^{2+} and Ca^{2+} ions are essential. Using this process, it prohibits glucose, N-acetyl- D-glucosamine, D-galactose, N-acetyl-D-galactosamine, fructose, sucrose, D-maltose, D-trehalose, and 2+2+ 2+ lactose of formulas and can not inhibit the hemagglutinating activity of the lectin and Mn , Ca , and Mg ions. This results in the restoration of the hemagglutinating activity of the lectin. Using thermal stability and pH stabilization processes, it is determined and indicated that the hemagglutinating activity of the lectin has changed with the change of protein conformation. This aids in the production and manufacturing of seeds, nutritional supplements, pharmaceuticals, environmental products, and biotech products having the purified lectin product of the method of the present invention incorporated therein.

[0030] Step 143, the lectin solution is placed in centrifuge tubes wherein the centrifuge tubes are placed in water baths wherein the water baths are at various temperatures. It is contemplated within the scope of the present invention that the temperatures of the water baths are both warmer and colder than that of the environment in which the centrifuge tubes are present. The centrifuge tubes are completely submerged in the water bath throughout the process and sealed to prevent the evaporation of water or loss of solution. While no particular temperature ranges are required, it is contemplated within the scope of the present invention that a series of

thermal treatments needs to be carried out at 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C, wherein the thermal treatments are executed for intervals within a range of five to thirty minutes. Ensuing the termination of the thermal treatment, the centrifuge tubes are immediately cooled in cold water and stored at 4 °C. It should be understood within the scope of the present invention that alternate storage temperatures could be employed

[0031] In the preceding detailed description, reference has been made to the accompanying drawings that form a part hereof, and in which are shown by way of illustration specific embodiments in which the invention may be practiced. These embodiments, and certain variants thereof, have been described in sufficient detail to enable those skilled in the art to practice the invention. It is to be understood that other suitable embodiments may be utilized and that logical changes may be made without departing from the spirit or scope of the invention. The description may omit certain information known to those skilled in the art. The preceding detailed description is, therefore, not intended to be limited to the specific forms set forth herein, but on the contrary, it is intended to cover such alternatives, modifications, and equivalents, as can be reasonably included within the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals wherein the method comprises:

creating a fine powder, wherein the fine powder is created from a seed, root, plant or legume, said fine powder being created utilizing a 50 mesh sieve;

mixing the fine powder, wherein the fine powder is mixed with phosphate buffered saline to create a mixture of fine powder and saline;

filtering the mixture, wherein the mixture is filtered for a crude extract;

purifying the crude extract, wherein the crude extract is purified utilizing an aqueous magnetic two-phase solution;

collecting and dialyzing lectin and proteins, wherein the lectin and proteins are collected and dialyzed against deionized water;

purifying the lectin into a lectin lyophilized powder, wherein the lectin is purified utilizing gel chromatography;

filtering the lectin,

determining hemagglutinating activity;

formulating lectin lyophilized powder into a lectin solution;

analyzing for sugar specificity;

mixing equal volumes of a sugar solution and the lectin solution, wherein the mixture of sugar solution and lectin solution is stored at ambient temperature for up to two hours;

detecting hemaglutination, wherein the mixture of lectin solution and sugar solution is diluted to detect hemagglutinating activity;

preparing a simulated gastric fluid, wherein the lectin solution is mixed with the simulated gastric fluid having a pH of 1.2 in a water bath at 37 °C to commence a digestion reaction;

dialyzing the lectin solution, wherein the lectin solution is dialyzed against phosphate buffer solution to remove ethylene diaminete tracetic acid;

confirming the hemagglutinating activity is lost, wherein the lectin solution is determined to be absent of hemagglutinating activity;

mixing the lectin solution with at least one metal salt, wherein the lectin solution displaying no hemagglutinating activity is mixed with different metal salt solutions; and

centrifuging the lectin solution;

2. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein the lyophilized lectin powder is mixed with phosphate-buffered saline by agitation for a period of ten to twelve hours.

3. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein during mixing of the lyophilized lectin powder the mixture is subjected to alternate temperatures that are different than that of the environment in which the mixture is located.

4. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, t, wherein the aqueous magnetic two-phase solution is formed by mixing an amount of ammonium sulfate, polyethylene glycol 600 and NaCl.

5. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein the step of dialyzing the lectin solution further employs utilization of a dialysis bag having 500-10000-Da molecular weight cutoffs so as to remove salt and polyethylene glycol.

6. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including the step of performing gel filtration, wherein five to ten milliliters of the

filtered lectin solution is applied to filtration media utilizing gel filtration chromatography.

7. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including the step of diluting the lectin solution, wherein ensuing formulation, a portion of the lectin solution is two-fold serially diluted with stroke-physiological saline.

8. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including the step of terminating a pepsin reaction, wherein the pepsin reaction is terminated by adding Na_2CO_3 solution with a pH of 11.0 after a specified time period.

9. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including the step of terminating a trypsin reaction, wherein the trypsin reaction is terminated by utilizing a boiling point temperature.

10. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including the step of determining pH stability, wherein pH stability is conducted by dissolving lyophilized lectin powder in buffers of desired pH to formulate a solution and wherein the solution is stored at 4 °C for a period of twelve to twenty-four hours.

11. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including a step of studying hemagglutinating the lectin solution, wherein

during the studying of the hemagglutinating a Glycine–HCl buffer solution at pH 2.0, a citrate buffer solution at pH 3.0 to 5.0, a phosphate buffer at pH 6.0 to 8.0, and a glycine–sodium hydroxide buffer solution at pH 9.0 to 11.0 are utilized to maintain the pH.

12. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein the method further includes a step of performing thermal treatments, wherein the thermal treatments are conducted at 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C, and wherein the thermal treatments are executed for time intervals within a range of five to thirty minutes.

13. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein during the method of the present invention the lectin solution fully reacts with Ethylenediaminetetraacetic acid and the hemagglutinating activity of the lectin solution is terminated.

14. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, wherein during the method of the present invention utilization of Mn^{2+} and Ca^{2+} is performed so as to prohibit glucose, N-acetyl- D-glucosamine, D-galactose, N-acetyl-D-galactosamine, fructose, sucrose, D-maltose, D-trehalose, and 2+2+ 2+ lactose of formulas.

15. The method for detoxification of lectin so as to be utilized in materials such as but not limited to nutraceuticals and pharmaceuticals as recited in claim 1, and further including a step of restoring hemagglutinating activity, wherein the hemagglutinating activity is restored utilzing Mn^{2+} and Ca^{2+} ions.

ABSTRACT OF THE DISCLOSURE

The method of the present invention is operable to provide isolation, purification and characterization of lectin from seeds, roots, bark, leaves, tubers, plants, fruits, vegetables, beans, nuts, and oils in order to assist with the characterization of the lectin and its activities in products. Detoxification of negative characteristics of lectin and protection of positive nutritional benefits which include but are not limited to ant-fungal, anti-viral, and anti-bacterial properties of the lectin while enhancing the important physiological roles, including insecticidal action, antibacterial activity, and antifungal effects, antihuman immunodeficiency virus, antitumor activity, and analgesic activity of lectin.
The present invention employs an aqueous magnetic two-step solution and further utilizes fractionation for lectin detoxification while providing protection of the the nutritional, antihuman immunodeficiency virus, antitumor activity, analgesic activity, insecticidal activity, anti-viral, anti-fungal, and immunity benefits of lectin.