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EXTRACTING THE LECTIN SUBSTANCE FROM PEA FLAKES AND ANALYZING IT

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ABSTRACT:

Lectins are proteins that bind to specific carbohydrates. They are found in most plants, aswell as in humans, microorganisms, animals, and fish. Therefore, they are found in many of the foods we eat. The most concentrated forms appear to be in edible seeds such as thosefound in cereal grains and legumes. The edible seeds of the legume plant (Leguminosae) may be referred to as pulses, legumes, dried beans, or beans.Lectin levels in plants and food crops can vary significantly depending on environmental conditions such as drought and salinity. Lectin compounds may have evolved in plants as asurvival mechanism, reducing the chance the they will beconsumed in large amounts.

Keywords: Lectin, PBS is a physiologic solution of phosphate buffer, Pea flakes, Extract, Louri Method.

RELEVANCE OF WORK:

Currently, there is a great demand for medicinal preparations from natural raw materials all over the world. Because drugs, developed on the basis of biologically active substances, which are separated from natural raw materials - plant, animal organisms, are considered harmless to the human body than drugs obtained by synthetic means. Therefore, we set a goal to isolate lectin on the basis of local plants.

Lectins are proteins that combine with specific carbohydrates. Vegetable lectins are proteins that are specially bound to carbohydrates, and they are recognized as a kind of sugar. The biological functions of lectins include cell-to-cell interaction, interaction of pathogens, and innate immunity reactions. Lectins are found mainly in leaves, vegetative tissues, roots and seeds. These are glycoproteins that bind with special mono or oligosaccharides without changing the covalent structure. They are usually in the seeds of some leguminous plants. They account for 3% of the weight of the fertilized egg. Lectins appear in living organisms, but plant lectins are the first studied proteins. [5]

They are in most cases exist in microorganisms, animals and fish. Therefore, most of them are contained in foods that we consume. The most concentrated forms are in the content of seeds, cereals, and legumes. [6]

Lectins are found in most plants, including willow, corn, tomatoes, beans, bananas, peas, lentils, soybeans, mushrooms, rice and potatoes.[7 , 8]. Lectin belonging to the

class of glycoproteids is a drug that has its place in the media as well as in pharmaceuticals. Lectin accumulates in large quantities in the composition of all leguminous plants (including plants such as soybeans, beans, peas, mung beans). It is used in medicine for cleaning antibodies, diagnosing cancer patients, in the detection of cancer cells, and in the study of mutant cells. [9]

PURPOSE OF THE STUDY:

Extract the lectin substance from the pea pulp and determine its physical chemical properties.

OBJECT OF THE STUDY:

Pea flakes

Practical Aspect:

The seeds of peas in an amount of 120 g were smashed and degreased with the help of Sokslet apparatus[1]. The resulting sample was dried, measured in 100 g and extracted in a refrigerator at 40 C for 1 day at 400 ml PBS.

PBS is a physiologic solution of phosphate buffer [2].

Salt	Concentration (mmol/L)	Concentration (g/L)
NaCl	137	8.0
KCl	2.7	0.2
Na_2HPO_4	10	1.42
KH_2PO_4	1.8	0.24

The resulting extract was centrifuged at a speed of 7000 turnover/minute for 20 minutes, separating the supernatant. The resulting supernatant was initially precipitated in a refrigerator at 40 C for 1 day using 30% of the ammonium sulfate. Then, at a speed of 7000 rpm, it was centrifuged for 20 minutes, separated from the sediment, and the supernatant part was drowned in the refrigerator at 40 C for 24 hours using 90% of the ammonium sulfate. Then, at a speed of 7000 revolutions/minutes, centrifugating for 20 minutes and the sediment was separated. The resulting sediment was desalinated with the help of dialysis bags at 1000(Da) in distilled water for 2 days. The samples obtained were frozen using nitrogen liquid, dried in Lyophilic dryer and the amount of water-soluble proteins was determined using the Louri Method [3]. 1(30%) accounted for 14.25%, 2(90%) accounted for 62.8%.

