韓作誌(Korean J. Crop Sci.) 38(1) : 66~71(1993)

Simplified Procedure of Amylose Analysis by Rapid Flow Autoanalyzer RFA-300

Hae Chune Choi, Yong Hee Son, and Soo Yeon Cho*

自動分析機 RFA-300을 利用한 아밀로스 分析法

崔海椿, 孫永姬, 趙守衍

ABSTRACT : Several trials and errors were repeated to develop a simplified recipe of amylose analysis using a Rapid Flow Autoanalyzer(Alpkem, RFA-300). The amylose content of rice samples analyzed by the Rapid Flow Autoanalyzer were compared with those of Williams' and Juliano's assay. The results by the simplified recipe of RFA amylose analysis were highly correlated with those by Williams' and Juliano's method($r=0.95^{**}-0.97^{**}$). The relative amylose content of defatted rice starch was higher than those of non-defatted rice flour, showing very close correlations between those analyzed by three method.

INTRODUCTION

Rice starch is composed of two major structurally distinct components;amylose and amylopectin. Amylose content or amylose to amylopectin ratio is the most important determinant affecting the cooking and eating qualities of milled rice. It is usually expressed as the percentage of milled rice dry weight rather than as starch basis.

Amylose has the important ability to form a stable blue complex with iodine within the axis of its own helical structure(Rundle et al., 1944). The ability of amylose to interact with iodine, forming the specific blue complex, was used extensively in the characterization of starch and physico-chemical determination of the amylose concent(Larson et al., 1953 ; Halick and Keneaster, 1956 ; Hall and Johnson, 1966).

McCready and Hassid(1943) first suggested a simple method for seperating pure amylose and amylopectin, and quantitative determining procedure for amylose assay of potato starch. Williams et al. (1958) adapted the method of McCready and Hassid for amylose determination of a number of southern U. S. A. rice varieties.

Hot-water-soluble amylose has been used as a cooking quality indicator for both rice flour (Halick and Keneaster, 1956 ; Hall and Johnson, 1966) and whole-grain milled rice

* Crop Experiment Station, R.D.A., Suwon 441-100, Korea

〈92. 11. 30 接受〉

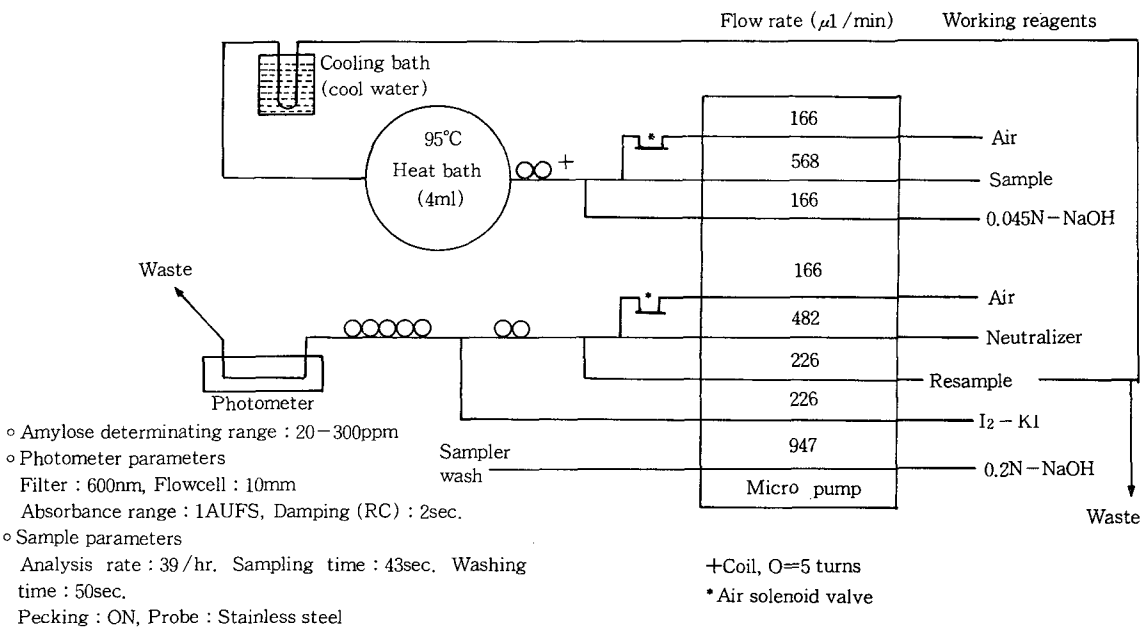
(Kurasawa et al., 1962). Juliano et al. (1968) expressed a fear of low-estimating the amylose content for the rice samples of high gelatinization temperature and high amylose by adapting the starch-iodine blue test at 77 ℃. Juliano(1979) also suggested in his review of amylose analysis that the water-soluble amylose should be an adequate indicator of total amylose content of milled rice in programs in which high-amylose, hard gel-consitency rice are absent. Morrison and Laignelet(1983) recommended an improved colorimetric procedure for determining apparent amylose and total amylose in cereal with lipid-free starch since the lipid is a hindrance factor to amylose-iodine complex forming.

