Research on method for determination of amylose content in rice

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Abstract

A simple and rapid method for determination of amylose content in rice was established in this research. The effects of defatted solvent, defatted time, chromophoric reaction acidity, chromophoric reagent concentration, dispersed condition (time, temperature, method, alkalinity and the analytical results were investigated. The optimal conditions were determined, including defatted the polished rice by refluxing with methanol (or petroleum ether) for 2 hr (brown rice for 4hr) in a Soxhlex extractor, dispersed sample at 85℃ for 10 minutes in a 100 ml beaker by 9.0 ml of 1 mol/l sodium hydroxide, chromophoric reaction acidity of 0.5 ml 1 mol/l acetic acid (or hydrochloric acid), 1.0 ml iodine solution as chromophoric reagent. Under the selected conditions, the linear regression equation was $Y = 0.0015 + 0.01299X$ ($r = 0.9999$), the relative standard deviation was 0.3% with the coefficient of variation less than 1.61% ($n = 10$) This research provided scientific basis for revising the quality standard of the state.

Introduction

Amylose content is a important index for appraising the food quality and the edible technological quality of rice. It provides important scientific basis for putting rice resources to rational use as well as selecting and breeding rice variety. The research was based on the ISO 6647 and in which comprehensive comparison tests were carried out among ISO 6647 and GB 7648, etc. The optimization of the operation condition of the determination of amylose content was specified. According to the result a recommended method for determination of amylose content was advanced and the scientific basis for revising the quality standard of state was provided.

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Materials and Methods

Materials

Round-grained non-glutinous rice (R. N. R.), long-grained non-glutinous rice (L. N. R.), glutinous rice (G. R.), published round-grained non-glutinous rice (P. R. N. R.), published long-grained non-glutinous rice (P. L. N. R.), published glutinous rice (P G. R.)

Methods

The recommended method was advanced by and comparing with the ISO6647 – 1987E, GB7648 – 1987, etc. optimizing operation condition.

Results and Discussion

Comparison and selection of the operation conditions

Selection of the defatting method, reagent and time

In this research different categories of samples were studied. Tables 1 and 2 were comparison test of defatting the test samples with different reagents by different methods for different times.

Table 1. Comparison of defatting method and reagents.

Method*	Reagent	P R.N. Rice		P.L.N. Rice		P.G. Rice
		S_1	S_2	S_1	S_2	S_1
A	methanol	0.380	0.385	0.320	0.325	0.100
B	Petroleum	0.360	0.370	0.310	0.320	0.100
C	petroleum	0.302	0.355	0.270	0.260	0.100
D		0.342	0.360	0.270	0.275	0.123

*A - dagatted before dispersed, B - defatted before dispersed, C - defatted after dispersed, D - undefatted

Some conclusions were obtained from Table 1 as follows:

(a) The absorbance was on the low side obviously because of the interference of fat when the test samples were determined directly.

(b) The result was on the low side obviously, because of volumetric change of the test solution owing to mutual dissolution when the test samples defatted with petroleum ether twice after dispersed were determined.

(c) The result by defatting with petroleum ether was a little less than with methanol. So methanol was taken for the defatting reagent in this research

Table 2 shows that the interference of fat was eliminated when the rice was defatted by refluxing with methanol for 2 hr in a Soxhlet extractor or brown rice with methanol for 4 hr, it was unnecessary for 16 hr.

Table 2. Comparison tests of different times and reagents.

Sample	2 hours		4 hours		24 hours	
	Methanol	Petroleum ether	Methanol	Petroleum ether	Methanol	Petroleum ether
P.R.N. Rice	0.323	0.310	0.336	0.320	0.355	0.325
P.L.N Rice	0.318	0.310	0.318	0.312	0.320	0.310
Brown Rice	0.230	0.250	0.280	0.270	0.270	0.260

Selection of dispersion method and condition

Two methods of dispersion were compared (see Table 3). One was that the test samples were dispersed directly in a 100 ml volumetric flask, another was that the test samples were dispersed in a beaker, then the suspension was transferred quantitatively to a 100 ml volumetric flask and diluted to volume mark with water and mixed fully.

The determined deviation of dispersing samples in the volumetric flask directly were bigger than dispersing in beaker.

The reason is that amount of the test sample is few and the test sample is apt to lose or adhere to wall when it was transferred into volumetric flask. So this method is adopted.

Table 3. Comparison of dispersion methods.

No.	Method	Absorbance				Average	Max. Deviation
1	A	0.334	0.344	0.332	0.330	0.335	0.014
	B	0.340	0.336	0.335	0.335	0.336	0.005
2	A	0.300	0.320	0.316	0.318	0.314	0.020
	B	0.310	0.305	0.312	0.315	0.311	0.010
3	A	0.352	0.350	0.370	0.350	0.357	0.020
	B	0.335	0.360	0.360	0.360	0.359	0.005

In this research the test samples were dispersed at 70, 85, or 100℃ for 10 min., as well as at ambient temperature for 24 hrs. The result is shown in Table 4.

