

Extraction and characterization of proteins assignment

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Abstract The activity of invertase and the quantification of albumin and casein were performed and analyzed after extraction of the said proteins from their respective sources. Isolation of proteins was initiated by the breakage of the cell wall / membranes in three different ways.

Homogenization of invertase, albumin and casein were achieved via grinding process, addition of 1M acetic acid and acidification by 0. 1M hydrochloric acid correspondingly. Extraction of invertase and casein involved precipitation through the utilization of 95% ethanol.

The obtained crude extracts for invertase and casein were 0. 86 g and 0. 9092 g respectively. In contrast, albumin's isolation depended on its solubility in saturated ammonium sulfate salt. Two trials were performed and the resulting weights of the precipitates were 19. 32 g. for precipitate 1 and 2. 80 g. for precipitate 2. The activity of invertase was ascertained through Benedict's Test. Test tubes containing either a combination of glucose and fructose or sucrose exhibited an orange coloration??? indicative of a weak positive result.

Quantification processes for albumin were determined using Warburg-Christian and Bradford Assays. Both method involved spectrophotometry. The resulting protein concentrations in Warburg-Christian were 1. 0042 mg/ml for precipitate 1 and 0. 7427 mg/ml for precipitate 2. The Bradford Assay for precipitate 1 (test tubes 7, 8 and 9) yielded 7. 47, 8. 97 and 8. 28 ? g/ml and for precipitate 2 (test tubes 8 and 9) gave 6. 44 and 4. 73 ? g/ml in that order. Casein's concentration was deduced through the Warburg-Christian Method yielded 0. 265 mg/ml.

Discussion of Data and Results Invertase??? also known as sucrase, saccharase or α -fructofuranosidase??? is an efficient enzyme in the hydrolysis of sucrose. Despite abundance in molds, bacteria, plants and higher animals; the most common source of invertase has remained to be yeast, in which “ invertase exists in more than one form??? the lightweight (135 kDa) intracellular invertase enclosed within the rigid cell wall and the heavyweight (270 kDa) extracellular variety that coats the outside of the cell. ” It is because of the existence of the rigid cell wall separating the two types of invertases that sand has to be mixed during the grinding process to ensure breakage of wall and to obtain a large amount of isolated invertase. The addition of hexane creates a non-polar environment for the enzyme as well as for other intracellular components. Subsequent pouring of water in portions not only introduces a polar medium but also minimizes the interference of proteases, species which cleave peptide bonds within polypeptide chains (endopeptidases) or sequentially digest amino acids from the C or N terminus (exopeptidases), on the isolation of invertase.

Cheesecloth filtration, repetitive centrifugation and discarding of sediments are performed afterwards to remove unwanted components such as the added sand or the ruptured cell walls. The precipitation of invertase is made possible by immersion of the final supernatant in an ice bath and pouring of 95% ethanol. The method is based fundamentally on the principle regarding dielectric constants. Upon introduction of the alcohol, “ the dielectric constant is lowered and the attractions between the ionizable groups of the enzyme are increased.

Consequently, interactions among the proteins present are lessened and the solubility of the invertase in the medium is decreased. " Centrifugation is again employed to serve a similar purpose. However, instead of discarding the sediments as before, it is the supernatant that must be removed as most, if not all, of the invertase species in the solution are assumed to have undergone complete precipitation. Lastly, the precipitates are both weighed and analyzed of the physical properties (Experimental Results: weight = 0.85 g and physical properties = white powdery precipitate) before dissolving in 0. M acetate buffer with pH 5 as the enzyme is known to " exhibit relatively high activity over a broad pH range 3.5 to 5.5 with the optimum pH near 4.5" or " 4.6" The activity assay for invertase was investigated by preparing solutions with components as stated in the table of the Laboratory Manual on page 12. The prepared test tubes are placed in a water bath maintained at 37?? C. However, temperature may exceed 37?? C as it has been known that " enzyme activity reaches a maximum at 55?? C. " Upon reaching equilibrium, 2ml of invertase extract is introduced to the first, third and fourth test tubes.