This experiment was conducted to establish a simplified procedure of amylose assay using a Rapid Flow Autoanalyzer. For undertaking our breeding project of high-quality rice effectively, there must be prepared the efficient method to check the amylose content quickly for large number of breeding materials.

MATERIALS AND METHODS

The materials used in this experiment were forty five rice varieties including eighteen japonica and twenty seven Tongil-type or indica rice. The rice samples were polished by Mcgill miller No. 2 and then 100-mesh rice flour was made by Udy cyclone sample miller. For seven rice varieties, both rice flour and defatted rice starch were prepared.

Tow different procedures of amylose analysis suggested by Williams et al. (1958) and Juliano(1971) were employed. Newly simplified recipe of amylose assay utilizing the Rapid Flow Autoanalyzer(Alpkem, RFA-300) was established by modifying the Juliano's method. The results of amylose analysis by RFA were compared with those through two



∘ Amylose determinating range : 20−300ppm
∘ Photometer parameters
Filter : 600nm, Flowcell : 10mm
Absorbance range : 1AUFS, Damping (RC) : 2sec.
∘ Sample parameters
Analysis rate : 39/hr. Sampling time : 43sec. Washing time : 50sec.
Pecking : ON, Probe : Stainless steel

Fig. 1. Flow diagram of amylose analysis by Rapid Flow Autoanalyzer(Alpkem, RFA−300).

previous amylose assays.

The modified amylose assay with one kernel also was set up and undertaken for thirteen rice samples. The detail recipe of RFA amylose analysis is as follows :

The working solutions for running the RFA amylose assay are 0. 045 N sodium hydroxide solution, iodine /acetic acid reagent, neutralizer and sampler washing solution. 0.045 N solution of sodium hydroxide were prepared by mixing 45㎖ of 0.1N sodium hydroxide stock solution, 55㎖ of deionized water and two drops of Triton X−405. Iodine /acetic acid reagent was made from 8 ㎖ of I_2−KI solution (KI 20g and I_2 2g being soluted in 1ℓ deionized water), 5㎖ of 1 N acetic acid, 87㎖ of deionized water and two drops of Triton X−405.

Stock neutralizer was prepared by soluting 1.5㎖ of glacial acetic acid and 1.5g of citric acid in 1ℓ of deionized water. Working solution of neutralizer was used after mixing two drops of Triton X−405 with 100㎖ of stock neutralizer. 0.2 N sodium hydroxide solution was used for washing the sampler.

