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MUCINASE IN YAM.

By

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薯蕷中のムチナーゼに就て

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Yam yields a mucilaginous substance when grated and extracted with water. The chemical nature of this slime was, for the first time, studied by J. Ishii¹⁾, and later by Prof. K. Oshima and the author²⁾ who proved conclusively that the yam slime belongs to a class of mucins.

As the occurrence of mucinase or mucin coagulating enzym is not yet known in the vegetable kingdom while its presence in the animal kingdom has been reported by Roger, Tremolliens and others³⁾, it seemed of great interest to me to investigate whether or not the mucinase is present in the yam which contains mucin.

As material for my study, the tubers of *Dioscorea Batatas* Doene were taken and grated as thoroughly as possible, mixed with five volumes of water and then allowed to stand for several hours until starch granuls and other substances settled at the bottom of the vessel. The thick liquid thus obtained was used for the following experiment.

1. Elucidation of enzymic nature.

At the beginning of the experiment, three series of bottles were prepared, each containing a definite volume of the thick liquid of mucin. To the two series were added a few drops of dilute solution of calcium chloride and toluol, while to the other only toluol was added. The one series of the former and the later were kept in a thermostat at 30° C for several hours. The other series of the former were kept in an ice box for the same hours.

Among the series of bottles kept in a thermostat, the following phenomena

1) Bull. Coll. Agric., Imp. Univ. Tokyo, 2, (97-100.)

2) Jour. Coll. Agric., Tohoku, Imp. Univ., Sapporo, Japan. IV, 6, 1911 (243-249) and V, 11, 1913 (58-72).

3) Oppenheimer, C.—Die Fermente und ihre Wirkungen, spezieller Teil. 3. Aufl. 1909 (336-)

were observed.

In the series of bottles containing calcium chloride solution, the formation of abundant flocculent precipitates which gave a protein reaction with Millon's reagent was observed. In the other series the formation of such precipitates could not be found, but the liquids became slightly turbid. Also the bottles kept in an ice box formed no such precipitates. These phenomena prove the presence of a mucin-coagulating enzym in yam. After repeating the same experiment with cow milk, this action was distinguished from that of chymase (the milk coagulating enzym.)

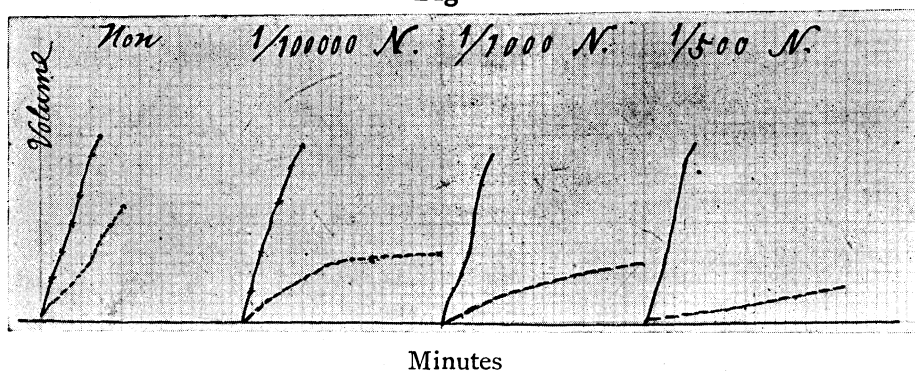
The following experiment was then undertaken to determine the effective amount of calcium chloride for acceleration of enzymic actions. For the determination of the degree of coagulation of mucin the following method was adopted in which the filtering velocity of an enzym-containing liquid is compared with the control test. The filtering velocity was determined by measuring the volume of the filtrate in a certain number of minutes through the area of a circle (dia. 17,5 mm) of a hard filter (R. F. P. 575, Carl Schleicher & Schüll.) under—50 mm Hg pressure. The results were as follows:

Table 1.

Concentration of CaCl- solution	Minutes								Note.
	1	2	3	4	5	6	10	20	
	Volumes (ccm)								
o	4,0	7,0	10,0	12,5	16,5	18,5	—	—	Samples were kept in an ice-box and then taken for the control.
1/100000 N.	3,5	7,0	10,0	12,0	16,0	18,0	—	—	
1/1000 N.	3,0	5,0	10,0	12,0	15,0	17,0	—	—	
1/500 N.	2,5	6,0	10,5	15,0	19,0	—	—	—	
o	2,0	3,0	4,0	5,5	6,5	7,5			Samples were kept in a thermostat at 30° C for 18 hours.
1/100000 N.	2,0	2,5	3,0	3,5	4,0	4,5	—	—	
1/1000 N.	0,5	—	1,5	—	2,5	—	4,5	6,5	
1/500 N.	—	—	—	—	—	—	1,5	3,5	

For comparison the experimental data are shown graphically on a rectangular coordinate system taking the abscissa for the time and the ordinate for the volume. In the following figure, the solid line is taken for the sample kept in an ice-box, and the dotted line for that kept in a thermostat.

Fig. 1.



From the foregoing table and figure, I perceived that 1/10000 normal concentration of calcium chloride has a distinctly accelerative power on mucinase, and this power was increased perceptibly in 1/1000 normal concentration. Also I could distinguished this power from the coagulating action of calcium chloride.

