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제품의 규격과 성능, 인증 및 적용 조건을 확인하기 위한 자료입니다.

ABOUT SNAPSHOOT

제품 공급을 넘어, 현장에 맞는 해결책으로

스냅슛은 산업현장의 작업 환경과 요구 조건을 바탕으로
적합한 제품과 시스템을 제안합니다. 현장 실사를 통해 설치 조건을 확인하고,
공급부터 시공·설치 및 사용 교육까지 필요한 과정을 함께합니다.

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AMYLOSE/ AMYLOPECTIN

PRODUCT INSTRUCTIONS

SKU: 700004262
K-AMYL

05/26

FOR THE MEASUREMENT OF THE AMYLOSE AND AMYLOPECTIN CONTENTS OF STARCH

(100 Manual Assays per Kit)



Megazyme by NEOGEN

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INTRODUCTION:

Many of the properties of cereal starches that determine their suitability for particular end-uses are dependent upon their amylose/ amylopectin ratios. These properties include gelatinisation and gelation characteristics, solubility, the formation of resistant starch, and, for rice, the cooking and textural characteristics of whole grains.[1-5] Thus, the measurement of the amylose content of starches is an important quality parameter for starch processing.

Amylose is most commonly determined in cereal starches by the potentiometric, amperometric or colourimetric measurement of the iodine binding capacity of the amylose with the resultant formation of amylose-iodine inclusion complexes.[6-10] However, these methods are subject to uncertainties. Amylopectin-iodine complexes also form, and these reduce the concentration of free iodine measured by the non-colourimetric methods and may absorb at similar wavelengths to amylose-iodine complexes in colourimetric methods.

These complexes lead to an over-estimation of the amylose, requiring corrections to be applied. Many of the other problems experienced in the use of these methods are detailed by Gibson *et al*.[11]

The specific formation of amylopectin complexes with the lectin concanavalin A (Con A) offers an alternative approach to amylose measurement in starches that is not subject to these uncertainties.[12-13] Under defined conditions of pH, temperature and ionic strength, Con A specifically complexes branched polysaccharides based on α-D-glucopyranosyl or α-D-mannopyranosyl units at multiple non-reducing end-groups with the formation of a precipitate. Thus, Con A effectively complexes the amylopectin component of starch but not the primarily linear amylose component.

The procedure described in this booklet[13] is a modification of a Con A method developed by Yun and Matheson (1990).[13] It uses an ethanol pre-treatment step to remove lipids prior to analysis [modified from Morrison and Laignelet (1983)[7]].

PRINCIPLE:

Starch samples are completely dispersed by heating in dimethyl sulfoxide (DMSO). Lipids are removed by precipitating the starch in ethanol and recovering the precipitated starch. After dissolution of the precipitated sample in an acetate/salt solution, amylopectin is specifically precipitated by the addition of Con A and removed by centrifugation. The amylose, in an aliquot of the supernatant, is enzymatically hydrolysed to D-glucose, which is analysed using glucose oxidase/peroxidase reagent. The total starch, in a separate aliquot of the acetate/salt solution, is similarly hydrolysed to D-glucose and measured colourimetrically by glucose oxidase / peroxidase (GOPOD). The concentration of amylose in the starch sample is estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample to that of the total starch sample.

This procedure is applicable to all pure starch samples and to cereal flours.

ACCURACY:

Repeated analyses of a set of samples yielded repeatability (within the laboratory) with relative standard deviations of < 5% for pure starches and ~ 10% for cereal flours.

KITS:

Kits suitable for performing 100 assays are available from Neogen. The kits contains:

Bottle 1: Freeze dried Con A.
Store below -10°C. See individual label for expiry date.

Bottle 2: Amyloglucosidase (2 mL) [200 U on *p*-nitrophenyl β-maltoside (i.e. 3,300 U on starch at pH 4.5 at 40°C)] plus fungal α-amylase (500 U on Ceralpha Reagent at pH 5.0 and 40°C).
Store at 4°C. See individual label for expiry date.

Bottle 3: **GOPOD Reagent Buffer.** Buffer (50 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.09% w/v).
Store at 4°C. See individual label for expiry date.

