菊粉酶活测定

一、　实验目的

测定菊粉酶的酶活。

二、　实验方法

① 菊粉酶酶活的测定方法：

1．试剂准备

1.1．DNS 溶液（3，5-二硝基水杨酸试剂）的配制：

将 6.3 克 DNS 加水于 45 度水浴溶解；

缓慢加入 262 mL 2M NaOH 溶液，边加边搅拌，直至溶液澄清透明；

称取 182 克酒石酸钾钠，溶于 50 mL 水中；

将 DNS 和 NaOH 的混合溶液缓慢加入酒石酸钾钠中，边加边搅拌；

逐步加入 5 g 苯酚和 5 g 无水亚硫酸钠直至溶解

冷却至室温，定容至 1000 mL，于棕色瓶中贮存 7-10 天备用。半年内有效。

1.2．标准果糖溶液（1mg/mL）的配制：

精密称取 25.00mg 果糖于适量蒸馏水中溶解，定容至 25mL 容量瓶中

1.3．5%菊粉溶液的配制：

1.3.1．pH4.6 HAc-NaAc 缓冲液：

配置方法：

1）2MNaAc 母液：称取 272.16g 乙酸钠溶于适量蒸馏水中，定容至 1000mL

2）2M HAc 母液：称取 120.10g 乙酸，溶于适量蒸馏水中，定容至 1000mL

3）取 24.5mL2MNaAc 溶液和 25.5mL2MHAc 溶液混合，定容至 1000mL，即得 pH 4.6 的 0.1MHAc-NaAc 缓冲液

1.3.2．精密称取 5.00g 菊粉于适量 pH4.6 HAc-NaAc 缓冲液，定容至 100mL 容量瓶中。

2．果糖标准曲线的制作（DNS 法）：

取 6 支干净的试管，按下表加入各试剂，反应后以 0 号管为对照，调零，测量各管的 OD_{520} nm：

以果糖质量（mg）为横坐标（X），以 OD520nm 为纵坐标（Y），获得果糖标准

曲线。Y=aX+b

获得标准曲线及回归方程。

编号	1	2	3	4	5	6
果糖溶液 (mL)	0	0.4	0.8	1.2	1.6	2
醋酸缓冲液 (mL)	2	1.6	1.2	0.8	0.4	0
DNS 溶液 (mL)	2	2	2	2	2	2
沸水浴 5 分钟（精略计时），冰水冷却，定容至 25 mL						
OD520						

3．菊粉酶活力的测定：

3.1．样品准备：

如果是酶粉（粉剂），称取 1 克酶粉剂溶于 100 mL 缓冲液中，充分混匀（注：稀释了 100 倍）。

3.2．反应

取 100 uL 稀释液加入 900 ul 5%菊粉溶液，混匀，50 ℃ 水浴反应 5 min（精确计时）；

立即取出沸水浴 5 min（精确计时）灭活；

从反应液中取出 50 ul，1.95 ml 缓冲液，加入 2.0 ml DNS 溶液；

沸水浴中 5min（精确计时），冷水冷却

用容量瓶定容至 25ml；

测 OD_{520} nm 值

对应果糖标准曲线计算得样品反应液中的含糖量 A(mg)。

3.3．设置对照：

菊粉酶稀释液，沸水 5 min 灭活，

取 100 uL 加入 900 ul 5%菊粉溶液，混匀，25ºC 水浴反应 5 min（精确计时）

立即取出沸水浴 5 min（精确计时）灭活，冷水冷却；

从反应液中取出 50 ul，加入 1.95ml 缓冲液，1.5ml DNS 溶液；

沸水浴中 5 min（精确计时），冷水冷却，用容量瓶定容至 25ml, 作为对照，调零。

4．酶活计算：

酶活单位定义(U)：每分钟产生 1 微摩尔还原糖所需要的酶量。

每毫升发酵上清液中菊粉酶活为：

$$U=\frac{A\times 1000}{180\times 5}\times N$$

U：酶活单位；

A: 反应液中产生果糖的浓度（mg/mL），即回归方程 Y=aX+b 中的 X 值；

180: 果糖的分子量；

5：水解反应时间（本试验为 5 分钟）

Ｎ：总的稀释倍数。

INULINASE ACTIVITY QUANTIFICATION

Aim:

To check the inulinase activity of the powder or liquid inulinase formulations.

Methods and Meterials:

The DNS method was used to determine the enzyme activity in the product, and fructose was used as the substrate.

1．Preparation of the reagents

1.1．Liquid DNS (3,5-Dinitrosalicylic acid) agents preparation:

Solution A-- Dissolved 6.3 g DNS in the water at 45 ^{o}C. Add 262 mL 2M NaOH solution, and stir till the solution becoming clear.