Samples running for amylose analysis were prepared as weighing 50 mg of 100-mesh rice flour in 100 ㎖ volumetric flask. Then 0.5㎖ of 95% ethanol was added, taking care to wash down any flour adhering to the sides of the flask, followed by 4.5㎖ of 1 N sodium hydroxide. The suspension was gelatinized in 30℃ incubator for two hours and filled up 100㎖ in volume with distilled water and mixed well. Amylose content of an aliquot of this dispersion was determined with the Autoanalyzer.

One kernel rice sample was weighed and put into 40㎖ volumetric flask added 2㎖ of 1 N sodium hydroxide for one kernel assay by Autoanalyzer. Then it was kept in 30℃ incubator for 24 hours to be gelatinized. The gelatinized suspension was filled up 40 ㎖ with distilled water and shaken for well dispersing. An aliquot of this dispersion was run on the Rapid Flow Autoanalyzer and the analyzed results was revised according to each kernel weight. The running condition of the Rapid Flow Autoanalyzer for amylose determination was presented in detail in Fig. 1.

The pure amylose of Wako's and Sigma's products were used for standard caliberation Pure amylose of 100mg was weighed in 100㎖ volumetric flask and added 1㎖ of 95% ethanol and 9㎖ of 1 N sodium hydroxide solution. The suspension was gelatinized in 30℃ incubator for two hours and filled up 100㎖ volume with deionized water and shaken well to be equalized. The running standard amylose solutions were made from 20 ppm to 340 ppm with 20 ppm interval.

RESULTS AND DISCUSSION

The results of simplified amylose analysis by Rapid Flow Autoanalyzer (RFA) were compared with those obtained by Juliano's recipe(1971) and the method of Williams' et al. (1958). The amylose content of nonglutinous rice varieties by the RFA procedure ranged from 16.3 to 28.6%.

The analyzed results by RFA were very closely correlated with those obtained by Juliano's and Williams' method(Fig. 2 and Fig. 3). The high-amylose rice samples revealed more deviations from regression between the results of RFA and Juliano's method, indicating probably more varietal difference of high-amylose rice in gelatinization temperature or amylose solubility (Maningat and Juliano, 1978). The amylose contents analyzed by RFA exhibited about 1.0 or 2.0% point

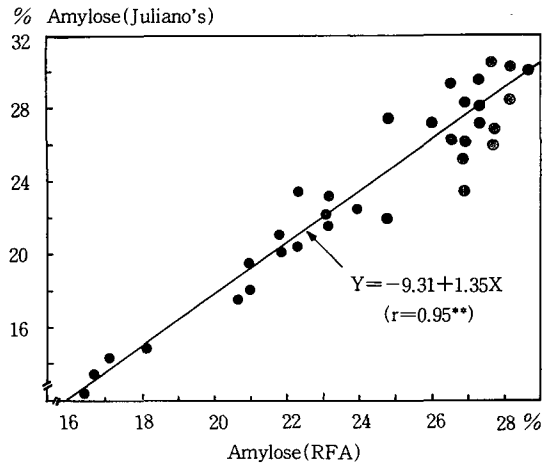


Fig. 2. Relationship between results of amylose analysis by Rapid Flow Autoanalyzer and those by Juliano's recipe.

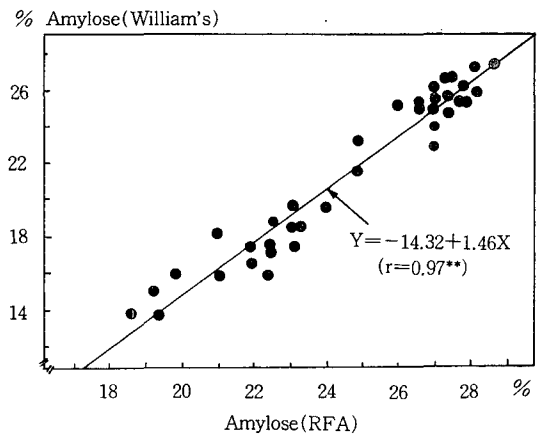


Fig. 3. Relationship between results of amylose analysis by Rapid Flow Autoanalyzer and those by Williams' recipe.

