

# EVALUATION OF FIAstar™ FOR THE DETERMINATION OF AMYLOSE IN MILLED RICE

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Amylose is regarded as the single most important characteristic for predicting rice cooking and processing quality. The amylose content is also a key factor in the selection of new rice varieties in breeding programmes. The following report is about an evaluation made at the Guangdong Rice Research Institute (GDRRI), Guangdong Academy of Agricultural Sciences, in China in 2001/2002.

**Abstract:** Compared to standard methods [1-3] for the determination of the apparent amylose content in rice and the micro-determination proposed by Chen Yi et al. [4], the FIA method from FOSS [5] showed very good results for the determination of amylose content in rice. 720nm was used as measuring wavelength and 880nm as reference wavelength. The FIA method for amylose is using very small amounts of reagents and samples, combined with high analysis speed (>1sample/min) in macro (standard test sample weight of 100mg) and micro (10mg test sample weight) determinations.

## FIA METHOD:

The dispersed sample is injected into a carrier stream of diluted sodium hydroxide and merged with the iodine color reagent and an acetate buffer stream. The color of the iodine-starch complex is measured in a flow cell.

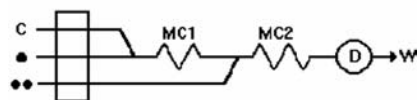


Fig. 1: Flow scheme for the determination of Amylose (for details see [5]).

## PROCEDURE:

### Preparation of check samples / standard samples / reference samples:

- Prepare a group of samples that represents all amylose levels to be en-



FIAstar™ 5000 1-channel configuration without autosampler.

countered in the test samples (at least 3 samples with low, medium and high content of amylose are needed; better is to have 4-5 samples including waxy rice samples and samples with very low amylose content). Mill and grind the samples in the same way and under the same conditions as the test samples.

- Determine the amylose content by the standard reference method using mixed amylose/amylopectin standards and by competent technicians multiple times to obtain stable and reliable values. It is a recommended way to send the samples to different authoritative laboratories for test, in order to balance the results because of differences that very often appear when comparing the results or to use certified reference material.
- Divide the sample flour into suitable amounts and pack well in sealed containers.

These “reference samples” are used as calibration standards in the FIA method.

### Preparation of rice samples for analysis

- Prepare test samples to powders the same way as reference samples. All

samples must be ground to pass a 100 mesh (about 150 µm) sieve.

- Place reference samples and test samples in paper bags in the same room for at least 2 days for moisture content equilibration.

*NOTE:* In this simplified routine method “defatting” is not done, as “defatted” standards, the amylose content of which has been determined by the reference method, are used.

### Dispersion of samples

- Weigh 100.0 mg of sample into 100 ml volumetric flask. (For micro determination weigh 10.00 mg of sample into 10 ml volumetric tube).
- Add 1.0 ml of 95% ethanol (100 µl for Micro), carefully washing down all sample adhering to the side of the tube, and slightly shake the sample in order to wet all of the sample.
- Add 9.0 ml 1 N NaOH to the sample (0.90 ml for Micro), slightly mix the sample, avoid any sample not immersed in solution. Keep samples at room temperature (higher than 10°C) for 18-28 hrs without shaking to disperse. Alternatively, heat the sample container 10 min exactly in fully boiling water and cool down completely at room temperature.

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- Make up to 100 ml volume (10 ml for micro-test) with distilled water and mix vigorously. It is recommended to vortex before adding water and vortex again after making up to volume.

#### Analysis of amylose:

- Start the FIAstar 5000 and get ready for analyzing samples.
- Transfer the well mixed samples into sample cup or directly connect to FIA sampling tip. First make or check the calibration using the reference samples, then measure the

FOSS is launching an amylose package on basis of the new ISO 6647 standard for the determination of amylose in rice. The package comprises:

#### Cyclotec™ 1093 Sample Mill:

- Providing fine, uniform particle size distribution, absolutely necessary for dispersion of the starch and for repeatability of results
- Cyclone mill as recommended in ISO standard
- Only cyclone mill on the market, that is available with a 0.3 mm screen which avoids additional sieving step to select test samples of correct particle size

#### Soxtec™ 2043, 2045 or 2050/55 extraction unit

- For convenient defatting of ground samples
- Can defat 2-6 samples simultaneously
- Space saving

#### FIAstar™ 5000 Flow Injection Analyzer

- Fast, automatic analysis with high sample throughput
- Avoids manual steps of dilution, reagent additions and photometric measurement and result calculation
- Accurate, reproducible results independent of operator

#### Know-how and support

- FOSS supplies certified standards for calibration of reference method to ensure the accuracy of the method
- The ISO standard was developed under FOSS project leadership
- The FOSS Application Lab belonged to the three top laboratories for the analysis of amylose in rice in an international collaborative study.