In accordance with Table 4, the results were identical on the whole of samples dispersed at 85 and 100℃ for 10 to 15 min. and at ambient temperature for 24 hrs.

Table 4. Comparison of dispersion temperature and dispersion times.

No.	Absorbance			
	Ambient(24 hr)	70℃	85℃	100℃
4	0.230	0.230	0.230	0.240
5	0.290	0.290	0.280	0.290
6	0.270	0.275	0.270	0.270

Comparison was carried out that different volumes of the 1 mol/L sodium hydroxide solution used for dispersing the test samples, the result is shown in Table 5.

Table 5. Influence of volumes of alkali (1mol/L NaOH) for the test sample dispersing.

Sample	Absorbance		
	5.0 ml	9.0 ml	15.0 ml
7	0.354	0.358	0.360
8	0.285	0.290	0.290

Table 5 showed that the results were identical on the whole of accordant when 9.0 ml or 15.0 ml of 1.0 mol/L NaOH was added as dispersing reagent.

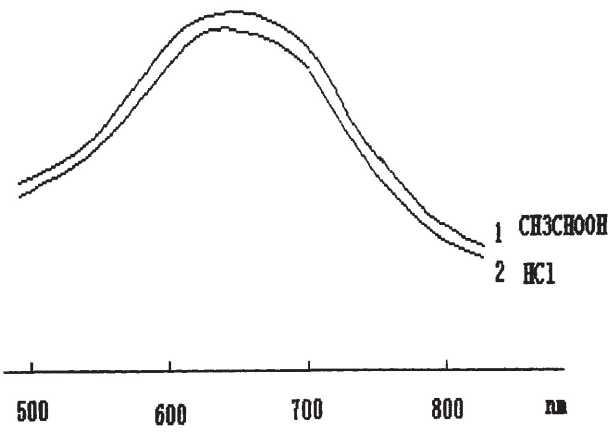

Fig. 1. Absorption spectrum under different acidity.

Selection of the acid and the acidity in chromophoric reaction

1.0 ml of 1 mol/L acetic acid or 1.0 ml of 1 mol/L hydrochloric acid is added to potato amylose standard solution, then carry out chromophoric reaction. The absorption spectrum was determined. Figure 1 showed that their absorption spectrum was accordant.

Comparison were carried out that different volumes of

acetic acid was added respectively to test samples solution, and hydrochloric acid to amylose and amylopectin standard solutions (Figure 2).

Figure 2 show that the absorbance of test samples solution and standard solution are basically steady when 0.3 ml to 1.0 ml 1mol/L aceticacid or 1 mol/L HCl is added in chromophoric reaction. Therefore, 0.5 ml of 1 mol/L acetic acid or hydrochloric acid was used.

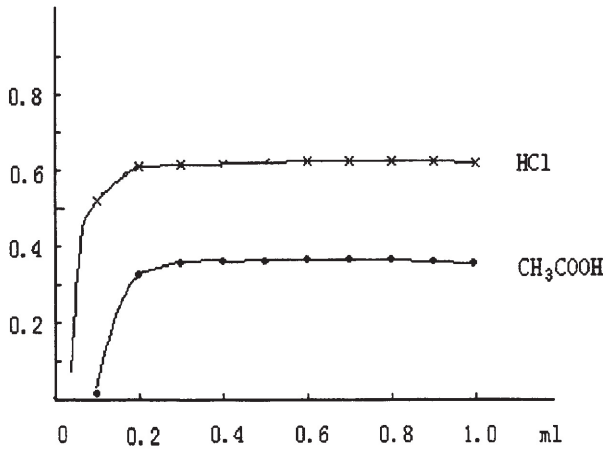

Fig. 2. Effects of the volumes of acids.

Selection of the volume of iodine solution as the chromophoric reagent

Different volumes of iodine solution were added respectively to mixture with 1.0 ml of 1 mg/ml amylose standard solution and 1.5 ml of 1 mg/ml amylopectin standard solution. Then these solutions were diluted to 50 ml. The results of comparison are shown in Fig 3 Figure 3 shows that the absorbance was basically steady when the volumes of iodine solution changed from 0.8 to 1.5 ml. Therefore, 1.0 ml of iodine solution as chromophoric reagent is adopted in this research.