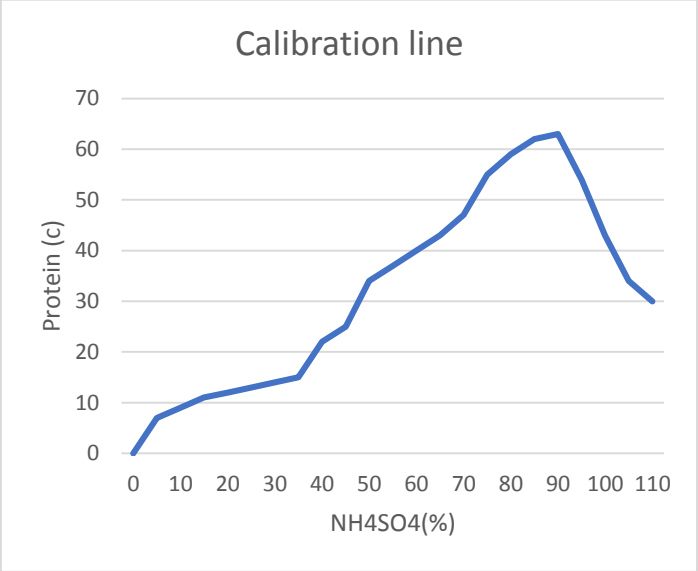
The most common methods used in determining the amount of protein is the Louri method, which differs from other methods with extreme accuracy and convenience. The Louri method is based on the property of proteins to form colored ointments under the influence of biuret and folin reagents. Complex color intensity depends on the amount of protein in the solution under investigation, which is determined using a photocolorimeter. This method makes it possible to detect a very small amount of proteins contained in extremely diluted solutions.[6]

Reagents: reagent A is a mixture of 0.1 n of sodium carbonate salt, 0.2% solution of sodium hydroxide, reagent V - mixture of 60ml of a solution with 1ml of B reagents. It is mixed before use. The tube 50g sodium wolframat, 12.5 g Sodium molybdate and 350 ml distilled water is added to a tube with a foil reaction volume of 1L. After cooling, 25ml 85% orthophosphate acid and 50 ml hydrochloric acid is added to the tube. Boil for 10 hours, connecting a reversible refrigerator to the tube. Then again 75g lithium sulfate, 25ml water 2-3 drops of bromine are added and boiled. After the mixture cools, the volume is reached to 500ml using water. The solution should be orange in color as crisp.

The order of the work: 0.1-0.2 ml of the investigated solution containing 5-10 mcg of protein was taken into the test tube. On top of it

is added 1ml V reagent and left for 10 minutes, stirring thoroughly. Quickly added from 0.1 ml of Folin reagent on the mixture, and leave it for 30-40 minutes. The yellow color in the test tube gradually changes to blue. The intensity of the color of the solution is measured in a photocolorimeter (using a red light filter) or a spectrophotometer at a wavelength of 750nm (the thickness of the bun is used from 1sm cuvettes). The amount of protein in the sample under examination is determined by the calibration line prepared using pure protein.

Drawing up the calibration line: first of all, protein solutions with a certain concentration are prepared. For this, a number of standard solutions are also prepared using the prepared protein solution. The amount of protein in them is 10, 20..... and so should be 100mkg. Crystalline proteins, for example , take 100mg of animal albumin from casein or egg albumin and dissolve in 100ml of 0.1 N of corrosive sodium . The volume is increased by 10ml of a protein solution prepared for 10 of a measuring probe, that is, 1 ml for the first probe, 2 ml for the second probe, etc.is poured into the 10th probe from a 10ML protein solution.Solutions in the test tube are delivered to the line with distilled water. In the end, the protein content in the test tubes goes from 0.1 mg to 1mg. Solutions in the test tubes are mixed thoroughly. To determine the amount of protein, 0.1 -0.2 ml of each probe is taken and the above-mentioned reagents are added. When a stagnant color is formed, color intensity is determined using a FEK or spectrophotometer. Each of the prepared solutions must return Louri reactions at least 3 times, and depending on the average data obtained, a line of calibration is drawn.



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