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Variation in Activity of Pepsin Extracted from Buffalo Stomach Mucosa

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Abstract. Pepsin was extracted from the buffalo's mucosa in an acidic medium by incubating at 40 °C for 48 h and dried in an air blanket at 50 °C. Conditions for the maximum yield of pepsin were optimized. Changes in pH, temperature and incubation time affect the yield of pepsin. It has been noted that the time of the year in which extractions were made under optimized conditions was an important factor which affected the yield as well as activity of pepsin. Studies showed that maximum yield 11.5% was in February 2009 and minimum 10.3% in May 2009. It was further studied that the activity of the pepsin extracted in February was higher i.e 110 U/mg as compared to the activity of the enzyme extracted during the month of May which was 102.6 U/mg. The purpose of the study was to consider the conditions of the slaughter houses to attain maximum yield of pepsin with maximum activity.

Keywords: pepsin extraction, enzyme activity, stomach mucosa, buffalo

Introduction

Pepsin is a digestive protease (Dunn and Fink, 1984) which is released by the chief cells in the stomach and degrades food proteins into peptides (Andreeva, 1994). Pepsin was discovered in 1836 by Theodor Schwann who also coined this enzyme's name from the Greek word pepsin, meaning digestion (Fruton, 2002). It was the first animal enzyme to be discovered, and in 1929 it became one of the first enzymes crystallized by Northrop (1946).

Pepsin is expressed as a pro-form zymogen pepsinogen, whose primary structure has additional 44 amino acids. In the stomach, chief cells release pepsinogen. This zymogen is activated by hydrochloric acid (HCl), which is released from parietal cells in the stomach lining. The hormone gastrin and the vagus nerve triggers the release of both pepsinogen and HCl from the stomach lining when food is ingested (Klomklao *et al.,* 2007). Pepsin digests up to 20 % of ingested carbon bonds by cleaving preferentially after N-terminal of aromatic amino acids such as phenylalanine, tryptophan and tyrosine (Bovey and Yanari, 1960). Peptides may be further digested by other proteases (in the duodenum) and eventually absorbed by the body. Pepsin is stored as pepsinogen so it will only be released when needed, and does not digest the body's own proteins in the stomach's lining (Northrop, 1946).

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Pepsin functions best in acidic environments, particularly those with a pH of 1.5 to 2 (Andreeva, 1994) and will denature if the pH rises up to 5 (Dee *et al.,* 2006). When the pH is adjusted back to 6.0 activity returns (Johnston *et al.,* 2007). It should be stored at very cold temperatures (between-20 °C and -80 °C) to prevent autolysis (self-cleavage). Autolysis may also be prevented by storage of pepsins by reductive methylation (Tanji *et al.,* 1988).

Pepsin is a multipurpose enzyme and is of great importance in food industry (Aukkanit and Garnjan-agoonchorn, 2010) and has therapeutic value (Barsky *et al.,* 1984.

Animal tissues like pancreas, liver, small and large intestine are available daily in very large amount from slaughter houses in the cities. These tissues are source of many other enzymes that are of great importance (Dunn and Fink, 1984). The stomach mucosa of buffalo is a rich source of pepsin. In this context, the objective of this study was to analyze the effect of pH, temperature and time of incubation in the activity of pepsin extracted from buffalo stomach mucosa. Its maximum yield and minimum loss in its activity within different months of a year was also estimated.

Materials and Methods

Collection of buffalo stomach. The buffalo stomachs were obtained from animal slaughter house, Lahore immediately after slaughtering the animals, during

February to May 2009. The collected tissues were kept in ice water and transported to the Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan as early as possible and were stored at -20 °C for further processing.