After a second equilibration period, 2ml of 10% NaOH is added subsequently to each test tube to stop the occurring reaction. Finally, 1ml of Benedict's reagent is poured to test for the presence of sugars with free anomeric carbons (reducing sugars). (Experimental Results: orange coloration in test tubes 1, 2 and 3 and no visible reaction in test tube 4). The experimental results are in accordance with the theoretical results as supported by the following: (a) " A positive Benedict's test is observed as the formation of a brownish-red cuprous oxide precipitate.

A weaker positive test is the appearance of a yellow or an orange coloration;" (b) Test tube 3 contains two reducing sugars, glucose and fructose, and is therefore expected to yield a positive outcome; (c) Test tube 1 contains a non-reducing sugar, sucrose. But because the test tube contains an appreciable amount of water for hydrolysis and it has been determined that " invertase catalyzes the hydrolysis of the disaccharide sucrose to invert sugar, a mixture of glucose and fructose," a positive result is again anticipated; (d) Test tube 2 is similar to test tube 1 except for the absence of the enzyme.

But since the test tube also contains enough water to hydrolyze sucrose to its corresponding monosaccharides, a positive outcome is deduced; (e) The hydrolysis of sucrose is also " catalyzed by acids. " Indeed even in the absence of invertase, test tubes 1 and 2 are anticipated to exhibit color change; and (f) Test tube 4 is expected to yield a negative result as neither sucrose nor a combination of glucose and fructose is present. Lastly, may it be added as additional information that in the hydrolysis of sucrose, inversion in the optical activity occurs (i. . from dextrorotatory to levorotatory). The extent of isolating the albumin was to use its solubility depending on their amino acid composition and salt concentration in the solution, as well as the ionic strength and temperature of the solvent used. With salt at low concentrations present, stabilization of several charged groups present on the protein molecule happened and thus attracting protein into the solution enriching its solubility. The phenomena is commonly called salting-in. s the salt concentration is increased, there is a point in which maximum protein solubility is reached and further increase in

concentration will result in less water available to solubilize the protein. Having not enough water molecules to interact with the proteins or the “dehydration” of the protein environment, they precipitate and this is called salting-out. To isolate albumin from eggs, the salting-out principle was used, specifically, the isolation used the method of decreasing salt concentration. Two different groups worked in albumin isolation. 20 mL of fresh egg white were used and placed in a beaker.

While stirring the sample with a stirring rod, 2.0 mL of 1.0 M Acetic acid was added to disrupt the cell membrane so that the cell contents were released. The process is called homogenization. Then by filtration using cheesecloth, the crude protein was separated from its cellular components and contaminants. While filtering, the cheesecloth was pressed against the sides of funnel or beaker by a rod, squeezing out the filtrate and breaking the cell membranes. The filtrate were collected in a 250-mL beaker, and along the sides of beaker, equal volume of salt, saturated $(\text{NH}_4)_2\text{SO}_4$ solution, was added slowly in the filtrate.

Due to its high solubility, does not usually denatures proteins and relatively inexpensive, $(\text{NH}_4)_2\text{SO}_4$ was used for this process. Upon addition of saturated $(\text{NH}_4)_2\text{SO}_4$ solution, large number of water molecules bind to the SO_4^{2-} decreasing the amount of water available for binding to proteins. As much the protein content as possible was first precipitated using concentrated saturated $(\text{NH}_4)_2\text{SO}_4$ solution. Then the mixture was let to stand for about 30 minutes, after that, it was centrifuge and the precipitate

was discarded. The resulting precipitate was the protein that was not hydrated by binding to water molecules.

Also different proteins precipitate at different salt concentrations and that fact was majorly exploited to eliminate contaminating protein in the crude extract. The supernatant was transferred to a 250-mL Erlenmeyer flask that was immersed in an ice bath. 1: 1 saturated $(\text{NH}_4)_2\text{SO}_4$ solution (50% solution) was added until turbidity persisted in order to extract selectively the albumin that was soluble in concentrated saturated $(\text{NH}_4)_2\text{SO}_4$ solution. To complete the precipitation, the mixture was let to stand for 15 minutes, and after that the mixture was subjected to centrifuging.

