Accurate Estimation of Sweetpotato Amylase Activity by Flour Viscosity Analysis

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Sweetpotato flour (SPF), prepared from 44 genotypes adapted to Philippine conditions, showed wide variation in Rapid Visco-Analyzer (RVA) pasting characteristics due to its variation in composition and endogenous amylase activity. The RVA pasting parameters of peak viscosity determined in water (PV1) and that determined in 0.05 mM $AgNO_3$ (used as an amylase inhibitor) (PV2) were successfully used to estimate α-amylase activity. The correlation of the ratio (PV2−PV1)/PV1 to α-amylase activity was 0.96 ($p < 0.01$, $N = 44$). Swelling volume measurements were not found to be suitable for prediction of α-amylase activity.

Keywords: *Rapid Visco-Analyzer; viscoamylograph; sweetpotato; Ipomoea batatas; amylase activity; peak viscosity; swelling volume*

INTRODUCTION

To widen its utilization, sweetpotato can be processed into flour and starch which are less bulky and more stable than the very perishable fresh root. Sweetpotato would be in greater demand if it could be used in staple food products that have wide consumption, such as bread and pasta/noodles, which are used as rice substitutes in the Asia-Pacific region. Increased use of sweetpotato would also help to offset the increasing wheat importation into developing countries, which constitutes an economic drain for many economies (Fellers and Bean, 1988; De Ruiter, 1975).

Utilization of sweetpotato is greatly affected by its high endogenous amylase activity, and selection of genotypes according to amylase activity is practiced in plant breeding (Collins, 1982). In products such as flakes, mashes, and purees endogenous amylase activity may be used to advantage in the development of desirable sensory qualities such as texture, consistency, and flavor (Walter and Hoover, 1984; Walter and Purcell, 1976). McArdle and Bouwkamp (1986) have shown that sweetpotato mashes may be adequately fermented following heat-induced mash saccharification. Sweetpotato flours have also been used successfully in place of exogenous enzymes to increase saccharification in sorghum brewing (Etim and EtokAkpan, 1992). However, the endogenous enzyme activity of the sweetpotato root varies from season to season and changes continuously under storage (Deobald et al., 1971) and according to genotype [e.g., Collado et al. (1997)], thus making it difficult to control starch hydrolysis by the native enzyme (Szyperski et al., 1986).

Pasting properties have often been found to correlate well with the quality of various products such as Japanese noodles from wheat flour (Oda et al., 1980). Little work has been reported on the effect of endogenous amylase in sweetpotato on the pasting properties of its flour, although this clearly affects utilization in products such as wheat−sweetpotato composite flour noodles (Collado and Corke, 1996). Because pasting properties are readily affected by amylase activity, viscoamylography can be used in the assessment of amylase activity in sweetpotato flours from different genotypes and can aid in the evaluation of measures to control it in food products.

We studied the physical characteristics of the sweetpotato flour (SPF) from a wide range of advanced breeding lines and varieties agronomically adapted to Philippine conditions. The aim was to develop a Rapid Visco-Analyzer (RVA)-based viscoamylograph procedure to quantitatively estimate amylase activity in sweetpotato flour. We also discuss the classification of Philippine sweetpotato varieties into groups based on amylase activity and sugar content.

MATERIALS AND METHODS

Preparation of SPF. The 44 sweetpotato genotypes used were supplied by the Asian Sweetpotato and Potato Research and Development program, Philippines. The roots were harvested after 3 months of cultivation in Tarlac, Philippines, and were processed into flours at the Institute of Food Science and Technology, University of the Philippines, Los Baños. The roots were washed, peeled, sliced thinly, soaked in 0.1% sodium metabisulfite, dried in a convection dryer at 50 °C, and then ground (Cyclone sample mill, Udy Corp., Fort Collins, CO) into a flour that can pass through a 60−80 mesh screen (212 μm aperture). The flours were sealed in polyethylene bags and shipped to Hong Kong. All experiments were conducted in Hong Kong. This set of materials was previously used in a study of genetic variation in flour color (Collado et al., 1997).