2. Relation of enzymic to acidic coagulation.

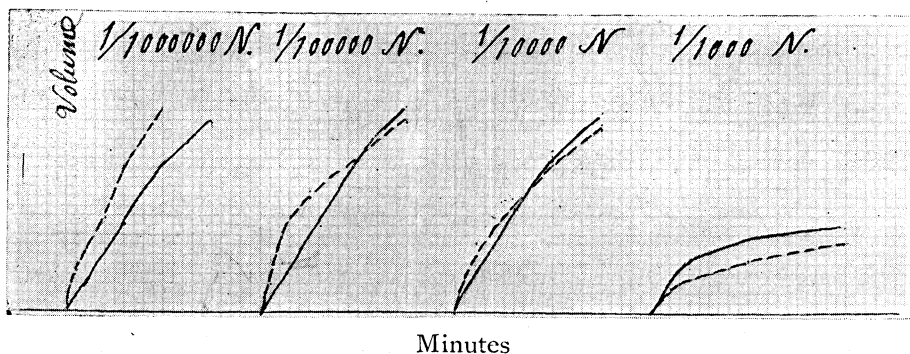
It has already been shown that a mucin solution coagulates in the presence of a small amount of acetic acid. In my previous study (1. c.), I coagulated the sample of mucin from tubers of yam through the addition of acetic acid. Therefore, it seemed to me of much interest to distinguish the coagulation by acetic acid from that by enzymic action and to know the relation of both phenomena. The experiments were undertaken in the same way as previously explained and gave the following results:

Table 2.

Concentration of acetic acid	Minutes							Note.
	1	2	4	6	10	15	20	
	Volumes ccm							
1/1000000 N.	1,5	3,0	6,5	9,0	15,5	19,0	26,5	Samples were kept in an ice-box and then taken for the control test.
1/100000 N.	1,5	3,5	6,5	9,5	16,0	22,5	29,5	
1/10000 N.	2,5	5,0	7,0	10,0	16,0	21,5	30,0	
1/1000 N.	1,5	—	5,0	—	7,5	—	9,5	
1/1000000 N.	5,0	—	11,0	14,0	18,0	23,5	—	Samples were kept in a thermostat at 30° C for 18 hours.
1/100000 N.	3,0	—	10,0	12,5	15,0	20,0	—	
1/10000 N.	4,0	—	9,5	10,0	14,0	19,0	—	
1/1000 N.	1,5	—	3,5	4,5	—	5,5	—	

The following figure was traced in the same manner as figure 1.

Fig. 2.



From the results, I can say that there is a clear distinction between the coagulations by acid and by enzyme, because this power does not increase by rising temperature as the enzymic action. At the same time, I perceived of that acetic acid has not any accelerating power on enzymic action.

CONCLUSIONS.

- 1) A mucin coagulating enzyme or mucinase, is found in the tubers of yam and it was distinguished from chymase. (The milk coagulating enzyme.)
- 2) The action of mucinase is accelerated by the presence of calcium chloride, the influence of which is perceived in $1/100000$ normal concentration, a distinct increase is observable in $1/1000$ normal concentration. This phenomena was distinguished from coagulating power of calcium chloride.
- 3) There is a clear distinction between the coagulations by acid and by enzyme, and acetic acid has no accelerating power on enzymic action.

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## 摘 要

曩に石井學士は薯蕷粘質物のムチンに類することを述べたり、次て大島博士並に著者は東北帝國大學農科大學紀要第四卷第六號に薯蕷中の粘質物は動物界に廣く分布するグリコプロテードの一種ムチンと全く同様の組成を有するものなることを報告せり。

其後著者は又同第五卷第十一號に於て、薯蕷汁液は下の如き諸種の酵素作用を有することを報ぜり。即ち澱粉の糖化、蛋白質の分解、アミノ酸の分解、糖原質の分解、接觸並に酸化等の諸作用を營む酵素是なり。

而してムチン凝固酵素即ちムチナーゼは既に二三の學者により動物界に存在することを報告せられしと雖も、植物界にありては未だ其存在を證せしものあるを聞かず、之著者の研究を企てし所以なり。

常用の方法に由り薯蕷よりムチン液を採集し之を三種に區別せり。第一第二は原液に微量の鹽化石灰液とトルオルを加へ第三は原液にトルオルのみを加へ第一及第三を五度の定溫器に數時間置き、第二を氷室に同一時間放置して後之を検査せり、其結果第一は多量の雲狀沈澱を生成せり、之をミロン氏試藥を用ゐて蛋白質反應を検するに積極的なり、然るに第二第三は僅に混濁せしに過ぎず、是ムチナーゼの存在を示すものにして酵素は鹽化石灰の存在に於て其力を顯はすことを知れり之と同時同酵素力は牛乳凝固酵素と全く相違することを認めたり。

仍て更に鹽化石灰の適量を試験せしに其結果十万分の一規定液に於て既に其好影響を認め得べく千分の一規定液に於て顯著なことを認む。次に酸による凝固と之を比較するに、酵素によるものは全く之と異なり、且つ酸の存在にありて酵素作用は見るべき影響を受けざることを認めたり。

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