Bottle 4: **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze- dried powder.
Store below -10°C. See individual label for expiry date.

Bottle 5: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Store sealed at room temperature. See individual label for expiry date.

Bottle 6: Starch reference sample (with specified content of amylose).
Store sealed at room temperature. See individual label for expiry date.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Dissolve the contents of **bottle 1** in 50 mL of **Con A Solvent (working concentration)** (see page 4 for preparation). Divide into aliquots of appropriate size and store in polypropylene tubes below -10°C between use and keep cool during use if possible. This is **Con A solution (solution 1)**. Stable for 2 years below -10°C.

2. Dissolve the contents of **bottle 2** in 20 mL of 100 mM sodium acetate buffer, pH 4.5 (see page 3 for preparation). Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and keep cool during use if possible. This is **amyloglucosidase/α-amylase enzyme mixture (solution 2).**
Stable for 2 years below -10°C.

3. Dilute the contents of the **GOPOD Reagent Buffer** bottle to 1 L with distilled water. This is **solution 3**. Use immediately.

NOTE:

1. On storage, salt crystals may form in the **GOPOD Reagent Buffer**. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This buffer contains 0.09% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

4. Dissolve the contents of the **GOPOD Reagent Enzyme** bottle in 20 mL of **solution 3** and quantitatively transfer this to the bottle containing the remainder of **solution 3**. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is **Glucose Determination Reagent (GOPOD Reagent)**.
Stable for 1 month when stored at 4°C or 12 months below -10°C.

Note: If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots. Do not freeze/thaw more than once. When the reagent is freshly prepared it may be light yellow or light pink in colour. The solution may develop a stronger pink colour upon storage at 4°C. The absorbance of this solution should be less than 0.05 when read against distilled water.

5 & 6. Use the contents of **bottles 5** and **6** as supplied.

BUFFERS AND SOLVENTS (NOT SUPPLIED):

1. Sodium Acetate Buffer (100 mM, pH 4.5)
Add 5.9 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to pH 4.5 by the addition of 1 M (4 g/100 mL) sodium hydroxide solution. Adjust the volume to 1L with distilled water.

2. Concentrated Con A Solvent (600 mM, pH 6.4 sodium acetate buffer)
Dissolve 49.2 g of anhydrous sodium acetate (Sigma cat. no. 71183), 175.5 g of sodium chloride (Sigma cat. no. S7653), 0.5 g of $CaCl_2.2H_2O$ (Sigma cat. no. C5080), 0.7 g of $MgCl_2.6H_2O$ (Sigma cat. no. M2670) and 0.7 g of $MnCl_2.4H_2O$ (Sigma cat. no. M3634) in 900 mL of distilled water. Adjust the pH to 6.4 by dropwise addition of glacial acetic acid and then adjust the volume to 1 L with distilled water.
Stable for 2 weeks at 4°C.

NOTE: When preparing this buffer mixture, it is essential that the pH is adjusted very carefully. If the pH drops significantly below 6.4 a precipitate forms and this will not redissolve on pH adjustment. Consequently, this buffer must be discarded, and a fresh batch prepared.

3. **Con A Solvent (working concentration)**
   Dilute 30 mL of Concentrated Con A Solvent to 100 mL with distilled water. Use on the day of preparation.

4. **Dimethyl sulfoxide (DMSO)**
   Analytical reagent grade (CAS Number: 67-68-5).

5. **95% Ethanol (v/v)**
   Analytical reagent grade.

EQUIPMENT (RECOMMENDED):

1. Glassware:
   - volumetric flask (25 mL);
   - glass test tubes (16 x 120 mm, 15 mL); [Plastic test tubes of the same dimension are also suitable for use].
   - screw capped sample tubes (Kimax®) (10 mL).
2. Micro-pipettors, to dispense 50-1000 µL (e.g. Gilson® Pipetman®).
3. Positive displacement pipettor, e.g. Eppendorf® Multipette®.
4. Eppendorf® microfuge tubes (2.0 mL capacity).
5. Boiling water bath.
6. Bench centrifuge (capable of 2,000 *g*).
7. Vortex mixer
8. Spectrophotometer (set at 510 nm).
9. Stop clock.
10. Analytical balance.
11. Microfuge (capable of 14,000 *g*).
12. Thermostated water bath set at 40°C.