Proximate Analysis. Moisture content, crude protein, total starch, crude fat, total free sugar, and fiber content of SPF were measured as described in Collado et al. (1997), and summary results for those traits from that paper (covering the same material) are reported here. The α-amylase content was analyzed biochemically using the Amylazyme α-amylase assay procedure (Megazyme International Ireland Ltd., Ireland) (Collado et al., 1997).

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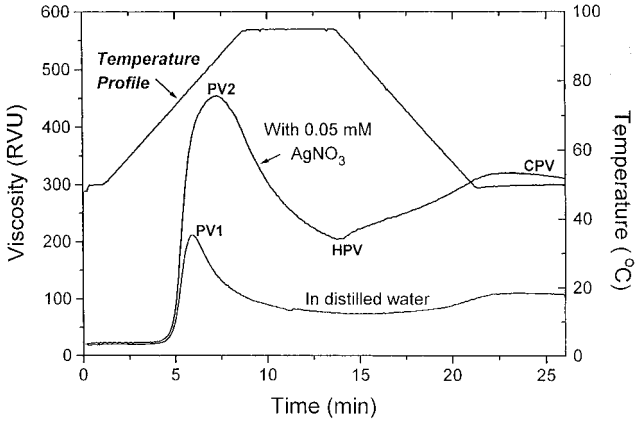

Figure 1. Typical Rapid Visco-Analyzer pasting profile of SPF in 0.05 mM $AgNO_3$ and in distilled water.

Pasting Properties. RVA viscoamylographs were determined from 4.00 g of flour (14% mb) in 24 g of distilled water at "as-is" pH (5.9−6.7) using a Rapid Visco-Analyzer model 3-D (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia). The RVA viscoamylograms of the SPF were also determined with a potent (Dixon and Webb, 1964) amylase inhibitor, $AgNO_3$, in which case 0.05 mM $AgNO_3$ was used instead of distilled water. This treatment did not affect the pH of the flour mixture. A programmed heating and cooling cycle was used at constant shear rate, where the sample was held at 50 °C for 1 min, heated to 95 °C in 7.5 min, held at 95 °C for 5.5 min, cooled to 50 °C in 7.5 min, and then held at 50 °C for 5 min. Duplicate tests were used in each case. The RVA pasting peak in distilled water was noted as PV1, whereas the other pasting parameters in distilled water were not recorded as they are too greatly affected by endogenous amylase activity. The RVA pasting parameters of SPF in 0.05 mM $AgNO_3$ were recorded as peak viscosity (PV2), hot paste viscosity (HPV) (the pasting viscosity after the holding time at 95 °C), and cool paste viscosity (CPV) (pasting viscosity at the end of the hold time at 50 °C) (Figure 1).

Swelling Volume. Swelling volume (Crosbie, 1991) was determined by weighing 0.450 g of SPF, db, into 125 × 16 mm Pyrex tubes to which 12.5 mL of water or 0.05 mM $AgNO_3$ was added. The tubes were placed in a mixing unit equilibrated at 25 °C for 5 min, after which time they were transferred to a 92.5 °C water bath and mixed in a prescribed mixing schedule for 30 min. Samples were cooled in ice water for 1 min, allowed to stand at 25 °C for 5 min, and centrifuged at 1000*g* for 15 min. The height of the gel was measured and converted to volume of gel per unit dry weight of the sample. Swelling volume in water was recorded as SV1 and in 0.05 mM $AgNO_3$ as SV2.

Statistical Analysis. All analysis was done in duplicate. Genotype means were calculated, and Pearson correlation coefficients among variables were calculated.

