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Pepsin extraction process from swine wastes

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Abstract

The pepsin extraction from swine stomachs allows the valorization of meat wastes. Three methods are assayed to extract the pepsin from swine stomachs. Method 1, where the pepsin extraction is made by dissolution in water of the pepsinogen contained in fundus, has the highest extraction yield. Methods 2, that also considers a pepsinogen concentration step by coagulation, and Method 3, that also considers a lyophilisation process, leads to lower amounts of pepsin, but with a higher purity. This study could be useful to optimize the industrial extraction of pepsin from swine stomachs.

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Keywords: Pepsin; extraction process

Nomenclature

AU	Enzymatic activity units
A	Enzyme activity, in AU per cm3
VE	Volume of pepsin solution on activity assays
A_{280}	Absorbance at 280 nm

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1. Introduction

The use of enzymes allows the development of efficient and economic industrial processes, since they are highly specific, versatile and effective catalysts. Biotechnology research leads to the finding of news enzymes and uses for them, which increases their industrial importance [1]. More specifically, the enzyme world market annual growth is estimated at about 6.5-10 % [2], and the demand of more stable and highly active and specific enzymes grows quickly.

Proteases, the enzymatic group which pepsin belongs to, are mainly used in food industry, although they are also employed in textile and cleaning. In such applications, they increase protein quality or allow recovering proteins from wastes [1, 3]. More specifically, pepsin is an aspartic protease, a family of gastric enzymes which catalyze the hydrolysis of peptides chains in the first step of their digestion. There are several kinds of pepsin, namely pepsin A, B and C, gastricin and chymosin [4]. All these enzymes are synthesized as a zymogen, an inactive form of the enzyme which is stable until the active form is required. In the particular case of pepsin, it is called "pepsinogen". Through activation of pepsinogen in acid media active pepsin is obtained [5].

Despite a lot of microbial processes to obtain proteases are known, their origin is mainly animal. For example, in adult pig stomachs, the predominant gastric protease is pepsin A, and there are small amounts of pepsin B and gastricin [6].

The aim of this work is the study and evaluation of several extraction processes of pepsin from swine stomachs, more specifically from fundus, the stomach area with the highest amount of this enzyme, approximately 90 % over the whole amount present in the stomach. This study will be useful for future attempts to optimize an industrial extraction process of pepsin from swine stomachs.

2. Materials and methods

2.1. Activity assays

The proteolytic activity of pepsin was determined by an assay based on the hemoglobin digestion [7]. The substrate consisted of 5 cm^3 of bovine hemoglobin solution (Sigma) (2 % w/v) in HCl 60 mM and the temperature was fitted to 37 ºC The volume of pepsin solution added to the substrate was 1 cm^3. At that moment the hydrolysis of hemoglobin starts. The duration of the enzymatic reaction (t) was 10 minutes. After this time, 10 cm^3 of trichloroacetic acid (5 % w/v) were added in order to stop the reaction. Once coagulated protein had precipitated (50 minutes), the supernatant was filtered (Millipore 0.45 μm HV) and the absorbance of the resulting solution was measured at 280 nm (CARY 100 Bio UV-visible spectrofotometer)

The enzymatic activity was correlated with the absorbance at 280 nm. One activity unit is considered as the increment in 0.001 units of absorbance per minute, so the enzymatic activity can be defined as:

$$A = (A280\text{-}A0)/(0.001 \cdot t \cdot VE) \quad (AU/cm3\ pepsin\ solution\ assayed) \tag{1}$$

A_0 is the absorbance of a sample prepared in the same way described above but without adding pepsin to the substrate.

2.2. Extraction of pepsin from swine stomach

Several pepsin extraction processes were studied:

- Method 1: extraction by dissolution in water of the pepsinogen contained in fundus.

- Method 2: the same as method 1, plus a pepsinogen concentration step by coagulation [8].
- Method 3: the same as method 2, plus lyophilisation of the concentrated pepsinogen.

Swine stomachs were used for the pepsinogen extraction. The stomachs were rinsed with water and then fundus, easily recognizable by its reddish colour, was separated. Fundus was frozen and subsequently grinded until a 3 mm particle size, approximately. Then it was mixed with 5 parts of water and 3 of ice and stirred for 1 hour. pH was kept at around 7-7.5 and temperature about 1.5 ºC. Finally, the mixture was centrifuged 5 min at 2800 g (Hettich Universal 320 R centrifuge). The solid phase was discarded and the pepsinogen solution (supernatant) was obtained (Solution 1).

In Method 2, coagulation of pepsinogen was achieved by lowering pH of the supernatant of Method 1 to 4 with a HCl solution (3 N). Under these conditions, pepsinogen was coagulated and was separated by centrifugation at 4000 g, being the supernatant discarded. Afterwards, pepsinogen was diluted with a dilution ratio of 1:10 (substrate: water), giving rise to another pepsinogen solution (Solution 2).

In Method 3 the concentrated pepsinogen obtained in Method 2 was lyophilized (Labconco Lyph-Lock6 lyophilizer) at 18ºC and 5 µmHg for 24 h. The final product (a brownish dust) was diluted 1:10 in water (Solution 3).

For each method, pepsinogen solution activation was required in order to obtain an active pepsin solution. This was achieved by lowering the pH to 2 with HCl 3 N. After 10 minutes pH was raised to 2.75 with $NaHCO_3$ 2 N. Finally the solution was decanted for 6 hours and Solutions 1, 2 and 3 were obtained.

