PREPARATION AND BIOCHEMICAL ASSAY OF PHARMACEUTICAL ENZYMES FROM PANCREAS (A SLAUGHTER HOUSE WASTE)

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Four different methods were tried for the extraction of Pancreatin from the pancreas of ox and buffallo (a slaughter house waste). Activity units of the enzyme constituents i.e. amylase, lipase and trypsin were determined in the various pancreatin powder preparations.

It was observed that the acetone precipitation method was most suitable. The product thus obtained exhibited maximum enzymatic activity and the method also proved fairly economical.

Introduction

All the enzymes, associated with the living cells of plants or animals, are proteinous in nature and defined as heat labile, biological catalysts possessing a specific power of catalysing reaction without being destroyed in the process or becoming part of the final product. In the last 30 years a great deal of work has been done in which classification, investigation, and mode of action of many enzymes have been performed. Since 1864, when pepsin was included in British Pharmacopoeia, the importance of enzymes in pharmacy and medicine has steadily increased. But there is still a good deal to learn about the medicinal aspects of enzymes, because of the 700[1] classified enzymes known to-day, relatively a few are important in medicine.

Pancreas of ox and buffalo which is a slaughter house waste in Pakistan is of great importance due to the presence of some important enzymes in its juice. Pancreas secretes internally Insulin and externally pancreatin. Biochemical assay shows that pancreatin consists of amylase, lipase and trypsin as the major constituents. These enzymes do not exist as a whole but are secreted as zymogen—in the duodenum in which pancreas secretion is discharged. Pancreatin in alkaline or neutral media, digests proteins, converts starch in to sugar and saponifies fats. It is used to check the destructive action of free hydrochloric acid in stomach.[2] Medicinally it is administered by mouth[3] to treat the pancreatic deficiency pancreatitis, fibrocystic disease of pancreas, aids in digestion and absorption of food of all types. It is also used in preparing predigested peptonized food for patients, desizing of textiles and leather bating. Trypsin (in capsule, dry powder or in ointments) is used to clean wounds, ulcers, abcesses, to remove eschar from second degree burns.[4]

The aim of the present work is to utilize the pancreas, a slaughter house waste, for the production of pancreatin, the importance of which in medicine, has been discussed.

Experimental

Preparation of Pancreatin.—Fresh ox pancreas were freed from adhering fat and external membrane. They were stored in deep freeze. Frozen pieces were dipped in liquid air and ground into a fine powder in a pestle mortar. The powder thus obtained was macerated with 15% alcohol and distilled water separately.

Maceration with 15% Alcohol.—The pancreas powder (837 g.) was macerated in 15 percent alcohol (1500 ml.) for three days and stirred twice a day for an hour at 0°C. The liquid extracted was squeezed, filtered and centrifuged at 8000 rpm at 0°C. to remove insoluble matter. The resulting liquid (1720 ml.) is a weak solution of enzymes mostly in the form of unactivated precursors together with soluble proteins and other unwanted material. The precipitation of enzymes is induced by the addition of high concentration of salts, such as $MgSO_4$, NH_4Cl and $MgCl_2$. All the procedures were conducted at temperature near 0°C. and repeated several times for complete precipitation. The precipitate was filtered and redissolved in 15 percent alcohol. After a number of precipitations the purified enzymes obtained were freeze-dried in order to have a minimum loss in potency and a final product with very low moisture content. The data in respect of the yields of pancreatin with concentrated solutions of magnesium sulphate, magnesium chloride and ammonium chloride is given in Table 1.

Acetone Precipitation Method[6].—Frozen pancreas pieces were powdered in the same way as in Method No. 1. The powder (279 g.) was macerated in 80 ml. of distilled water and kept continuously stirred for 6 hours at 0°C. Then it was squeezed through a cheese cloth. The extract was filtered and centrifuged at 15000 rpm.

TABLE 1.

S. No.	Pre-cipitant	Vol. of precipitant used (ml.)	Weight of pancreatin (g.)	Yield %
1	NH_4Cl	130	23	8.24
2	$MgCl_2$	300	26	9.31
3	$MgSO_4$	380	32	11.48

to remove the suspended matter. The resulting clear liquid (slightly brownish) 95 ml. was mixed with three times its volume of chilled commercial acetone (285 ml.). The enzymes precipitate so formed was centrifuged. The supernatant liquid was decanted off and the precipitate was again stirred in acetone. This process was repeated till the precipitate was quite dry. Finally it was washed with ether. After complete drying and mechanical grinding, the enzymes powder (15 g.) was stored in vacuum over $CaCl_2$ at 0°C. so that it may retain its activity for a long period. The percentage yield by this method was 5.39.

The pancreatin powder is buff-coloured, amorphous in nature, soluble in water forming a slightly turbid solution and insoluble in 95 percent alcohol and solvent ether. The proteolytic activity is rapidly destroyed in acid solution or by boiling the aqueous solution.

