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ISOLATION OF PEPSIN FROM BUFFALO AND ITS ACTIVITY

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Optimum conditions for the isolation of pepsin from the young buffalo were studied. Changes in pH, temperature and isolation time effected the isolation of pepsin. Pepsin was precipitated from the extract by adding sodium chloride (200 g/litre). Maximum pepsin activity (194 units/g) was observed when minced gastric tissues were suspended in acidic solution of pH 2.2 at 40°C for 18 hours. Pepsin activity in camel, cow, buffalo end chicken gastric tissues, was found to be 102, 143, 192 and 224 units/g respectively, while 174 units/g pepsin activity was observed in the cases of sheep and goat. The gastric tissue samples from different animals were kept at -10°C for six months and a gradual decrease in pepsin activity of 18-68% was observed during storage.

Keywords: *Pepsin; Buffalo; Isolation; Precipitation; Minced gastric tissues*

Introduction

Pepsin is a porteolytic enzyme present in variable amounts in the gastric tissues of nearly all the vertebrates (1-5). It has been estimated that the average amount of pepsin in cattle, sheep, swine and rabbit is 1300, 1200, 1490 and 1175 mg/g respectively(6). It has also been reported that younger animals have relatively more pepsin in their gastric tissues than the older ones(7). Pepsin has been used as a digestive enzyme in many pharmaceutical preparations. It has many useful industrial applications such as making cheese and protein hydrolysates (8-10). The literature regarding the optimum conditions for its isolation and purification is scanty. Pepsin is usually isolated by suspending minced gastric tissues in acidic solutions containing hydrochloric acid, sulphuric acid or phosphoric acid. Various methods for the isolation of pepsin from the gastric and other tissues of different animals, such as buffalo(11), swine (12), chicken and ducks(13), camel (14) and monkey (15), have been described by a number of workers. These methods are often lengthy, time-consuming, complicated and labourious. Moreover, the yield of pepsin by these methods is often very poor. Therefore, there is a need to develop a simple and proficient method for the isolating of pepsin from gastric tissues of various animals.

The present work was undertaken to optimize the conditions (such as pH, temperature and time of isolation) for the development of simple and proficient procedure of pepsin isolation from buffalo gastric tissues. Since the temperature in the summer season is high, gastric tissues were kept in deep freezers to avoid deterioration. The effects of cold temperature (-10°C) on the activity of pepsin during the storage period was also studied.

Materials and Methods

Collection of materials: Gastric tissues from young buffalo (almost 2 years old) and other animals were collected from the local slaughter house soon after assassinating the animals. The stomach contents were removed and washed with chilled water immediately. The washed tissues were put in an ice box and brought to the laboratory for processing. Garbage and fatty layers were removed before mincing. Minced tissues were packed in plastic bags and preserved in the freezer for later use.

Citric acid solution: pH 2.2 (5 g citric acid in 100 ml water)

HCl buffer: pH 2.2 (25 ml 0.2 M KCl and 3.35 ml 0.2 M HCl in 100 ml water)

HCl solution: pH 2.2 (8 ml 0.1 N HCl in 100 ml water)

Isolation of pepsin: Minced gastric tissues were mixed well with water of different pH containing 1.0% boric acid keeping the substrate-water ratio 1:1.00, 1:1.25, 1:1.50, 1:1.75 (w/v). The desired pH of the mixture was adjusted with 0.1 N hydrochloric acid by pH metre. The mixture was then incubated at different temperatures for various time periods with frequent stirring.

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Table 1. Effects of different solutions on the isolation of pepsin (Mean of three readings)

Extractants (pH 2.2)	Material: Extractant ratio	Temp. (°C)	Time (hr)	Units/g Tissue Weight
Citric acid solution	1:1.25	40	18	120
HCl-buffer	1:1.25	40	16	142
HCl-solution	1:1.25	40	18	206

After incubation, the contents were filtered and pepsin activity was estimated in the filtrate. HCl solution, HCl-buffer and citric acid solutions of pH 2.2 were also used for isolating the pepsin. Composition of these solutions have been outlined in Table 1.

Precipitation of pepsin: Sodium chloride and ammonium sulphate were used as precipitating agents for pepsin. Different amounts of the precipitating agents were added slowly with frequent stirring. The mixture was then allowed to stand for about 20 minutes for the precipitation to be completed. After filtration it was dried at ambient temperature.

Estimation of pepsin activity: The method described by Anson (16) was used for the determination of pepsin activity in the gastric tissues enzyme extract. The enzymatic reaction was started by the addition of 1 ml of 2.5% haemoglobin that had previously been acidified with HCl to pH 2.5 and incubated for 10 minutes at 37°C. The reaction was then terminated by adding 10 ml of 5% trichloro acetic acid. The mixture was then filtered through Whatmann No. 576 filter paper. Finally the optical density of the filtrate was measured at 280 nm on a spectrophotometer.