Solution B—Dissolved 182 g potassium sodium tartrate in 50 mL water.

Mix Solution A and Solution B together, and then add 5 g crystalline phenol and 5 g $NaHSO_3$ (no water) till them dissolved totally (yellow color).

The DNS work solution was kept at room temperature for about 7 days to make it stable and then could be used for the following inulinase activity determination. The work solution will be stable for half a year.

1.2．Standard fructose solution (1 mg/mL) preparation.

Dissolved 0.50 g fructose into the distilled water to make the volume to be 500 mL in volumetric flask. The final concentration was 1 mg/mL.

1.3．Preparation 5% inulin solution.

1.3.1．HAc-NaAc (pH4.6, 0.1 M) buffer.

1）2 M NaAc stock solution: Dissolved 272.16 g NaAc into distilled water and make the final volume was 1000 mL.

2）2 M HAc stock solution: Dissolved 120.10 g into the distilled water and make the final volume was 1000 mL.

3）Aliquot 24.5 mL 2M NaAc stock solution and 25.5 mL 2M HAc stock solution, and then mix together. Add distilled water to make the final volume as 1000 mL. The 0.1 M HAc-NaAc (pH 4.6) buffer was prepaed.

1.3.2. Add 5.00 g inulin into 0.1 M HAc-NaAc (pH4.6) buffer, and add the distilled water to make the final volume as 100 mL.

2．Making the standard curve of fructose by DNS method.

Take 6 tubes (with 25 mL volume), and preparing the glucose solution as the below list. And then make these tube went to the DNS reaction, and then the OD_{520} of every tube was recoded. The standard curve of glucose was depicted by using glucose amount as the X-axis, and the OD_{520} value as the Y-axis.

According to the OD520 value, the regression equation Y=aX+b was finally got.

Tube number	1	2	3	4	5	6
Fructose solution (mL)	0	0.4	0.8	1.2	1.6	2.0
HAc-NaAc buffer (mL)	2	1.6	1.2	0.8	0.4	0
DNS solution (mL)	2	2	2	2	2	2
Put into the boiling water for exact 5 min, cooled with water, then add water into the tube to make the final volume 25 mL						
OD520						

3．Determination of the inulinase activity.

3.1．Sample preparations.

For the powder inulinase formulation, we recommended you dissolved 1 g powder into 100 mL into the 0.1 M HAc-NaAc buffer buffer, stir to make the powder solved.

For the liquid formulation, you can take 1 mL liquid into 0.1 M HAc-NaAc buffer.

3.2. The steps for inulinase activity checking was following:

Take 100 uL diluted inulinase solution and added into 900 uL 5% inulin solution, mixed and incubated in 50 ºC water bath for exact 5 min.

Take out the reaction mixture and immediately put into the boiling water for 5 min to inactive the enzyme.

Aliquot 50 uL reaction mixture, and added into 2.0 mL DNS+1.95 mL 0.1 M HAc-NaAc, mixed, and then put into the boiling water for exact 5 min to make the DNS reacted with the reductive sugar. We can see that the color of the liquid becoming dark brown.

Cool the tube with water, and then add the distilled water into the tube to make the final volumn was 25 mL.

Then the OD value was determined at OD_{520} nM.

Then refereed the standard fructose curve and the regressive equation, the amount of the reducing sugar was calculated (A= mg/L).

3.3. The control experiment.

The inulinase supernatant was firstly put into the boiling water for 5 min to inactive the inulinase.

Described as above, 100 uL inactive enzyme solution was put into 900 uL 5% inulin solution, mixed and then put into 50 ºC water bath to react for exact 5 min.

Take out the tube and then put into the boiling water for exact 5 min to inactive the reaction again.

Aliquot out 50 uL reaction mixture, add 2.0 mL DNS+1.95 mL 0.1 M HAc-NaAc, mix together, and then put into the boiling water for 5 min to make the reducing sugar to react with DNS.

Cool the tube by the water to terminate the reaction, and then add distilled water into the tube to make the final volume as 25 mL.

As a control, the solution was taken out as the blank for OD_{520} determination.

Enzyme Unit defined:

One unit was defined as the amount of enzyme need to released one micro mole reduced sugar from the inulin in one minute. The activity of our products was described as the enzyme unite per gram powder or milliliter liquid.

The equation was listed as:

$$U = \frac{A \times 1000}{180 \times 5} \times N$$

U：inulinase activity；

A: The concentration of the fructose in the reaction mixture （mg/mL），This value comes from the regression equation Y=aX+b, A= X；

180: Molecular weight of the fructose；

5：Reaction time（5 min in this test）

N：Total dilution fold.