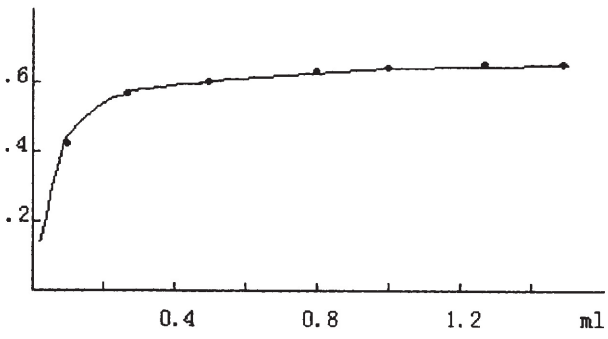

Fig. 3. Effects of the volumes of iodine solution.

Preparation of the calibration curve

Mix amylose and amylopectin standard suspensions (see Blank test in Procedure) and the 0.09mol/L NaOH 2.50 ml of each calibration solution was pipetted into 50ml test tubes, then color developed and colorimetric analysis. Prepare the calibration curve by plotting absorbance vs. the amylose content Result was expressed as a percentage by mass, in the milled rice on the dry basis. The linear regression equation was $Y = 0.0015 + 0.01299X$ ($r = 0.9999$). Result was shown in Fig. 2.

In addition, the method that defatted milled rice flours of predetermined amylose content may be used for the calibration in place of amylose and amylopectin suspensions was adopted in this research. That can omit tedious and time-consuming preparation and purification of amylose and amylopectin. The calibration curve was prepared by round-grained non-glutinous rice suspension (amylose content was 18.0%) and the linear regression equation was $Y = 0.0018 + 0.0127X$ ($r = 0.9996$). The other four samples' amylose contents (on the dry basis) calculated by this calibration curve were listed in Table 6

Table 6. Results by different calibration graph.

Method*	Amylose content (%)			
	Sample 9	Sample 10	Sample 11	Sample 12
A	23.8	16.8	17.8	17.5
B	24.0	17 5	18.0	18.0
Deviation	0.2	0.7	0.2	0.5

* Method A is preparation calibration curve by amylose and amylopectin standard suspension, and method B by rice flours of predetermined amylose content

Precision of the method

The deviation of amylose content mainly originated from test sample's weighing, piping solution and standard solution, and samples' adhesion to the walls of the flask in dispersing.

In this study, each of 10 samples of round-grained non-glutinous rice or long-grained non-glutinous rice were determined repeatedly for eight times by different operators in the same laboratory and different laboratories (Nanjing University of Economics, Sichuan Research Institute of Grain Storage of the Ministry of Internal Trade and the Central Laboratory of Grain Bureau, Jiangsu province). The results were listed in Table 7.

Table 7 showed that the deviation determined by different operators in the same laboratory (Nanjing Institute of Economics) was less than 0.4% was 80%. The maximum deviation was 0.6%, the maximum of relative deviation was 3.2%, mean of relative deviation less than 1.0% was 70%, the standard deviation repeatability (SD) was less than 0.3%, and the coefficient of variation of repeatability (CV) was 1 61%. 70% of the deviations determined by three laboratories were less than 1 0%, the repeatability was from 0.20 to 0.29, and the repeatability was less than 0.27. Therefore, the allowable error of two determination

of rice amylose content was provided in this research, it should not exceed 0.5% while amylose content of rice is less than 10.0%, and it should not exceed 1.0% while amylose content of rice more than 10.0%.

Table 7. Precision of the method for determination of amylose content.

Laboratory	Items	R.G.N Rice					L.G N.Rice				
		1	2	3	4	5	1	2	3	4	5
A	Mean	19.4	21.6	20.5	18.6	19.4	23.5	24.4	28.9	27.4	25.5
	Standard deviation (SD)	0.18	0.20	0 28	0.30	0.26	0.26	0.25	0.29	0.22	0.18
	Coefficient of variation (%)	0.96	0.91	1.37	1.61	1.34	1.10	1.02	1.00	0.80	0.71
	Max of difference	0.3	0.4	0.5	0.6	0 4	0.4	0.4	0.4	0.4	0.4
	Max. of relative deviation (%)	1.5	1.9	2.4	3.2	2.1	1.7	1.6	1.4	1.5	1.6
	Mean of relative deviation (%)	0.8	0.8	1.1	1 4	1.1	0.9	0.9	0.8	0.7	0.5
B	Mean	18.7	21.9	20.1	18.4	19.6	22.2	24.0	28.8	27.3	25.1
	Standard deviation (SD)	0.43	0.36	0.28	0.32	0.37	0.33	0.22	0.36	1.75	0.34
	Coefficient of variation (%)	2.28	1.66	1.41	1.74	1.86	1.47	0.91	1.26	0.64	1.36
	Max of difference	0.9	0.6	0.6	0.6	0.6	0.5	0.4	0.8	0.3	0.6
C	Mean	19.3	22.1	20.9	18.6	20.0	22.1	25.1	28.2	26.2	25.1
	Standard deviation (SD)	0.31	0.21	0 33	0.32	0.28	0.26	0.26	0.33	0.29	0.48
	Coefficient of variation	1.60	0.94	1.59	1.72	1.41	1.17	1.07	1.16	1.12	1.91
	Max. of error	0.6	0.2	0.5	0.5	0.4	0 3	0.4	0.6	0.5	0.7
Statistical Analysis	Mean	19.1	21.8	20.5	18.5	19.7	22.6	24.5	28.6	27.0	25.2
	error among laboratories	0.7	0.5	0.9	0.2	0.6	1.4	1.1	0.7	1.2	0.4
	Repeatability	0.26	0.22	0.20	0.25	0.25	0.23	0.20	0.27	0.26	0.27
	Reproducibility (%)	18.0	20.5	19.3	17.4	18.5	21.2	23.0	26.7	25.3	23.7

