

## PEPSIN FROM HOG STOMACH

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<b>SYNONYMS</b>	Pepsin
<b>SOURCES</b>	Commercial preparations of Pepsin contain proteolytic enzymes obtained from the glandular layer of hog stomach.
Active principles	Pepsin (acid proteinase), composed of Pepsin A (major component), Pepsin B and Pepsin C
Systematic names and numbers	None (EC 3.4.23.1) None (EC 3.4.23.2) None (EC 3.4.23.3)
Reactions catalyzed	The enzyme preparations hydrolyze polypeptides, including those with linkages adjacent to aromatic or L-leucine residues yielding peptides of lower molecular weight. Pepsin B and Pepsin C which are minor components have more restricted specificity.
<b>DESCRIPTION</b>	White to light tan, water soluble powders, amber pastes or clear amber to brown aqueous liquids
<b>FUNCTIONAL USES</b>	Enzyme preparation Used in the preparation of fish meal and other protein hydrolysates, and the clotting of milk in cheese making in combination with one of the rennet.
<b>GENERAL SPECIFICATIONS</b>	Must conform to the <i>General Specifications for Enzyme Preparations used in Food Processing</i> (See Volume Introduction)
<b>CHARACTERISTICS</b>	
IDENTIFICATION	
<u>Pepsin activity</u>	The sample shows proteolytic activity See description under TESTS
<b>TESTS</b>	
IDENTIFICATION TESTS	
<u>Proteolytic activity</u>	<u>Principle:</u> Coagulated egg albumin is digested with a pepsin solution and the residue of the undissolved albumen is compared with that from a test with a reference standard. <u>Definition of Activity:</u> The activity of pepsin is expressed as the multiple of its own weight with that of coagulated egg albumen which it can digest. The Food Chemicals Codex defines, that one pepsin unit digests 3,000

times its weight of coagulated egg albumen under the condition of the assay.

Procedure:

Mix 35 ml of 1.0N hydrochloric acid with 385 ml of water. Dissolve 100 mg of pepsin, accurately weighed, in 150 ml of this solution. Prepare a similar solution of N.F. Pepsin Reference Standard, accurately weighed. Boil one or more hen eggs for 15 min to provide coagulated albumen for the assay. Cool them rapidly to room temperature by immersion in cold water; remove the shell and pellicle and all of the yolk and at once rub the albumen through a clean, dry No. 40 sieve rejecting the first portion that passes through the sieve. Place 10 g of the succeeding well mixed portion in each of 3 wide-mouth bottles of about 100 ml capacity. Immediately add 35 ml of the dilute acid at one time or in portions and, by suitable means, thoroughly disintegrate the particles of albumen.

Place the bottles in a water bath at 52°. After the contents of the bottles have reached that temperature, add 5.0 ml of the acidified solution of pepsin to one bottle, 4.30 ml of the same solution and 0.7 ml of the dilute acid to another bottle, and 5.0 ml of the Reference Standard solution to the third bottle. At once stopper the bottles securely, invert them 3 times, and maintain them at 52° for 2 h and 30 min, agitating the contents equally every 10 min by inverting the bottles once.

Remove the bottles from the bath, pour the contents into 100 ml conically shaped measuring vessels, having diameters not exceeding 1 cm at the bottom and complying in other respects with the water and sediment tube ASTM Standard Methods D 96-68, graduated from 0 to 0.5 ml in 0.05 ml graduations; from 0.5 to 2 ml in 0.1 ml graduations; from 2 to 3 in 0.2 ml graduations; from 3 to 5 ml in 0.5 ml graduations; from 5 to 10 ml in 1 ml graduations; from 10 to 25 ml in 5 ml graduations; and with graduation marks at 50, 75, and 100 ml points.

Transfer the undigested egg albumen which adheres to the sides of the bottles to the respective measuring vessels with the aid of small portions of water until 50 ml has been used for each. Mix the contents of each measuring vessel, and allow them to stand for 30 min. The volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the solution of pepsin being assayed does not exceed the volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the Reference Standard solution, and the volume of the undissolved albumen in the measuring vessel corresponding to 4.30 ml of the solution of pepsin being assayed is not less than the volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the Reference Standard solution.

Note:

Other measuring vessels than the type described in this monograph may be used if they are of such design and graduation as to measure the residue accurately.