AAC %	590 nm	620 nm	720 nm
1,3	1401,0	986,2	278,8
16,0	2099,1	1776,1	898,7
19,0	2094,3	1828,2	990,2
24,5	2257,1	2067,1	1218,6
28,5	2360,1	2195,3	1373,6
R	0,9610	0,9860	0,999

Table 1: Apparent Amylose Content (AAC) and mAU for rice standards measured at different wavelengths.

test samples. It is recommended to run a check sample for recalibration every 10-20 samples.

### EVALUATION OF THE FIAstar™ 5000 METHOD

1. Analysis of the basic set-up of the FIA system: Based on the set-up introduced in [5], we tested different sizes (lengths or tube diameters) of tubing for pump tubes and for reaction coils, and found they did not affect the result very much, so we followed the proposed set-up. For injection loop volumes, we also noticed that 300 µl is a suitable volume for the usual concentrations of samples. 400 µl is too much for higher amylose contents and 200 µl is less sensitive at lower concentrations.
2. Many methods, including SFA methods for the determination of amylose content, state 620nm as wavelength for the measurement, while in some applications 590nm is selected. However, on FIAstar 5000, we found that 590nm or 620nm wavelength cannot reflect the same situation as the standard manual method. Instead 720nm shows a very good agreement with the standard method (Table 1, Fig. 1) and gives very reproducible results.

The reason for changing the wavelength to 720 nm is the interference of amylopectin and the very short reaction time for the color development in FIAstar 5000 [6]. When the reaction

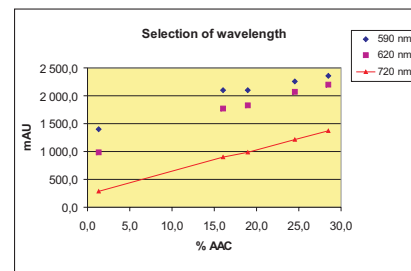


Fig. 1: Measured absorbance for rice standards at different wavelengths.

solution flows through the detector, the color is still umber, just a little bit blue. With the results obtained, we believe 720nm is a suitable wavelength for determining amylose using the FIAstar 5000 under the selected conditions. The method shows very good repeatability ( $\leq \pm 0.2$  absolute) for many repeating tests (up to 10 tests made from one and the same dispersed sample).

880nm and 1000nm can both be used as reference filter. In our experiment 880nm showed a little bit better results than 1000nm but not significantly.

3. Comparison of the results from manual method [2] and FIAstar method [5]. The results are shown in table 2. The Normal/Manual method follows the GB standard which is equivalent to the simplified amylose iodine-blue determination proposed by IRR / AACC [1], but using cold-dispersion. For the Normal/FIAstar method the same dispersed samples as for Normal/Manual method have been injected into FIAstar, using 720nm+ 880nm, 300 µl sample loop.

For the Micro/FIAstar method the simplified micro amylose determination by Chen Yi [4] was followed, samples were dispersed the same way as normal samples and then injected to FIAstar.

Six samples – including low, medium and high amylose contents – were tested on different days with three weighing and dispersion repetitions.

Sample	CNRRI		HBAAS	GDRI	
	1st Test	2nd Test	Own standard	CNRRI standard	Own standard
1	-	1,8	1,25	2,46	1.91
2	13,2	13,0	13,44	12,82	13.08
3	19,1	17,9	18,68	18,42	19.25
4	24,3	26,8	27,14	26,56	28.16
5	27,8	26,5	28,26	26,58	28.02

Table 4: Results obtained in different authoritative quality control labs for rice (% Amylose).

No.	Sample	10-Jan	10-Jan	29-Dec	29-Dec	10-Jan	29-Dec
		Normal	Normal	Normal	Normal	Micro	Micro
		Manual	FIAStar™	Manual	FIAStar™	FIAStar™	FIAStar™
1	1	14,99	15,54	14,97	15,30	14,58	14,87
2		14,76	15,53	14,51	15,37	14,17	14,72
3		14,97	15,58	14,67	15,32	14,23	13,81
4	2	13,83	14,48	13,71	13,88	13,27	13,68
5		14,04	14,38	13,75	13,85	13,10	13,32
6		13,71	14,55	13,72	13,81	13,72	13,03
7	3	21,58	21,66	22,60	21,75	21,52	21,08
8		21,70	21,61	22,40	21,90	20,65	21,13
9		21,87	21,80	21,27	21,89	21,22	22,88
10	4	19,39	19,78	18,93	19,40	18,17	18,92
11		19,75	20,09	18,76	19,35	18,56	18,73
12		19,46	20,36	18,68	19,42	18,82	18,91
13	5	28,52	29,23	28,15	28,75	27,59	27,44
14		28,24	29,15	28,33	28,47	29,79	27,23
15		28,38	28,77	28,20	28,39	27,61	27,25
16	6	26,69	26,50	27,21	27,54	28,10	27,10
17		27,02	26,79	27,44	27,27	27,81	26,78
18		27,26	26,79	27,58	27,46	27,02	26,92

Table 2: Results of amylose content in rice from manual method and FIAStar™ 5000.