PRECAUTIONS:

Starch samples must be pre-treated with ethanol as described to remove lipids. If samples are not treated with ethanol, the amylose contents in some samples may be under-estimated by as much as 50%.

ASSAY PROCEDURE:

A. Starch Pre-treatment

1. Accurately weigh starch or flour sample (20-25 mg to the nearest 0.1 mg) into a 10 mL screw capped Kimax® sample tube. Record the sample weight to the nearest 0.1 mg.

> **NOTE:** Include a reference sample with each batch. Duplicates of all samples are recommended.

2. Add 1 mL of DMSO to the tube while gently stirring at low speed on a vortex mixer. Cap the tube and heat the tube contents in a boiling water bath until the sample is completely dispersed (approx. 1 min). Ensure that no gelatinous lumps of starch are remaining.
3. Vigorously mix the contents of the sealed tube at high speed on a vortex mixer, return the tube to the boiling water bath and heat it for a further 15 min, with intermittent high-speed stirring on a vortex mixer.
4. Store the tube at room temperature for approx. 5 min and add 2 mL of 95% (v/v) ethanol with continuous stirring on a vortex mixer. Add a further 4 mL of ethanol, cap the tube and invert to mix. A starch precipitate will form. Allow the tube to stand for 15 min (or overnight if desired).
5. Centrifuge the tubes at 2,000 *g* for 5 min, discard the supernatant and drain the tubes on tissue paper for 10 min. Ensure that all of the ethanol has drained. Use the pellet in the subsequent amylose and starch determinations.
6. Add 2 mL of DMSO (with gentle vortex mixing) to the starch pellet. Place the tube in a boiling water bath for 15 min and mix occasionally. **Ensure that there are no gelatinous lumps**.
7. While removing the tube from the boiling water bath, immediately add 4 mL of **Con A Solvent (working concentration)** (see page 4) while vigorously mixing the tube contents. Quantitatively transfer the tube contents (by repeated washing with the **Con A solvent**) to a 25 mL volumetric flask. Dilute to 25 mL volume with **Con A solvent (working concentration)**. **This is Solution A.** If necessary, filter this solution through Whatman® No. 1 filter paper (this step will be necessary for whole flour samples).

> **NOTE:** This solution should be analysed within 2 h.

B. Con A Precipitation of Amylopectin and Determination of Amylose

1. Transfer 1.0 mL of **Solution A** to a 2.0 mL Eppendorf® microfuge tube. Add 0.50 mL of **Con A solution** (page 2, **solution 1**), cap the tube and gently mix by repeated inversion. **Avoid frothing of the sample.**
2. Allow the tube to stand for 1 h at room temperature. Centrifuge at 14,000 *g* for 10 min in a microfuge at room temperature.

NOTE:

1. Samples in Con A Solvent (i.e. **Solution A** as described in Section A above) cannot be left for extended periods because the amylose will tend to retrograde and precipitate.
2. The time required for effective Con A precipitation of the amylopectin (Step **B1** above) is 1 h at room temperature. However, these solutions should not be left for longer than 2 h as the amylose will tend to retrograde.
3. In this procedure, pre-treatment of the samples with ethanol has the added advantage of removing any soluble sugars in the sample that would otherwise interfere with the assay.

3. Transfer 1 mL of the supernatant to a 15 mL centrifuge tube. Add 3 mL of 100 mM sodium acetate buffer, pH 4.5. This reduces the pH to ~ 5. Mix the contents, lightly stopper (with a marble) and heat in a boiling water bath for 5 min to denature the Con A.
4. Place the tube in a water bath at 40°C and allow it to equilibrate for 5 min. Add 0.1 mL of **amyloglucosidase/α-amylase enzyme mixture** (page 2; **solution 2**) and incubate at 40°C for 30 min. Centrifuge the tube at 2,000 *g* for 5 min.
5. To 1.0 mL aliquots of the supernatant add 4 mL of **GOPOD Reagent**. Incubate at 40°C for 20 min. Incubate the **Reagent Blank** and the **D-Glucose Controls** concurrently.