The resulting supernatant was discarded and the precipitate collected was the crude albumin extract. The crude albumin precipitates mass were measure and found to be 19. 32 and 2. 8 grams for the first and second precipitate respectively. From the resulting precipitates, 20 mL of 10% (w/v) albumin solutions were prepared using 0. 9% NaCl in two different test tubes and store in a cool place for determination of protein concentration. The temperature was regulated to as low as possible to minimize heat denaturation and the pH was carefully regulated to avoid denaturation rought about by the fluctuation in pH. Because proteins tend to denature at dilute solution, the protein concentration was kept as high as possible. Casein may be extracted from milk by acidification and the crude extract purified. In this experiment, a 25mL sample of fresh milk was acidified with 0. 1 M HCl. The acid was added to the fresh milk sample until flocculent precipitates were observed. Just enough acid was added to the fresh milk

sample to avoid the dehydration of the sample which would lead to the denaturation of casein. The pH was measured during the acidification, and a pH of 4.8.

At this pH level, casein and many other proteins are released from binding cell structures and cellulose. The solution with precipitates was centrifuged and the supernatant was discarded. Then, the residue was washed using 95% ethanol. Washing with ethanol makes sure there are no sugars left in the sample that may greatly interfere with the assay. The ethanol washings were removed through centrifugation and decantation. The precipitate, which by then was a dirty white, sour-smelling grainy residue, was finally washed with acetone and dried under the fume hood. After the solution was dry, it was weighed.

Using this weighed crude casein extract, a 20mL 1% weight by volume solution in 0.01M NaOH medium was prepared. This preparation would be used for the assays. The Warburg-Christian assay takes advantage of the strong absorption of nucleic acids tryptophan and tyrosine in the 280nm wavelength (in the UV range). The advantages of this method are that it is nondestructive, direct and sensitive. It is nondestructive because the proteins are not subjected to any calorimetric reaction. It is direct because it is the component nucleic acids of the proteins itself that absorbs, and not some other substance which reacts with the protein.

Finally, this assay is sensitive because it can detect samples with concentrations as low as 20 μ g/mL. One disadvantage of the method is that different proteins absorb differently at 280nm. Because of this, the assay

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cannot distinguish one protein from the other in a protein mixture. But as long as only a single protein predominates in a given sample, the assay would work. Perhaps the most considerable disadvantage of absorbance measuring at 280nm is the interference of nucleic acids. But Warburg and Christian have independently come up with ways to correct for this discrepancy. It was noted that nucleic acids generally absorb stronger near the wavelength 260nm. Because of this, there are at least two ways to approximate the protein sample concentration from the absorbance in both 280nm and 260nm. One way is through the use of the equation below.

$$\text{Protein concentration} = 1.55A_{280} - 0.76A_{260}$$

Here, A_{280} and A_{260} are the absorption of the sample at 280nm and 260nm, respectively. Their coefficients in the equation above were determined experimentally. The other method for correcting the discrepancy due to nucleic acid interference is by means of a table comparing the ratio A_{280}/A_{260} with the experimentally determined first-order approximations of the %nucleic acid impurities.

For example, in this experiment, $A_{280}/A_{260} = 0.888$. From Table 3 in Experiment 1 of Biochemistry Laboratory Manual (2007 edition), this corresponds to about 5% nucleic acid impurity. Thus, the sample prepared in this experiment is approximately 95% pure casein extract, according to this correction method. Of course, the correction methods cannot fully obliterate the errors in the assay. However, as it stands, the advantages of the Christian-Warburg assay easily overthrow the disadvantages whenever first-order approximations are enough (which is most of the time).

It was also necessary to determine the protein concentration from the extracted crude proteins. Casein concentration was determined via the Warburg-Christian assay and the egg albumin concentration was determined in two ways, the Warburg-Christian assay and the Bradford Dye Binding Protein assay or simply the Bradford assay. In protein concentration determination using the Warburg-Christian assay, two 20 mL of 1% albumin from the stock extract were prepared using distilled water. The absorbances of each solution were measured at 280 and 260 nm with distilled water acting as the blank.

