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PRODUCTION OF PEPSIN FROM CATTLE GASTRIC TISSUE

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The optimal conditions for the extraction of pepsin from cattle gastric tissue have been determined. The yield of pepsin is maximum when minced tissue adjusted at pH 2 is incubated at 40° for 24 hr. Pilot plant experiments and material balance have been described for the production of pepsin. The protein concentrate obtained as a by-product may be used as a source of protein in poultry feed.

Key words. Pepsin, Gastric tissue, Cattles.

Introduction

Pepsin, an animal proteolytic enzyme is present in the gastric juice of all vertebrates [1-4] as an inactive precursor, pepsinogen. The conversion of pepsinogen to pepsin is catalysed by hydrogen ions or by pepsin itself. Pepsin has many industrial applications. It is used in medicine as a digestive aid, in the manufacture of cheese and in the preparation of protein hydrolyzates [5-8].

On the industrial scale, pepsin is usually manufactured from gastric tissue of pig, cattle and sheep [9-12]. The aim of the present study was to prepare pepsin from cattle gastric tissues which are abundantly available in the slaughter houses of the major cities of the country and are not, at present, being put to any proper use and thus go waste.

The affect of different variables, such as pH, temperature and incubation time on the yield of the enzyme on the laboratory scale was studied. Attempts were also made for the larger scale production of the enzyme on the basis of the study carried out at laboratory scale.

Materials and Methods

Gastric tissues of cattle (ox and buffalo) were collected from the local slaughter house soon after the cattle were slaughtered. The cleaned tissues were minced, packed in plastic bags and placed in a freezer for later use.

Preparation of pepsin. Minced gastric tissue (250 g) was mixed with water (250 ml) well stirred and adjusted to the desired pH with hydrochloric acid and incubated at the desired temperature for the chosen time with frequently stirring. The mixture was then filtered and the filtrate was processed further to obtain pepsin by precipitated with sodium chloride to a final concentration (200g/litre) of the original filtrate. The salt was added slowly with continuous stirring and the mixture was allowed to stand for about 1/2 an hr to complete the precipitation. The mixture was filtered and precipitate of crude pepsin collected, spread on glass plates, dried under fan at room temperature (32-35°) and weighed.

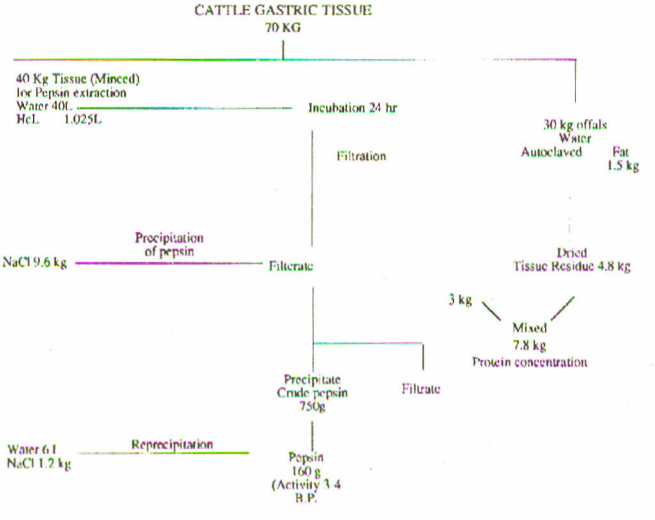
The dried crude pepsin was redissolved in water, filtered and pepsin was reprecipitated with salt (200 g/litre) which was found to have 3 to 4 times the pepsin activity of the crude obtained earlier.

Assay of pepsin activity in the tissue extract. Amounts of pepsin in the tissue extract was determined by the B.P. method[13]. The volume of the tissue extract required to digest completely 12.5 g of egg albumin in 4 hr was determined. From this the amount of pepsin per ml of the extract was determined.

Autoclaving. The offals were minced, mixed with water and autoclaved for 1 hr. Fat was skinned off from the top, water drained and tissue dried at 60-70°.

Proximate analysis of protein concentrate. The dry weight was obtained by drying the sample at 106° for 6 hr. Nitrogen was determined by Markham micro method using selenium as catalyst [14]. For the estimation of fat, the sample was finely powdered and extracted with ether. For the estimation of ash the sample was placed in Muffelled furnace at 550-580° for 6 hr. Fibre was estimated by acid alkali digestion method [15].

Microbiological examination of purified pepsin and protein concentrate was performed by the method described in a book published by Merck [16].



Flow sheet for the production of pepsin from cattle gastric t issue.

Results and Discussion

Temperature, pH and time are the main variables which could affect the conversion efficiency of pepsinogen to pepsin and hence the yield of the latter. Experiments carried out to find the affect of these variables at laboratory scale are described below.