higher values in medium or low-amylose samples but about 1.0% point lower values in high-amylose one as compared with those by Juliano's assay (Fig. 2), while it was 2.0~5.0% point higher values compared with those by Williams' recipe with gradiently higher evaluation to low-amylose rice (Fig. 3). The over-estimation of amylose content especially in low-amylose rice through the RFA procedure might be affected by higher inter-

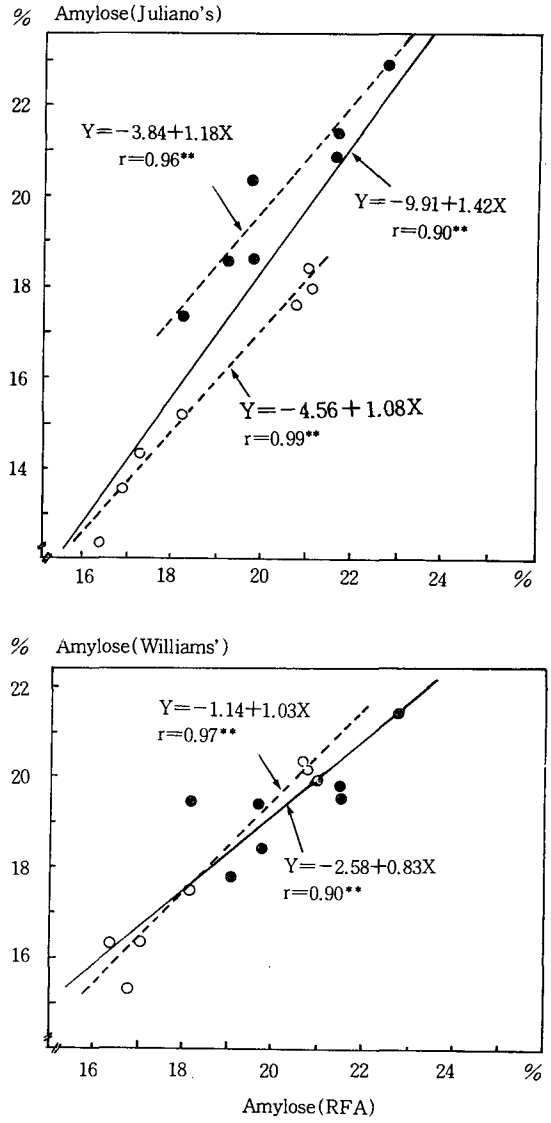


Fig. 4. Comparison among three kinds(RFA, Juliano's and Williams' method) of amylose analyses by the analyzed amylose content in starch(•) and flour(○) of seven rice cultivars.

ference of amylopectin-iodine reacted color as compared with other two methods(Juliano, 1979).

The results of amylose analysis by the RFA recipe were highly correlated with those by

other two methods in both rice flour and defatted starch samples (Fig. 4). The analyzed results through three methods were more accurately coincided with each other in defatted rice starch than in the non-defatted rice flour samples. The regression lines of rice flour and starch were almost overlapped at around 18% point of amylose content as compared between the results of RFA and those by the method of Williams' et al., while those were dispersed in comparison between the results of RFA and those by Juliano's assay (Fig. 4). The amylose contents of defatted starch samples was about 2.0% point higher than those of non-defatted rice flour samples through any method mentioned above.

The results of amylose analysis for single kernel by RFA method were highly correlated with those by Juliano's assay with 100mg sample of rice flour (Fig. 5). Single kernel analysis by the RFA can be useful for genetic analysis for progeny seeds from heterozygotic plant and dosage effect of reciprocally backcrossed seeds or physiological study on rice kernel development.