NOTE:

The **Reagent Blank** is prepared by adding 1.0 mL of 100 mM sodium acetate buffer (Buffer 1; page 4) to 4.0 mL of **GOPOD Reagent** and incubating at 40°C for 20 min.

D-Glucose Controls (duplicate) comprise 0.1 mL of D-glucose standard solution (1 mg/mL), 0.9 mL of 100 mM sodium acetate buffer and 4.0 mL of **GOPOD Reagent.** This value is not used in the calculation, however we suggest that it is performed to ensure that there are no problems with this part of the assay.

6. Read the absorbance of each sample and the D-glucose controls at 510 nm against the reagent blank.

C. Determination of Total Starch

1. Mix 0.5 mL of **Solution A** with 4 mL of 100 mM sodium acetate buffer, pH 4.5.
2. Add 0.1 mL of **amyloglucosidase/α-amylase enzyme mixture** (page 2; **solution 2**) and incubate the mixture at 40°C for 10 min.
3. Transfer 1.0 mL aliquots (in duplicate) of this solution to glass test tubes, add 4 mL of **GOPOD Reagent** and mix well. Incubate at 40°C for 20 min. This incubation should be performed concurrently with the samples and standards from Section B above.

CALCULATION OF AMYLOSE CONTENT (%):

Amylose, % (w/w)

$$= \frac{\text{Absorbance (Con A Supernatant)} \times 6.15 \times 100}{\text{Absorbance (Total Starch Aliquot)} \quad 9.2 \quad 1}$$

$$= \frac{\text{Absorbance (Con A Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times 66.8$$

Where **6.15** and **9.2** are dilution factors for the Con A and Total Starch extracts respectively.

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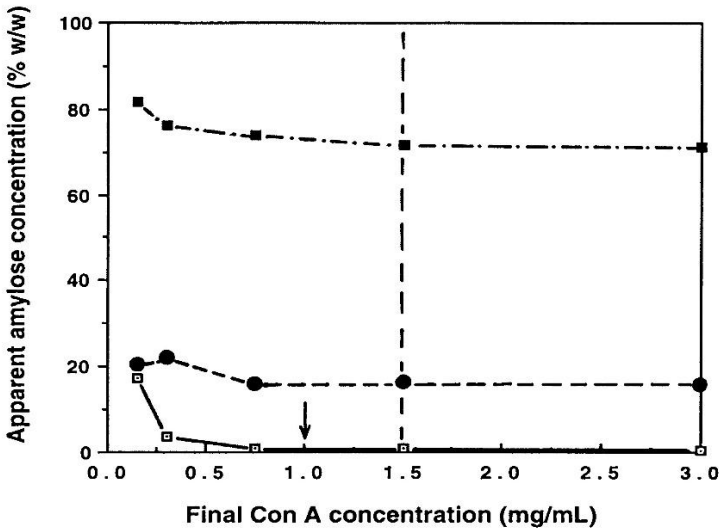


Figure 1: Effect of Con A concentration on the level of amylose determined in starch samples by the modified Con A procedure. ■ = high amylose maize starch (74.4% w/w amylose), ● = rice starch (16.9% w/w amylose), □ = maize starch (1.9% w/w amylose). The vertical dashed line represents the conditions used in the final assay format.

