

ENZYMATIC ACTIVITIES OF PORCINE STOMACHS AND PORCINE AND BOVINE PANCREASES

Lenka Zivkovic and Veselinka Djordjevic, Galenika-Institute, Beograd, 29 Novembar 111, Yugoslavia.

SUMMARY

Pepsin activity of porcine stomach glandular part is ranged from 252.7 to 377.3 F.I.P. units/g and it is about six to ten times greater than activity of whole stomach.

Amylase activities in porcine pancreases amount from 10,000 to 14,000 F.I.P. units/g, lipase activities from 12,000 to 16,000 and total protease activities from 600 to 750 units. In bovine pancreases amylase activities were found to be from 300 to 400 units, lipase activities from 350 to 450 and total protease activities from 230 to 250 units.

Changes in enzyme activities of long kept frozen pancreases were similar to changes of pepsin activities in stomachs after long frozen storage. No sample was found to contain free proteases up to 6 months frozen storage.

The investigation shows that the utility of these raw materials is approximately the same in 6 month period of frozen storage for production of insulin, pepsin, trypsin, chymotrypsin and pancreatine.

INTRODUCTION

Many slaughter by-products (endocrine glands, stomachs, small and large intestines etc) represent an important source of raw materials for enzyme and hormone production being used in medicine and veterinary practice. Enzymes are utilised in many industries and in analytic and diagnostic procedures (Deilweg and Hartmeier 1984; Szadkowska 1984; Caric et al. 1985; Djordjevic 1987; Kozatchenko et al. 1987).

The humanitarian significance of using by-products in production of medicines and pharmaceutical products is found in fact that by properly taken pancreases obtained insulin in sufficient to keep a person suffering from diabetes ten days (Anon 1975).

By testing enzyme activities of chymotrypsin from tissues of marine animals adapted at low ocean temperatures, it was found that chymotrypsin of dogfish was significantly more active than bovine one (Ramakrishna et al. 1987).

Having in mind the importance of a proper choice of by-products for their further processing, first of all for insulin and enzyme production, we investigated tissues that are the most abundant ones in enzymes. The purpose of this work was to find the initial enzyme activity of porcine and bovine pancreases and of various porcine stomach parts as well as to define keeping properties of raw materials for processing in the pharmaceutical industry.

MATERIALS AND METHODS

Porcine pancreases of Landrace pigs, approximately 6 months old, weighing about 100 kg and pancreases of Simmental cattle, approximately 18 months old, weighing about 400 kg as well as porcine stomachs, were taken out immediately following slaughtering, washed for a short time in cold water and immediately frozen at -2°C. The testing of their initial properties was performed 24 hours later. In the course of -20°C storage of pancreases, enzyme activities were checked after 30, 90, 180 and 210 days.

The initial pepsin activity was tested in whole stomach and in its individual parts: the glandular part of the stomach, the stomach without its glandular part, pylorus and duodenum. In mentioned investigations, 20 pigs were included i.e. 4 samples of whole stomachs obtained from 8 pigs of which each two stomachs were minced and homogenised; out of 12 pigs, individual parts of stomachs of 3 pigs were combined into a sample for testing and so 4 experimental samples were prepared.

Changes in enzyme activities of glandular part of stomachs and pancreases in comparison to the initial enzyme activity were investigated on 30 pigs and 30 cattle.

The initial activity was determined individually on all samples. Pancreases and glandular parts of stomach of 30 animals were cut into pieces and average samples were made; four average samples were sealed in containers and each taken for testing after certain storage time at -20°C.

In pancreases, both porcine and bovine, activities of amylase, lipase, total and free proteases were determined initially, after 24 hours of keeping the frozen tissues at -20°C, as well as after 30, 90, 180 and 210 days of keeping them at -20°C.

For determining the activity of amylase, lipase, protease and pepsin in raw materials, the procedure was developed in our laboratory and applied in experiments followed by F.I.P. methods used for the determination of enzyme activities in pharmaceutical preparations.

Table 1. Pepsin activities in different porcine stomach parts (in F.I.P. units/g, expressed on dry matter)

Part	S a m p l e s			
	1	2	3	4
Whole stomach	126	157	191	252
Stomach glandular part	1,260	1,323	1,449	1,512
Stomach without glandular part	out of detection limit			
Pylorus	out of detection limit			
Duodenum	out of detection limit			

Table 2. Pepsin activity of porcine glandular part (in F.I.P. units/g)

N	min.	max.	X	C _v
30	252.7	377.3	292.3	12.52

Table 3. Enzyme activities of porcine and bovine pancreases

	Activities (F.I.P. units/g) of		
	Amylase	Lipase	Total protease
<u>Porcine pancrease</u>			
N=30			
minimum value	10,000	12,000	600.7
maximum value	14,000	16,000	750.3
mean value	12,300	13,697	682.1
Correlation coefficient, C_v	6.39	9.07	9.66
<u>Bovine pancrease</u>			
N=30			
minimum value	300.7	350.1	230.6
maximum value	400.3	450.5	250.2
mean value	349.2	401.4	240.5
Correlation coefficient, C_v	7.98	7.19	2.69

Pepsin activity determination.- 2 g of homogenised samples of an average sample of minced tissue is mixed with 200 ml of 0.03 N HCl, pH 1.6±0.1 in Ultra-turax for 10 min at 4 to 5°C. The, 2 ml of the solution is to be diluted together with 10 ml of 0.03 N HCl, pH 1.6±0.1. The procedure is further continued according to F.I.P. method (Lauwers et al. 1974).