No	Manual vs FIA, day 1		Manual vs FIA, day 2		Normal Method		FIAStar™		Micro-method	
	C-D	SD	E-F	SD	C-E	SD	D-F	SD	G-H	SD
1	-0,55	0,39	-0,33	0,23	0,02	0,01	0,24	0,17	-0,29	0,20
	-0,77	0,54	-0,86	0,60	0,25	0,18	0,16	0,11	-0,55	0,39
	-0,61	0,43	-0,65	0,46	0,30	0,21	0,26	0,18	0,42	0,30
	-0,65	0,46	-0,17	0,12	0,12	0,08	0,60	0,42	-0,41	0,29
	-0,34	0,24	-0,10	0,07	0,29	0,21	0,53	0,38	-0,22	0,16
	-0,84	0,59	-0,09	0,06	-0,01	0,01	0,74	0,52	0,68	0,48
	-0,08	0,06	0,85	0,60	-1,02	0,72	-0,09	0,06	0,44	0,31
	0,09	0,06	0,50	0,36	-0,70	0,49	-0,28	0,20	-0,48	0,34
	0,07	0,05	-0,62	0,44	0,60	0,42	-0,09	0,06	-1,66	1,17
	-0,39	0,28	-0,47	0,33	0,46	0,33	0,39	0,27	-0,75	0,53
	-0,34	0,24	-0,59	0,42	0,99	0,70	0,74	0,52	-0,16	0,12
	-0,90	0,63	-0,74	0,52	0,78	0,55	0,94	0,66	-0,09	0,06
	-0,71	0,50	-0,60	0,42	0,37	0,26	0,48	0,34	0,16	0,11
	-0,91	0,64	-0,14	0,10	-0,09	0,06	0,68	0,48	2,56	1,81
	-0,39	0,27	-0,19	0,13	0,18	0,13	0,38	0,27	0,35	0,25
	0,19	0,13	0,33	0,23	-0,52	0,37	-1,04	0,73	1,00	0,71
	0,23	0,17	0,17	0,12	-0,42	0,30	-0,48	0,34	1,03	0,73
	0,47	0,33	0,12	0,08	-0,32	0,23	-0,67	0,47	0,09	0,07
Max Difference										
	0,91	0,64	0,86	0,6	1,02	0,72	1,04	0,73	2,56	1,81

Table 3: Differences between methods and testing times (Analysis of table 2).

## DISCUSSION:

As it is not easy to obtain a good repeatability with the iodine-blue method for the determination of amylose when using pure amylose and amylopectine standards for calibration (so called standard reference method), people normally just use pure amylose for calibration when testing the reference samples, and then use the reference samples as standards to test unknown samples (called simplified standard method or routine method). Standard samples and test samples are then milled, ground, dispersed, color reacted and measured under the same conditions, using the same procedure, so the results for the amylose content using the simplified method show always a better repeatability than the standard reference method. So, we performed the test of FIAStar 5000 using the simplified method as we have stable standard samples.

We have a long experience, showing that several key points can have a big effect on obtaining a correct result:

1. Sample preparation: Test samples are recommended to have similar milling grade, as the uniformness of the grind and the fat content can

cause more than 2% difference. To have the same grade of grind and a fine enough flour is essential, because 1N NaOH cannot fully disperse rice starch and also destroy amylose by “over dispersing”. This can very easy bring more than 2% difference to the result, especially for some hard rice samples (we recommend to shake the dispersed sample again when pipetting or dispensing).

2. We often found that results varied very much between laboratories (table 4). It is very important to have ring tests between labs, especially between authoritative labs. Other labs can then use reference material certified by these authorities.

## CONCLUSIONS

FIAStar allows for the fast, automatic analysis of amylose in rice with a high sample throughput. It is also suitable for micro-analysis of a few grains.

As manual steps of dilution, reagent additions and photometric measurement and result calculation are avoided, accurate, reproducible re-

sults, independent of operator are obtained.

In addition valuable laboratory time is freed for other tasks.

## REFERENCES:

- [1] American Association of Cereal Chemists approved method 61-03
- [2] Chinese standard GB7648-87
- [3] Juliano, B.O., A simplified assay for milled rice amylose, *Cereal Science Today*. Oct 1971, vol 16, no 10, p. 334-338, 340, 360
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- [5] FOSS Application Note AN 5290: Determination of amylose content in rice with FIAStar 5000.
- [6] Jürgen Möller, New standard for the determination of amylose in rice, *Proceedings of the CCOA/ ICC symposium in Shanghai, June 13-16, 2004 and the AACCC/TIA joint meeting in San Diego, September 19-22.*