Processing. Extraction of pepsin from buffalo stomach mucosa was done according to the method of Zia-ur-Rehman and Shah (1997) with slight modifications. The stomachs were cut opened and washed with ice cold water to clean it from other waste products. The inner lining of mucosal part was obtained by cutting with sharp knife at 10 °C and minced in meat mincing machine. Two batches were set, each batch containing 1 kg wet weight of stomach mucosal lining. The linings were then suspended in 1.5 L of water. Afterward the water was acidified with 2N HCl and homogenized. The pH of homogenate was adjusted to 2.0 with HCl. The tissues of first batch of 1 kg wet weight were incubated at 40 °C for 24 h and the other batch was incubated at 40 °C for 48 h for the conversion of pepsinogen into pepsin. After completion of time, the extract was squeezed using muslin cloth. Saturated NaCl was added to the semitransparent liquid obtained after squeezing the extract. Mixture of each batch was allowed to settle down to extract maximum amount of precipitate that was filtered by using Whatman 40 filter paper. The precipitate obtained was dried in an air blanket at 50°C in tunnel oven so as to collect the pepsin. The dried pepsin was grinded to obtain amorphous powder and was preceded for analysis.

Analysis. The activity of enzyme was estimated according to Anson (1938), using hemoglobin as a substrate. One unit of enzyme can cause an increase in optical density by 0.001 at 280 nm at 40 °C.

$$U/mg = \frac{(A_{280nm} \text{ of sample} - A_{280nm} \text{ of blank}) \times 1000}{(10 \text{ mins}) \text{ (mg. Enz/mL of reaction mixture)}}$$

$A_{280\,nm}$ ---- absorbance at 280 nm
10 min--- incubation time

Results and Discussion

Reproducibility of the extraction method. Reproducibility of the method used for the pepsin extraction from buffalo stomach mucosa was also studied to gain maximum yield with maximum activity.

Effect of pH. The influence of pH on the extraction of pepsin was studied. It was observed that the yield of pepsin greatly influenced by the change in pH (Fig.1). Maximum yield was obtained at pH 2.0 and with the increase in pH from 2.0 to 3.5 a decrease of 13 % in the yield of pepsin was noted and this decrease in yield reached to 40% when pH raised from 2.0 to 4.5.

Effect of temperature and incubation time. Effect of incubating temperature and time is shown in Fig. 2 and 3, respectively. Maximum yield of pepsin was obtained when mucosal lining was incubated at 40 °C for 48 h. Above and below this time and temperature yield of pepsin decreased tremendously. Results are slightly contradictory to the findings of Saeed and Ford (1999), who reported maximum yield at 40 °C for 18 h incubation.

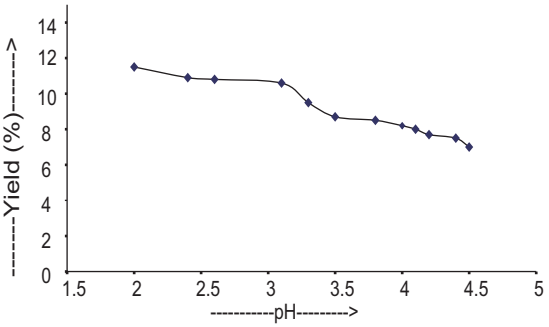

Fig. 1. Effect of pH on the yield of pepsin.

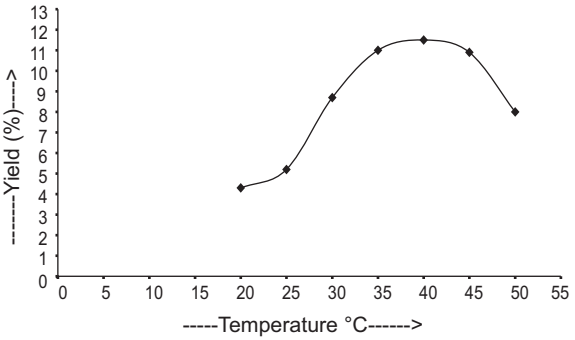

Fig. 2. Effect of incubation temperature on the yield of pepsin.

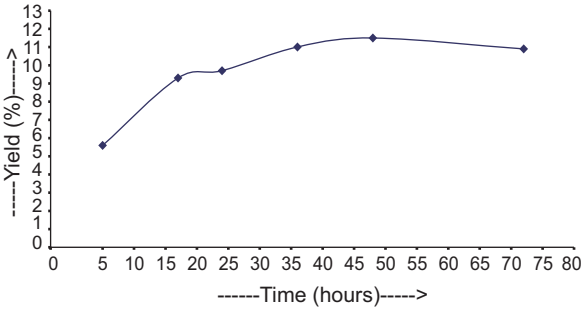

Fig. 3. Effect of incubation time on the yield of pepsin.