Determination of amylase activity.- 1.5 g of minced, homogenised sample is mixed with 100 ml phosphate buffer pH 6.8 in Ultra-turax at 4-5°C. Then 1 ml of solution is diluted with 10 ml of phosphate buffer, pH 6.8. Further procedure is performed by F.I.P. method (Lauwers et al. 1974).

Determination of lipase activity.- 1.5 g of minced, homogenised sample is to be mixed with 200 ml of cooled solution of 0.02 M CaCl₂ (2.94 g of CaCl₂·2H₂O in 1 L of water, pH adjusted to 6.0 to 6.2) in Ultra-turax in the course of 10 min at 4 to 5°C.

In case of bovine pancrease, the activity of free proteases is determined directly in a solution by F.I.P. method (Lauwers et al. 1974). Total proteases are determined by F.I.P. method following diluting of 10 ml of solution with 25 ml of solution of 0.02 M CaCl₂ (pH 6.0 to 6.2).

In case of porcine pancrease, for analysis of free proteases 10 ml of solution is diluted, whereas 5 ml of the same solution is diluted for analysis of total proteases with 25 ml of 0.02 M solution of CaCl₂ (pH from 6.0 to 6.2). Further procedure is performed according to the F.I.P. method.

The results of all enzyme activities have been expressed in F.I.P. units/g of raw tissue.

RESULTS AND DISCUSSION

The results of a comparative testing of pepsin activity in individual parts of porcine stomach (Table 1) indicate approximately 6 to 10 times greater pepsin activity in the glandular part of stomach than in the whole stomach while pepsin activity of the stomach without its glandular part, as well as a pylorus and duodenum were out of detection limit. The pepsin activity of the glandular part of stomach of 30 examined pigs of Landrace race was 353.7 to 377.3; average value was 292.3 F.I.P. units/g of raw tissue (Table 2). The value of the variation coefficient mainly points out the uniformity of pepsin activity in the stomach glandular part of 30 examined pigs. In 18 samples (60%) pepsin activity ranged 250-300 and only in 3 samples (10%) it was over 350 F.I.P. units/g.

Besides hormone - insulin, pancrease secretes enzymes important for pharmaceutical industry - proteolytic ones (trypsin, chymotrypsin and carboxypeptidase), amylolytic ones (amylase) and lipolytic ones (lipase).

Pancrease enzyme activity testings on pigs and cattle indicate (Table 3) that the activity of amylase in porcine pancrease is from 10,000 to 14,000 F.I.P. units/g, of lipases - from 12,00 to 16,000, and of total proteases from 600 to 750 units. No evidence of free proteases was established in tested pancreases. Correlation coefficients for determining enzyme activity point out the uniformity of

Table 4. Enzyme activities during frozen storage at - 20°C (in F.I.P. units/g)

Enzyme activity of	Initial	Storage time (days)			
		30	90	180	210
<u>Stomach-glandular part</u>					
Pepsin	292.3	292.0	291.3	290.9	280.2
<u>Porcine pancrease</u>					
Amylase	12,299	12,298	12,299	12,295	12,280
Lipase	13,697	13,696	13,694	13,697	13,600
Protease total	682.1	682.2	681.4	682.7	680.9
<u>Bovine pancrease</u>					
Amylase	349.2	349.0	347.4	348.6	335.2
Lipase	401.4	401.0	401.2	401.5	395.0
Protease total	239.9	239.0	239.4	239.2	238.9

amylases, lipase and protease activities of pancreases of 30 tested pigs.

In bovine pancreases, amylase activities are from 300 to 400 units, lipase activities from 350 to 450, and total protease activities from 230 to 250 F.I.P. units/g. Free proteases were not present. Pancreases of young cattle show uniformity regarding amylolytic, lipolytic and proteolytic activities on the basis of the correlation coefficients.

By mean values of examined enzyme activities (Table 3), it appears that approximately amylase and lipase activities are higher thirtyfive times and total protease three times higher in porcine than in bovine pancreases.

As pepsin is a very sensitive enzyme, especially during processing procedures starting with slaughtering the animal, the quality of glandular tissue of the stomach is of great importance as well as keeping up high enzyme activity until processing. Testing pepsin activity changes in the course of a long term storage of the glandular part of stomach in frozen state, it was found that at -20°C storage there were practically no changes for 180 days (Table 4). Changes, although insignificant ones, start immediately after than.

Testing results of enzyme activity during a long-time storage of pancreases in frozen state (Table 4) indicate that at -20°C practically there are no changes of examined enzyme activities until 180 days. After that, i.e. after 210 days free proteases are found. Due to that as well as to

the decrease of other enzyme activities the initial quality of raw materials is lowered.

According to performed investigations, the quality of raw materials - porcine stomach glandular parts and porcine and bovine pancreases - for use in insulin and enzyme production and in processing for pharmaceutical purposes is approximately the same up to six month storage at -20°C and after that it is lowered.

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