Studies on Barley Malt

Some Investigations upon Zymogen Amylase

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The ph-curve of amylase activity revealed by zymogen amylase which is awaken by papain, is in accorrd with that of free amylase at the optimum point. But the optimum pH range of zymogen amylase is narrow both on scid and alkaline sides. Thermostabillity of zymogen amylase is higher than free amylase, since the former maintained almost its sctivity even when it was treated for 20 min. at 70°C.

We found interesting results in several experiments in which germinated green malt was kept in a closed vessel, containing small amount of toluene as disinfectant, and then left the green malt to autolyze. When this treatment was carried out at the begining of germination, zymogen amylase was decreased, while free amylase was increased, whereas if it was treated at the later stage of germination, free amylase was decreased and zymogen amylase was remarkable increased. A sistosubstance for amylase was frequently found in the autolysed green malt.

Even with non-germinated barley which was lost germinating power by soaking in water at 40°C with toluene, acceleration of saccharifying power and formation of dextrinogen-amylase were observed both in free and zymogen forms, when it was left to autolyse.

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Separation of Phosphatases of Takadiastase by Paper Chromatography

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We studied phosphatase action on thiaminetriphosphate by the partition paperchromatography, using filterpaper Toyo Roshi No. 50, (40×40 cm).

The papers were irrigated with 80 % EtOH for ascending chromatography in an all-glass aparatus, usually for 17 hrs. The spots were revealed by Dragendorff's reagent. Rf values were as follows: thiamine 0.50, thiaminemonophosphate (TMP) 0.33, cocarboxylase (TDP) 0.13 and thiaminetriphosphate (TTP) 0.06.

Two lines, apart 5 cm respetively from two adjacent edges of the paper, were drawn and the intersecting point was regarded as the origin. At first a dialysed 50 % Takadiastase solution was spotted at the origin, and developed with 2 % NaCl solution in the refrigerator

for 6~7 hrs. Then 75 mg. of TTP, 0.75 ml. of AcOH buffer (pH 5.5) and 0.75 ml. of glycerol were applied on the developed line of enzymes. After keeping it in the saturated water vapour at 37°C for 1 hr., they were developed with 80 % EtOH at right angles with the developed line of enzymes. The spots of thiamine appeared in two places, where Rf values were 0 and 0.3~0.4, and that of TMP was 0~0.7. The spots of TTP and TDP were observed on the whole applying line. These results suggest that TTP can be hydrolysed to TDP spontaneously. Organic pyrophosphatase which hydrolyses TDP to TMP develops in the value of Rf 0~0.7, and phosphomonoesterase which hyrolyses TMP to thiamine has the values of Rf 0 and 0.3~0.4. The purified pyrophosphatase (Utzino and Suzue, This Bulletin. **32**, 103 (1954) hydrolysed TTP to TMP (Rf=0~0.7) but not to thiamine. The purified urine phosphomonoesterase (Utzino and Suzue, *ibid*, **31**, 385 (1953)) did not hydrolyse TTP to TMP and thiamine.

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