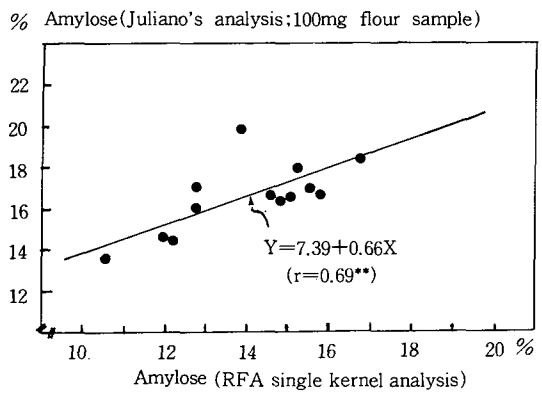


Fig. 5. Comparison between amylose contents by RFA single kernel analysis and those by Juliano's recipe(100mg rice flour sample).

摘 要

自動分析機(Rapid Flow Autoanalyzer) RFA-300을 利用한 쌀의 아밀로스 分析法을 確立하였다. 自動分析機로 분석한 아밀로스 함량을 Williams나 Juliano 등의 방법에 따른 분석결과와 비교하여 본 결과 매우 높은 直線的인 相關關係($r=0.95^{**}-0.97^{**}$)를 나타내었다.

自動分析機를 利用한 分析法으로 얻어진 아밀로스함량은 Juliano등의 분석법에 의해 얻어진 결과에 비해 低·中 아밀로스 품종에서는 1.0-2.0%가 높게, 高 아밀로스품종에서는 1%정도 낮게 측정된 반면 Williams등의 분석법에 따른 검정결과에 비해서는 2.0-5.0% 정도로 높은 함량을 나타내었는데 검정치가 低아밀로스품종 쪽일수록 증가정도가 약간 큰 경향이었다. 세가지 분석방법간에 相互 直線的인 相關을 보이면서 쌀가루에 비해 녹말시료에서 아밀로스함량이 약간 높은 경향이었다.

LITERATURE CITED

1. Halick, J.V., and K.K. Keneaster. 1956. The use of a starch iodine blue test as a quality indicator of white milled rice. Cereal Chem. 33:315-319.
2. Hall, V.L., and J.R. Johnson. 1966. A revised starch-iodine blue test for raw milled rice. Cereal Chem. 43:297-302.
3. Juliano, B.O., A.V Cartano, and A.J. Vidal. 1968. Note on a limitation of the starch-iodine blue test for milled-rice amylose. Cereal Chem. 45:63-65.
4. Juliano, B.O. 1971. A simplified assay for milled-rice amylose. Cereal Sci. Today 16:334-340.
5. Juliano, B.O. 1979. Amylose analysis in rice- a review. In proceedings of the workshop on chemical aspect of rice grain quality:251-260, IRRI.

6. Kurasawa, H., I. Igaue, and T. Hayakawa. 1962. Studies on the eating quality (especially stickiness) of non-way rice. 3. The stickiness indication of rice by a starch-iodine blue test [in Japanese, English summary]. Agr. Bull. Niigata 14:93−100.
7. Larson, B.L., K.A. Gilles, and R. Jenness. 1953. Amperometric method for determining the sorption of iodine by starch. Anal. Chem. 25:802−804.
8. Maningat, C.C., and B.O. Juliano. 1978. Alkali digestibility pattern, apparent solubility and gel consistency of milled rice. Staerke 30:125−127.
9. McCready, R.M., and W.Z. Hassid. 1943. The seperation and quantitative estimation of amylose and amylopectin in potato starch. J. Amer. Chem. Soc. 65:1154−1157.
10. Morrison, W.R., and B.Laignelet. 1983. An improved colorimetric procedure for determin- ing apparent amylose and total amylose in cereal and other starches. J. Cereal Sci. 1:9-.
11. Rundle, R.E., J.F. Foster and R.R. Baldwin. 1944. On the nature of the starch-iodine complex. J. Amer. Chem. Soc. 66:2116−2120.
12. Williams, V.R., W.T. Wu, H.Y. Tsai, and H.G. Bates. 1985. Varietal differences in amylose content of rice starch. Agr. Food Chem. 6(1):47−48.