Effect of pH. Minced tissue (250 g) was placed into each of 4 beakers, and 250 ml of water was added. The pH of each sample was adjusted to 1,2,3 annd 4 respectively with dilute hydrochloric acid on pH meter and incubated at 40° for 24 hr. After the incubation the contents were filtered and pepsin activity was assayed in the filtrate. The results given in Table 1 indicate that the conversion of pepsinogen to pepsin varied appreciably at different incubation pH. Highest conversion (8.9 mg/ml) was obtained at pH 2 and least at pH 1 (4 mg/ml).

Effect of temperature. The effect of temperature on the yield of pepsin was studied at different temperature ranging from 30 to 60°. The weight of tissue and volume of water was taken as above and keeping the pH of contents at 2 and incubation time 24 hr. The results are given in Table 2. Clearly, the maximum yield of pepsin (8.5 mg/ml) was obtained at 40°. Higher temperature of incubation decreased the yield of pepsin and it was only in traces at 60°.

Effect of time of incubation. Minced tissue (250 g) was taken in each 5 beakers, 250 ml water added, pH adjusted to 2 and contents incubated at 40° for different interval of time ranging from 8 to 48 hr. The contents of one beaker were removed after 8, 16, 24, 32, 40 and 48 hr and pepsin activity was assayed in the filtrate. The results are given in Table 3. The yield increased as the time was increased and was maximum (8.9 mg/ml) after 24 hr With further increase in incubation time the yield of pepsin decreased gradually.

From the results obtained with the above experiments, it is concluded that incubation of tissue at pH 2 at 40° for 24 hr gives the maximum yield of the enzyme.

Proximate composition of protein concentrate. Protein concentrate obtained from offals was mixed with tissue residue left after the extraction of the enzyme from tissue and analysed. Proximate analysis of the protein concentrate is given in Table 4. From the results it is evident that protein concentrate contains 68.4% protein and 13.8% fat. Probably it can be utilized as a protein ingredient for poultry feed.

Microbiological examination of the protein concentrate showed it to be free from any pathogenic micro-organism and the product contained overall bacterial counts 7,000 gm.

Pilot plant production of pepsin. The parameters obtained on laboratory scale experiments were utilized for the production of pepsin on the pilot plant scale. The details of the experiment are given in flow sheet diagram. Minced tissue (40 kg) was taken in a drum, 40 litre of water was added, the pH was adjusted to 2. The contents were thoroughly mixed and incubated at 40° for 24 hr. During incubation the contents were stirred frequently. After the incubation the contents were filtered through cheese cloth to remove the bigger tissue particles. Smaller particles were removed by sedimentation 64 litres of extract were obtained. Pepsin was precipitated with sodium chloride (200 g/litre). The mixture was allowed to stand for 1/2 an hr to complete the precipitation. Precipitated pepsin was recovered by filtration. Precipitate was spread in

TABLE 1. EFFECT OF PH ON THE YIELD OF PEPSIN.

pH	Volume of filtrate (ml)	Pepsin (mg/ml)
1.	350	4.0
2.	340	8.9
3.	345	6.7
4.	330	5.3

Tissue 250 g; water 250 ml; Temperature 40°; Time 24 hr.

TABLE 2. EFFECT OF TEMPERATURE ON THE YIELD OF PEPSIN.

Temperature °C	Volume of filtrate (ml)	Pepsin (mg/ml)
30	260	6.7
40	350	8.5
50	340	4.0
60	335	Traces

Tissue: 250 g; Water; 250 ml; pH: 2; Time: 24 hr.

TABLE 3. EFFECT OF TIME OF INCUBATION ON THE YIELD OF PEPSIN.

Time (hr.)	Volume of filtrate (ml)	Pepsin (mg/ml)
8	340	3.3
16	350	6.7
24	350	8.9
32	340	5.0
40	330	4.0
48	325	2.5

Tissue: 250 g; water: 250 ml; pH: 2; Temperature: 40°

TABLE 4. PROXIMATE ANALYSIS OF PROTEIN CONCENTRATE.

	%
Moisture	5.60
Nitrogen[14]	10.96
Protein (Nitrogen value x 6.25)	68.40
Fat	13.80
Fibre[15]	3.20
Ash	5.40

stainless steel trays and dried under fan at room temperature (32-35°). Crude pepsin (750 g) was obtained. Crude pepsin was then purified by reprecipitation. The purified pepsin (160 g) so obtained had an activity 3-4 times.

The purified pepsin was tested for its microbiological purity. No pathogenic microorganism could be detected in the sample of pepsin. Overall bacterial counts was found to be approximately 5,000 per gm.

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