Effect of NaCl. Pepsin extracted from buffalo stomach mucosa in an acidic media was precipitated from the filtrate by adding sodium chloride (NaCl). Maximum pepsin was precipitated after the addition of 250 g NaCl to the filtrate (Fig. 4). Beyond this amount the addition of NaCl did not show any increase in the yield or activity of the enzyme.

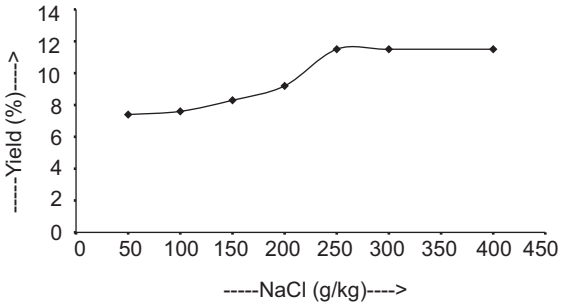

Fig. 4. Effect of NaCl on the yield of pepsin.

Effect of storage time. The minced mucosa of the buffalo was stored at -20 °C for 20 days. It was observed that the yield as well as the activity of the enzyme showed a slight decrease after 20 days of storage Table 1. The pepsin extracted from the fresh mucosa tissues showed maximum activity, while after storage it showed a decrease of activity, approximately 0.9 U/mg in February and March and 3.89 U/mg in May.

Effect of extraction in different months. The lower temperature in February seemed to be favourable for the maximum yield of pepsin. The maximum activity of pepsin was observed in the sample processed in the month of February i.e. to 110 units/mg enzymes (Anson, 1938). Poland and Bloomfield (1929) determined the activity of pepsin by using standard 2% solution of edestin and were found in the range of 100 to 110. These results were in agreement with the findings of Piper and Fenton (1965), who studied the activity of pepsin by radioiodinated serum albumen method. Tanji *et al.* (1988) demonstrated that pepsin activities were appreciable in preparation made from the fundus region of the stomach which was similar to present findings.

The minimum activity of the enzyme pepsin was observed during the month of May i.e. ranged from 102.6 to 87 units/mg in the samples. Niazi *et al.* (1997) studied extraction and isolation of pepsin from the stomach mucosa of buffalo and also noted the activity of this enzyme using hemoglobin as a substrate. Moreover, the precipitation method eliminated expensive and time consuming step of dehydration under vacuum. It was found that the stomach of buffalo slaughtered in big cities, if properly processed in time, would yield large amount of pepsin and save the expenses on its import. By improving the atmospheric conditions of the slaughter houses and controlling the temperature, pepsin yield as well as its activity can be improved.

Conclusion

The study proposes the use of slaughter house waste as a useful source for extraction of pepsin. The extraction

Table 1. Yield and activity of pepsin extracted from buffalo stomach mucosa

Months	Temperature of slaughter house (°C)	Days of storage at -20 °C	Yield (%)	Amount (g/kg)	Activity U/mg
February		1	11.50	115	110
	20	10	11.43	114.3	110
		20	11.20	112	109
March		1	11.30	113	109
	25	10	11.11	111.1	108
		20	10.94	109.4	108
April		1	11.30	113	103
	30	10	10.80	108	102
		20	10.29	105.1	99
May		1	10.30	103	102.6
	35	10	10.26	102.6	100
		20	10.26	102	87

steps are remarkably simple and do not involve costly chemicals and unique instrumentation. Yield as well as activity of enzyme is dependent on the temperature. Not only the temperature at which stomach mucosa stored before processing is important, but atmospheric temperature in which animals are slaughtered is also important to gain maximum yield with increased activity of the enzyme. The results of this study clearly showed the potential and versatility of this method, which could be applied to manage slaughter house waste and save foreign exchange.

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