Protein concentrate: The offal was also minced, mixed with water and autoclaved for 30 minutes. Fat was skimmed off from the top, excess of water was removed by filtration through cheese cloth and the tissues were dried at 60±5°C. Protein concentrate was obtained by mixing the autoclave dried offal with the residue left after isolation of pepsin from gastric tissue. Chemical analysis of protein concentrate was carried out according to AOAC method (17),

Microbiological examination: Pathogenic microorganisms such as *E. Coli*, *Salmonells sp.* and *Clostridium sp.* were isolated and identified by the methods described in the Handbook of Microbiology published by Merck (18).

Results and Discussion

Optimum conditions for the isolation of pepsin: Isolation of pepsin from the gastric tissues of the animals were greatly influenced by the change in pH, temperature of the medium and the time period of incubation. The pH of the solution plays a vital role in the isolation of pepsin. The results indicated that the conversion of pepsinogen to pepsin was highly modified at different incubation pH values (1.2, 1.7, 2.2 and 2.7). Highest activity of pepsin was found to be at pH 2.2 (Table 2). In case of buffalo, maximum activity of pepsin in the extract was found to be 194 units/g of fresh tissues weight when minced gastric tissues were suspended in acidic solution of pH 2.2 at 40°C for 18 hours (Table 2). At pH 2.7, the activity of pepsin indicated a marked decline at all temperatures and incubating periods.

Variable amounts of pepsin were obtained from the minced gastric tissues at the temperatures ranging from 30-45°C. Maximum activity of pepsin in the extract was found to be 90 units/g at 40°C on mixing the tissues with acidic solution of pH 1.2 after 18 hours. Similarly, highest pepsin activity was also observed at 40°C, when pH of the solution was raised from 1.2 to 2.7. However, reduction in pepsin activity was observed when the temperature was further raised from 40 to 45°C (Table 2).

It was further observed that acidic solution of pH 1.2 isolated only 36 units/g of pepsin from the fresh gastric tissues after 6 hours. The amount of enzyme seemed to increase with the increase of the isolation period. The optimum time for the maximum enzyme isolation appeared to be 18 hours. Maximum pepsin activity was 70

Table 2. Effects of pH temperature and time period on the isolation of pepsin (Mean of three readings)

Isolation time (hr) *	Pepsin activity (Units/g fresh tissue weight)															
	pH 1.2				pH 1.7				pH 2.2				pH 2.7			
	30	35	40	45	30	35	40	45	30	35	40	45	30	35	40	45
6	36	42	67	58	131	141	146	140	144	155	170	153	130	131	144	134
12	50	59	78	61	132	144	150	141	152	158	180	161	132	142	148	136
18	70	74	90	71	140	147	158	145	161	172	194	170	148	150	161	144
24	71	72	83	68	143	162	170	162	158	175	191	168	160	171	172	151
30	71	74	85	71	144	174	182	171	152	166	171	160	154	167	170	153

*Isolation temperature in °C

Table 3. Effect of material extraction ratio on pepsin activity at pH 2.2(Mean of three readings)

Material: Extractant Ratio	Temperature (°C)	Time (hours)	Units/g tissues wt.*
1:1.00	40	18	171
1.1.25	40	18	214
1:1.50	40	18	156
1:1.75	40	18	132

Table 4. Effect of different salts on the precipitation of pepsin from the filtrate (Mean of three readings)

Precipitating agent	Amount added (g/Litre)	Crude pepsin yield (g/kg)	Pepsin activity (units/mg dry wt.)
Sodium chloride	50	8.22	948
	100	16.54	950
	200	19.18	974
Ammonium sulphate	50	4.43	861
	100	8.92	866
	200	11.21	882

units/g after 18 hours at 30°C at pH 1.2. No further increase in the amount of pepsin was observed after 18 hours of incubation period (Table 2).

Effects of different material-extractant ratios on the activity of pepsin (at 40°C for 18 hours) indicated a maximum pepsin activity (214 units/g) when this ratio was 1:1.25 (Table 3). A decline in the pepsin activity by about 38% was observed when the same ratio was increased to 1:1.75.

On the basis of the results outlined in the tables 1-3, it may be concluded that maximum amount of pepsin could be isolated by mixing the minced gastric tissues with HCl solution of pH 2.2 in 1:1.25 ratio at 40°C for 18 hours.