* A - Nanjing University of Economics; B - Central Lab of Grain Bureau, Jiangsu; C - Sichuan Grain Storage Research Institute

Method Recommended as a State Standard

Reagents

(a) Methanol,
(b) Ethanol,
(c) Sodium hydroxide solution (1 mol/L and 0 09 mol/L),
(d) Acetic acid or hydrochloric acid solution (1 mol/L),
(e) Iodine solution (0.2%),
(f) Amylose standard (1.0mg/ml suspension):
Weigh 100(0.5mg of defatted and equilibrium conditioned potato amylose into a 100 ml beaker, carefully add 1.0 ml of ethanol and 9.0 ml of 1 mol/L sodium hydroxide solution. Then, heat it for 10 to 15 min in a water-bath maintained at 85℃. After solution was cold, transfer it quantitatively to a 100 ml volumetric flask, dilute to volume mark with water and mix vigorously
(g) Amylopectin standard (1.0 mg/ml suspension):
Prepare from milled defatted and conditioned glutinous rice Its amylopectin content was known to be at least 99%. According to the procedure given in (f) to prepare suspension.

Apparatus

Rice huller, rice mill, micro-mill with sieve of 80 mesh, spectrometer and other usual laboratory equipment.

Procedure

Preparation of test sample

Grind appropriate milled rice in a micro-mill to very fine powder that will pass through the sieve of 80 mesh It was kept in a bottle fitted with a stopper. Rice should be hulled before milling.

Defat the flour by refluxing with methanol for 2 hr in a Soxhlex extractor (brown rice for 4 hr).

After defatting, spread the flour in a thin layer on a dish and leave for 2 days to allow evaporation of residual methanol and to make its moisture content equilibrium.

Preparation of the test solution

Weigh 100 (0.5 mg of the test sample into a 100 ml beaker, carefully add 1ml of ethanol using a pipette, washing wall of the beaker, pipe 9.0 ml of 1mol/L sodium hydroxide solution Heat the test solution in a water-bath maintained at 85℃ for 10 − 15 min Then cool solution to room temperature rapidly Alternatively, leave this solution at room temperature for 15 − 24 hr Transfer it

quantitatively to a 100 ml volumetric flask and make up to volume with water and mix.

Blank test

Carry out a blank test in parallel with the determination by the procedure mentioned above, using the same quantities of all the reagents as in the determination except using 2.5 ml of 0.09 mol/L sodium hydroxide solution instead of the test solution.

Preparation of the calibration graph

Mix the amylose and amylopectin standard suspensions and 2.0 ml of the 0.09mol/L NaOH solution in accordance with the table as follows:

Amylose in milled rice	Composition of mixture (ml)		
(m/m)% dry basis	Amylose	Amylopectin	0.09mol/l NaOH
0	0	18	2
10	2	16	2
20	4	14	2
25	5	13	2
30	6	12	2

These values have been calculated on the basis of an average starch content of 90% (m/m) in milled rice on the dry basis.

Pipe a 2.5 ml aliquot of each calibration solution into a series of five 50 ml test tubes. Add 0.5 ml of acetic acid and mix fully. Then add 1.0 ml of iodine solution, then make up to the mark with water and mix. Leave mixed solutions to stand for 20 min. Measure the absorbance at 620 nm against the blank using the spectrometer. Prepare a calibration curve by plotting absorbance vs. the amylose content. Result was expressed as a percentage by mass, in the milled rice on the dry basis.

Determination

Pipe a 2.5 ml aliquot of the test solution into a 50 ml test tube, and proceed according to the procedure mentioned above, starting with the addition of acetic acid. Measure the absorbance at 620 nm against the blank, using the spectrometer.

Expression of results

The content of amylose was expressed as percentage by mass on a dry basis and could be obtained by checking the absorbance from the calibration curve.

Take as the result the arithmetic mean of duplicate determinations to one place of decimals.

The permissible error of result of the twin determinations must not exceed 1.0% when amylose content of rice is above 10.0%.

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