Identification.—1 g. of the pancreatin powder was dissolved in water and its pH was adjusted to 8.0 by the addition of N/1 NaOH, using cresol red as indicator. The solution was divided into two equal parts and one part was boiled. A few shreds of congo red fibrin were added to each part maintaining them at a temperature of 38°C. for one hour. It was observed that after one hour the unboiled liquid stained red in the case of each sample while the boiled liquid remained colourless. This showed the presence of pancreatin in each preparation.

Biochemical Assay.—(a) Qualitative: The tests for the minimum activity of amylase, lipase and trypsin were performed according to the standards of British Pharmacopoeia and it was found that pancreatin powder prepared by any of the methods gave positive results.

(b) Quantitative: In order to determine the activities of the enzymes in units/g. the methods and procedures were adopted which are used for the determination of activity units of these enzymes in duodenum contents.[4]

For the determination of various enzymes activity, 0.05 g. of pancreatin powder was dissolved in 50 ml. of water and the respective procedures were carried out with 1 ml. of this solution. Finally the results were calculated in units/g.

(1) Amylase: Determination of amylase activity units was done by Langerlof Method.[4] The amylase activity is expressed in Langerlof units and is defined as the amount of amylase of which one thousandth part gives a reaction constant of 0.001 during the conditions of the test. The reaction constant may be calculated from the formula:

$$C = \frac{1}{t} \times \log \left(\frac{7.5}{7.5-a} \right)$$

where C is the reaction constant, t is time in minutes and a is the amount of maltose formed during the test in mg.

The amylase activity units were studied by using standard Langerlof Table.

TABLE 2.

S. No.	Pancreatin powder	Difference of reading in ml.	Langerlof unit/ml.	Lengerlof units/g.
1.	Acetone powder	0.400	3.532	3532
2.	NH_4Cl powder	0.250	2.148	2148
3.	$MgCl_2$,,	0.350	3.060	3060
4.	$MgSO_4$,,	0.240	2.145	2145

(2) Lipase: Lipase activity is expressed in Goldstein-Roe Units[4] which is a measure of the amount of short chain of fatty acids released, expressed in terms of 0.1N alkali.

Calculations: Activity is given by:

$$\text{Lipase activity} = \begin{pmatrix} M_1 \text{ of 0.1N KOH} \\ \text{required in test} \end{pmatrix} - \begin{pmatrix} M_2 \text{ of 0.1N KOH} \\ \text{required in blank} \end{pmatrix}$$

$$= \text{Goldestein Roe's units/ml.}$$

TABLE 3.

S. No.	Pancreatin powder	Difference of readings of 0.1N alc. KOH (ml.)	Goldstein units/ml.	Goldstein units/g.
1.	Acetone powder	2.86	2.86	2860
2.	NH_4Cl ,,	0.45	0.45	450
3.	$MgCl_2$	0.38	0.38	380
4.	$MgSO_4$	0.14	0.14	140

(3) Trypsin: The trypsin units were studied by using Langerlof method 4 and the standard tables.

TABLE 4.

S. No.	Pancreatin powder	Difference of readings of 0.1N KOH in ml.	Trypsin units/ml.	Trypsin units/g.
1.	Acetone powder	1.10	0.496	496
2.	NH_4Cl ,,	1.00	0.438	438
3.	$MgCl_2$,,	0.85	0.358	258
4.	$MgSO_4$,,	0.80	0.336	336

Discussion

A suitable method for the mincing of pancreas pieces was to be sought out before maceration, either with 15 percent alcohol or distilled water. This mincing could not be done in a meat mincer or by any other mechanical device as there was always possibility of enzyme contamination and loss of their activity. Of all the methods tried, the method of dipping of the fat-free pancreas pieces in a sufficient amount of liquid air, was found to be the most suitable. The frozen solid pancreas pieces were then ground in a pestle mortar to a fine powder. According to Table 1 the percentage yield of pancreatin powder was 11.4, 9.31, and 8.24 by using different volumes of higher concentrations of various precipitants i.e. $MgSO_4$, $MgCl_2$ and NH_4Cl, respectively. On the other hand the percentage yield by acetone method was only 5.39.

Table 2 shows amylase unit/g. This is evident from Table 2 that the units/g. of amylase were maximum in acetone powder and minimum in $MgSO_4$ powder. Similarly Tables 3 and 4 show that lipase units and trypsin units per g. were again maximum in the case of acetone powder while minimum in $MgSO_4$ powder. In addition, acetone precipitation method does not require freeze-drying of the final products as a dry powder was obtained by the repeated washing with acetone. Furthermore the time consumed for the complete extraction of enzymes from pancreas in acetone precipitation method was comparatively less. It is therefore a more economical method, particularly because acetone used for precipitation can be recovered. Although the percentage yield of the powder by this method is not as high as in the other cases, the activities of the enzymes constituents i.e. amylase, lipase and trypsin are maximum.

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