Isolation of pepsin using different solutions: The results outlined in table 1 indicated that the maximum pepsin activity was 206 units/g with HCl solution at 40°C after 18 hours while minimum activity of pepsin was with the citric acid solution. HCl-buffer also isolated pepsin, but the activity was comparatively less than the HCl solution. The lower activity indicated that, the presence of potassium ions in HCl-buffer had probably reduced the activity of pepsin to some degree. Therefore, HCl solution of pH 2.2 could effectively be used for the isolation of pepsin from the gastric tissues of the animals.

Precipitation of pepsin: Crude pepsin was precipitated from the filtrate by adding variable amounts of sodium chloride and ammonium sulphate. Sodium chloride was found to be a better precipitating agent than ammonium sulphate. Maximum crude pepsin (19.18 g) was produced from 1 kg of the fresh

Table 5. Effects of storage on pepsin activity of different animals gastric tissues (Mean of three readings)

Animals	Units/g tissue weight*						
**	0	1	2	3	4	5	6
Buffalo	192	184	180	166	87	74	69
Cow	143	134	114	84	71	68	58
Goat	174	167	164	160	151	138	110
Sheep	174	168	165	161	148	138	114
Camel	102	90	85	72	60	54	45
Chicken	224	208	203	201	196	196	180

**Storage time in monts

gastric tissue when sodium chloride at the ratio of 200 g/litre was added slowly to the filtrate with constant stirring (Table 4), while 11.21 g/kg crude pepsin was recovered with ammonium sulphate under similar conditions. The activity in crude pepsin (dried) was found to be 974 units/mg (Table 4).

Effects of storage on pepsin activity at freezing temperature: The minced gastric tissue samples of different animals were stored at -10°C in a deep freezer for six months. The samples were taken out at intervals of one month and analysed for pepsin activity. The results outlined in Table 5 indicated that pepsin activity in fresh gastric tissues of different animals varied from 102 to 224 units/g which decreased from 18to 68% during the storage period of six mounths at -10°C. These results are in concurrence with the findings of other workers (19,20) who also reported variable amounts and activity of pepsin in different species of ruminants, while Djordjevic *et al.*(21) observed a 20% decrease in the activity of pepsin from the dog's gastric tissues after storage at freezing temperature.

Fresh gastric tissues of buffalo contained 192 units/g of pepsin which decreased to 166 units/g after three months of storage at lower temperatures. However, pepsin degradation took place rapidly and its amount declined when the storage was continued for another three months. The sample contained only 69 units/g of pepsin at the end of six mounths, although it was stored at freezing temperature of -10°C (Table 5). Similar tendencies were also observed when cow gastric tissue samples were kept at -10°C for six months. Therefore, it is apparent that under these conditions, buffalo and cow gastric tissues should not be stored for a period of more than three months. Sheep and goat gastric tissues contained 174 units/g of pepsin. Its production was also decreased but at a slower rate, as compared with the production of pepsin from buffalo and cow gastric tissues. So it seemed feasible to isolate pepsin from sheep and goat gastric tissues, which were not stored more than four months at -10°C. These results are in agreement with the findings of other workers (22) who also reported that the sheep pepsin was more stable than cattle pepsin.

Fresh gastric tissues of camel contained 102 units pepsin/g which decreased to 45 units/g after storage for six months at -10°C. Among various animals' gastric tissues that were examined, chicken gastric tissues possessed a maximum pepsin activity i.e. 224 units/g which was also decreased to 180 units/g after storage for six months at freezing temperature. This means that chicken pepsin was more stable when compared with the pepsin obtained from other sources.

Utilization of protein concentrate: Protein concentrate was achieved as a by-product after the isolation of pepsin from gastric tissues of cattle. It contained 68.4% protein and 5.39% minerals, whereas 13.8% fat was also present

in this by-product. Microbiological examination of this product showed that it was free from pathogenic microorganisms such as *E.coli, Salmonella sp.* and *Clostridium sp.* and bacterial counts were found to be 7000 per gam. These results indicated that this could be utilized as a protein ingredient in poultry or other animals' feeds.

Conclusion

The optimum conditions for the isolation and precipitation of buffalo pepsin from young buffalo's gastric tissues were standardized and the following procedure was used; 100 grams of minced fresh gastric tissue were suspended in 125 ml solution of 1% boric acid (pH 2.2) and this pH was kept constant with 0.1 N HCl throughout the incubation period. Gastric tissues in acidic solution were placed at 40°C for 18 hours and were agitated periodically. This solution was then filtered through cheese cloth and 200 g/litre NaCl was added by stirring the filtrate to provide complete precipitation of pepsin. The activity of dried crude pepsin was found to be 974 units/mg.

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