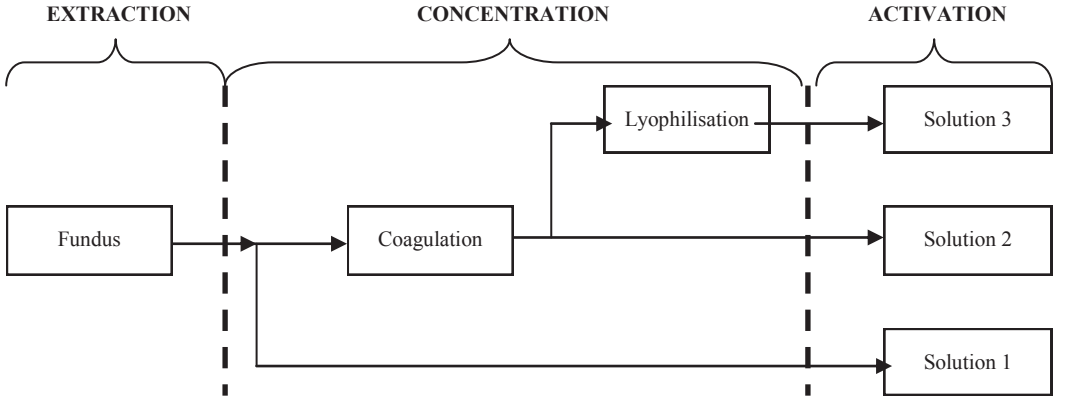

Fig. 1. Studied pepsin extraction processes

3. Results and discussion

The proteolytic activity of the Solutions 1, 2 and 3 against the inverse of the dilution factor are shown in Figure 2. All the solutions exhibit high proteolytic activity, and therefore, the assayed methods seem to be useful for the extraction of pepsin from swine stomachs. It is also necessary to point out that the proteolytic activity is due to the proteases present in the solution, although the predominant protease in fundus is pepsin [6].

In Figure 2, the slopes correspond to the enzymatic activity of Solutions 1, 2 and 3 (AU/cm3 solution) obtained in each method. As it can be seen the methods which include a concentration step (Methods 2 and 3) have a higher activity. These slope values are detailed in Table 1.

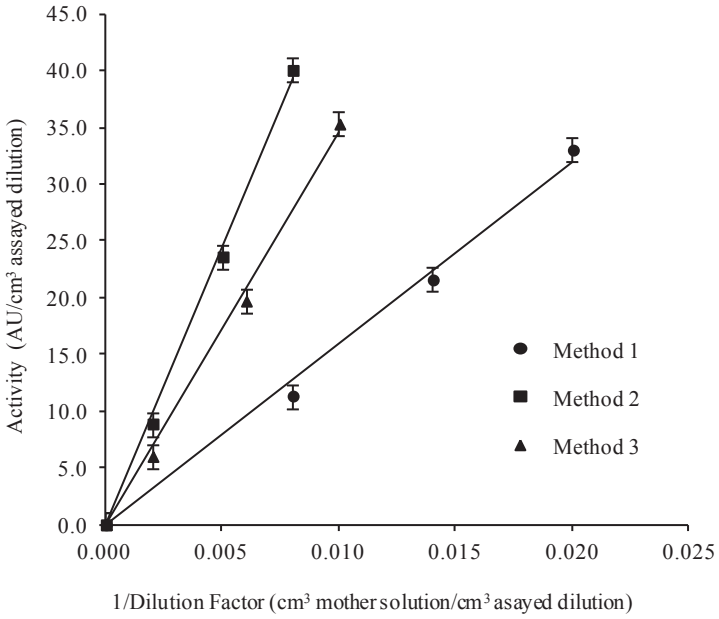

Fig. 2. Proteolitic activity of the obtained solutions from swine stomach

Table 1. Pepsin extraction processes results

	Slope (AU/cm3 solution)	R2	Enz. activity (AU/g fundus)	Extraction yield (%)	mg enzyme/g fundus (estimation)
Method 1	1597	0.994	12774	100.0	31.59
Method 2	4908	0.997	7681	60.1	18.99
Method 3	3458	0.998	5945	46.5	14.70

Another important factor is the enzymatic activity obtained per fundus mass unit, because it represents the extraction yield of the method. The values for this parameter are presented in Table 1. Method 1 shows the highest yield (it is considered as reference). In Methods 2 and 3, more concentrated pepsin solutions are obtained, although their yield is lower due to the additional steps in which pepsin has been involved. This way, the pepsinogen which does not coagulate in the concentration step causes a lower yield in Method 2. Also the lyophilisation process could affect to the activity because the dried pepsinogen is redissolved worse than the coagulated pepsinogen from Method 2. This additional step

could be responsible of the further decrease in yield of Method 3. However, these yields may be improved in the future to increase the pepsin obtained.

Methods 2 and 3 can be used if a concentrated pepsin solution is desired or an easier transport may be needed (the total volume is reduced to 98 and 99.5 % respectively). Furthermore, lyophilisation allows removing almost all the product water content, which leads to a better conservation of the final product.

Finally, Table 1 shows an estimation of pepsin production. The amount of pepsin obtained per mass of fundus is calculated by considering that the specific activity of the obtained pepsin is the same as the commercial one. Similarly to the results of extraction yield, higher amounts of pepsin are achieved with Method 1.

4. Conclusions

The three methods assayed allow the extraction of pepsin from swine stomachs. Method 1 has the highest extraction yield, whereas Methods 2 and 3 lead to lower amounts of pepsin, but with a higher purity of the final product. In addition, both methods can be used if a concentrated pepsin solution is desired or an easier transport may be needed. Method 3 would lead to a better conservation of the final product.

Acknowledgements

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