The resulting A_{280}/A_{260} are listed below: Precipitate A_{280}/A_{260} 10.9200.5551.6620.6650.3791.75 Table 1: A_{280}/A_{260} of albumin for % Protein determination by UV-Vis Spectrophotometry From the Table 3 of the laboratory manual, precipitate 1 had % Nucleic acid in the range of 0.25 and precipitate 2 had 0 % Nucleic acid. Therefore, the % protein for both precipitates 1 and 2 were at 99.75% and 100% respectively. Using the formula/equation provided by the laboratory manual, the resulting protein concentration for both precipitates 1 and 2 were 1.0042 mg/mL and 0.427 mg/mL respectively. On the other hand, the resulting absorbance for the extracted crude casein is listed below with the corresponding protein concentration evaluated using the equation: $A_{280}/A_{260} \text{ Conc. , mg/mL}$ 0.3810.4290.8880.265 Table 2: A_{280}/A_{260} of albumin and Protein Concentration by UV-Vis Spectrophotometry From the table 2, the nucleic acid in casein as found to be around 5 % and the percentage of protein was found to be around 95%. Below is the table for the tabulated Trials Albumin extract concentration Casein extract concentration ppt 11.0042 mg/mL 0.

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265 mg/mL ppt 20. 427 mg/mL Table 3: The resulting protein concentration of each extracts using the Warburg-Christian assay Another method studied to quantify albumin concentration was the Bradford assay. The method used a dye-binding process through the color change of the dye in different protein concentration. The Coomassie Brilliant Blue G-250 (structure below) dye in acidic media has its absorbance shifting from 465 nm to 595 nm. The shifting is due to the noncovalent electrostatic and van der Waals interaction of the proteins. Under acidic media, the dye is a red-colored compound in cationic, 2+, form.

When it binds with a protein, the dye becomes in a blue-colored stable form that is anionic. Figure 1: Coomassie Brilliant Blue G-250 structure. Reference; www.colby.edu/chemistry/CH367/laboratory/expt2.pdf The anionic dye is sensitive to proteins containing the following amino acids: Arg, Lys, Trp, Tyr and His. At 595 nm, the assay was checked in a spectrophotometer and measured the dye complex with the protein. The method can be use to quantify proteins having 1 ??? 20 ?? g/mL. This one step procedure formed a relatively stable complex that was colored and free from interferences.

Plastic cuvettes are used because the dye binds with quartz cuvettes. The table below was used in the assay of albumin. Volume of solutions in mL Test tube 1 2 3 4 5 6 7 8 9 Std BSA 0.0 20. 40. 60. 81. 0 Albumin Extract 0. 30. 50. 7 Distilled water 9. 89. 69. 49. 29. 08. 89. 59. 39. 1 Bradford rgt 0. 20. 20. 20. 20. 20. 20. 20. 20. 2 Table 4: Solutions prepared for Bradford assay of albumin. Test tube 1 is the blank, test tubes 2 ??? 6 are the calibration points and test tubes 7 ??? 9 are the samples. The CBBG dye was added lastly.

After preparing the said solutions, the absorbance of each was read at 595 nm.

It was noted that the test tubes with the solutions must be read within an hour after the addition of dye. Calibration curve was constructed through plotting the absorbance against the BSA concentration. In the resulting calibration plot below, a second order curve suited the plot better than linear one. This is due to the absorption spectra of the two forms of CBBG dye that overlap. Figure 2: Calibration curve for Bradford assay BSA conc. , ?

g/mL Absorbance 20. 007 40. 007 60. 008 80. 009 100. 013 Table 5: Data Points for the Calibration Curve

The Bradford assay, responds primarily to Arg rather than the other aromatic amino acids so, it the standard to be used played a major role for this quantification. The albumin contains Arg in its quaternary structure, so the right standard to use must have Arg residue that is present in Bovine Serum Albumin or BSA. However, BSA had twice than normal response in the quantification method and may become unreliable. This constitutes a limitation for the Bradford assay. Another important thing in the assay was the buffer blank. Due to its non-linearity, the plot should include the absorbance at 0 concentration of BSA.

The blank primarily consisted of water and the Bradford reagent because of the fact that the interferences were neglected or not in effect. The table below shows the resulting concentration of albumin extract: ppt 1ppt 2 test tube absconc. , ? g/mL absconc. , ? g/mL 70. 0097. 470. 005 80. 0118. 970. 0086. 44 90. 018. 280. 0074. 73 Table 5: The resulting albumin extracts

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concentration The calc. conc. ' s represents the calculated concentration using the square fit plot. The given concentrations are the concentration presented by the groups that did the procedure here.