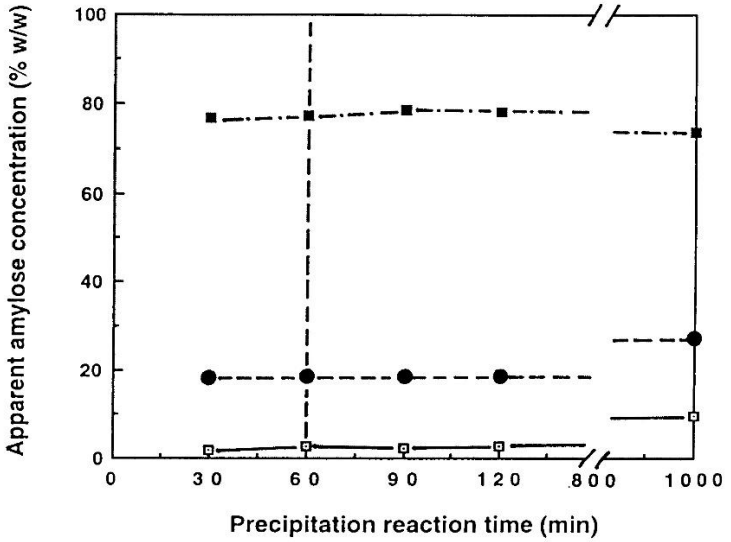


Figure 2: Effect of precipitation reaction time on the amylose determined in starch samples by the modified Con A procedure. ■ = high amylose maize starch (74.4% w/w amylose), ● = rice starch (16.9% w/w amylose), □ = maize starch (1.9% w/w amylose). The vertical dashed line represents the conditions used in the final assay format.

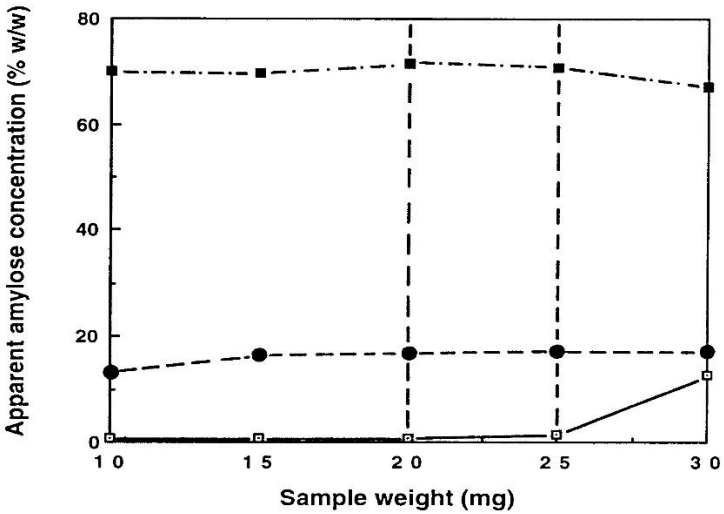


Figure 3: Effect of sample size on the amylose determined in starch samples by the modified Con A procedure. ■ = high amylose maize starch (74.4% w/w amylose), ● = rice starch (16.9% w/w amylose), □ = maize starch (1.9% w/w amylose). The vertical dashed lines represent the conditions used in the final assay format.

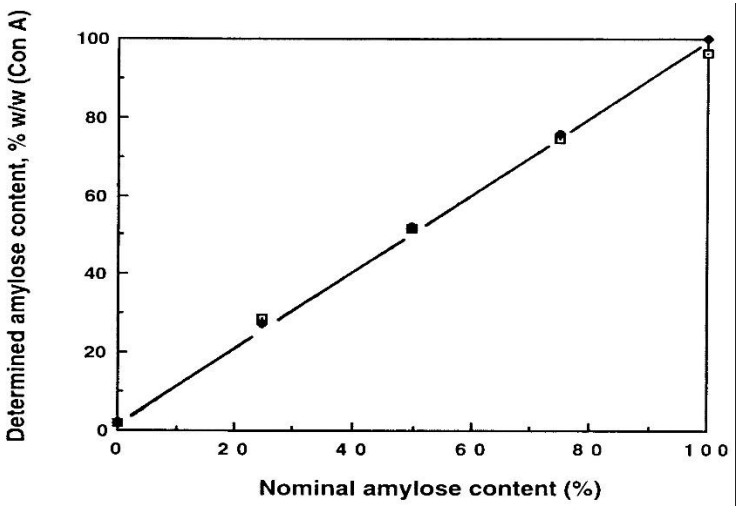


Figure 4: Mixed amylose/amylopectin standard curve for the modified Con A procedure. The regression equation is y = 0.956 x + 3.259 and the correlation coefficient 0.999. Nominal amylose contents are predicted values based on the proportion of ICN potato amylose (~ 100% w/w amylose) and waxy maize starch (~ 0% w/w amylose based on potentiometric iodine titration) in the mixed standard. Data points are means of duplicate determinations.

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