As shown in the table 4 above, the results did not have any significant pattern whether if it is increasing or decreasing. In ppt 2, the solution in test tube 7 did not enter the calibration curve, thus it was omitted. Comparing the obtained albumin extract concentration through Warburg-Christian assay and the concentration from the Bradford assay, it can be concluded that both methods are suitable in determining the concentration of albumin extract. Bradford assay is sensitive than Warburg-Christian assay and it is suitable in use for micro-assays.

Like the other assays, however, its response is prone to influence from non protein sources, particularly detergents, and becomes progressively more nonlinear at the high end of its useful protein concentration range. The response is also protein dependent, and varies with the composition of the protein. These limitations make protein standard solutions necessary. Other Methods of Protein Extraction , 1. Solids Separation (e. g. Centrifugation and Filtration) 2. Cell Disruption and Debris Separation (e. g. Homogenization, Bead Milling, Chemical and Enzyme Lyses and Debris Separation) 3.

Primary Separation Operations (e. g. Precipitation or Solubilization [via variation in temperature, pH, ionic strength, dielectric constants and etc.], Two-Phase Extraction, Diafiltration, Ultrafiltration and Nucleic Acids Removal) 4. Size-Dependent Separation (e. g. Gel Filtration) 5. Charge-Dependent Separation (e. g. Electrophoresis, Isoelectric Focusing, Ion-Exchange

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Chromatography, Chromatofocusing) 6. Hydrophobicity Separation (e. g. Hydrophobic Interaction Chromatography, Reverse Phase Chromatography) 7. Affinity Chromatography Other Methods of Protein Quantification 1.

Biuret Reaction ??? The reaction involves production of a purple coloration of strongly alkaline copper reagent with a particular protein. Disadvantages: (a) The method is fairly accurate as coloration varies from protein to protein, (b) It has low sensitivity, (c) Ammonium sulfate fractions interfere with the method by forming a complex with copper and (d) It is a type of destructive analysis. 2. Lowry Method ??? The method involves production of a strong, dark blue coloration of a combination of a strongly alkaline copper reagent and the Folin-Ciocalteu reagent with phenols in proteins.

Disadvantages: (a) Many compounds used in enzyme purification interfere with the method and (b) It is a type of destructive analysis. 3. Bicinchonic Acid (BCA) ??? The procedure is similar to the Lowry Method in which a reaction occurs between the strongly alkaline copper and a particular protein. Copper is then reduced and combines with bis-cinchonic acid to produce a purple coloration. Disadvantages: (a) A 30-minute period is advised to ensure correct color development and (b) It is a type of destructive analysis. References: Dunham, S.

Biochemistry 367 Laboratory Manual, 2004 edition, Experiment 2: Comparison of Protein Analysis. Colby College. Boyer, R. F, Modern Experimental Biochemistry, (Benjamin / Cummings Publishing Co. , Inc, 1993) pp. 77-88 Haldane, J. B. S, Enzymes, (Great Britain: Longmans, Green and Company Ltd. , 1930) p. 101 Harris, E. L. V. and S. Angal, Protein <https://assignbuster.com/extraction-and-characterization-of-proteins-assignment/>

Purification Applications: A Practical Approach, (NY, USA: Oxford University Press Inc. , 1995) Malcolm, A. D. B, Enzymes: An Introduction to Biological Catalysis, (Great Britain: William Clowes and Sons Ltd. , 1971) p. 9 Nam Sum Wang, Experiment No. 4: Enzyme Kinetics of Invertase Via Initial Rate Determination, (MD, USA: Department of Chemical and Biomolecular Engineering, University of Maryland) Scopes, R. K and C. R. Cantor, Protein Purification: Principles and Practice, (NY, USA: Springer-Verlag, 1994) Hydrolysis of Sucrose by the Enzyme Invertase, (Cape Canaveral) Website References: [http://www. glue. umd. edu/~NSW/ench485/lab6a. htm](http://www.glue.umd.edu/~NSW/ench485/lab6a.htm)
[http://matcmadison. edu/biotech/resources/proteins/labManual/chapter_4/section4_2. htm](http://matcmadison.edu/biotech/resources/proteins/labManual/chapter_4/section4_2.htm)
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