RESULTS AND DISCUSSION

Proximate Composition and α-Amylase Activity in SPF. On a dry weight basis, starch was the major constituent of the SPF with a mean starch content of 71.7% (Collado et al., 1997), whereas the total free sugar mean was 11.3%, the protein content mean was 3.9%, the fiber content mean was 10.3%, the crude fat mean was 0.6%, and the ash content mean was 1.89% (Table 1) (Collado et al., 1997). The α-amylase activity varied widely among the genotypes (Table 1) and had a mean level of 2.0 Ceralpha units/g. The lowest activity was observed in genotype VSP-6, with 0.26 Ceralpha ynit/g, whereas the highest was in genotype 46-12A, with 11.1 Ceralpha units/g. In sweetpotato, α-amylase has been identified as the key factor in starch degradation and sucrose synthase as the key factor in the sucrose accumulation differences among genotypes (Takahata et al., 1995). In another study, it was found that β-amylase was abundant throughout the root at all times, and its high levels did not directly affect starch degradation rates (Hagenimana et al., 1994). The maltose content of heated tubers is due to heat stability of β-amylase and gelatinization characteristics (Takahata et al., 1994). Because of the major role of α-amylase in controlling starch viscosity (due to rapid changes in molecular size caused by the endo-acting cleavage) and the high levels of measured α-amylase activity, we consider that the effect of β-amylase in our experiments was relatively insignificant.

Table 1. Mean and Range Values for Compositional and Physical Parameters of SPF from 44 Genotypes

	minimum	maximum	mean	SD[a]
α-amylase[b]	0.26	11.1	2.02	2.41
starch[b]	57.5	79.6	71.7	5.46
sugar[b]	6.8	22.7	11.3	3.33
protein[b]	1.0	5.3	3.9	0.79
fat[b]	0.06	1.90	0.60	0.38
ash[b]	1.28	2.53	1.89	0.28
PV1	40	309	156	66
PV2	344	678	521	87
HPV	182	273	232	23
CPV	288	411	348	31
SV1	10.6	18.8	15.7	1.8
SV2	14.9	21.7	18.1	1.4

[a] SD, standard deviation. [b] Previously reported in Collado et al. (1997).

Pasting Properties of SPF. The RVA viscoamylography in 0.05 mM $AgNO_3$ can be characterized as generally having a high peak viscosity relative to its hot paste viscosity and cold paste viscosity (Table 1; Figure 1). Furthermore, it was observed that there was greater variation in PV2 than in HPV and CPV. As shown in Table 1, PV2 had a mean of 521 RVU, which ranged from 344 RVU in genotype VSP-6 to 678 RVU in genotype Adams 3. The mean HPV of the SPF was 232 RVU, which ranged from 182 RVU in genotype VSP-6 to 273 RVU in genotype CN94625, whereas the mean CPV was 348 RVU, ranging from 288 RVU in genotype 88ws623 to 411 RVU in genotype Adams 3.

The RVA viscoamylographs of the SPF in 0.05 mM $AgNO_3$ from the different genotypes were correlated with its proximate composition. PV2 was significantly correlated to starch content ($r = 0.47$, $p < 0.01$), to total free sugar content ($r = -0.50$, $p < 0.001$), and to ash content ($r = -0.59$, $p < 0.001$). The HPV was significantly correlated to starch content ($r = 0.40$, $p < 0.01$), total free sugar content ($r = -0.42$, $p < 0.01$), and ash content ($r = -0.57$, $p < 0.0001$). Likewise, the CPV was significantly correlated to starch content ($r = 0.45$, $p < 0.01$), total free sugar ($r = -0.46$, $p < 0.01$), and ash content ($r = -0.64$, $p < 0.0001$).

RVA Parameters and Amylase Activity in SPF. The overall low pasting viscosities in the RVA amylograph in distilled water (Figure 1) are due to the endogenous amylase activity in the SPF. To test which parameter could be used to quantitatively estimate the α-amylase activity of SPF, correlation coefficients of PV1, PV2, PV2−PV1, and (PV2−PV1)/PV1 with α-amylase activity were determined (Table 2). It was noted that PV2−PV1 correlated less ($r = 0.45$, $p < 0.01$) with α-amylase activity than PV1 alone ($r = -0.68$, $p <$

Table 2. Correlation Coefficients of the RVA Peak and Swelling Volume in Distilled Water and 0.05 mM $AgNO_3$ with Flour Characteristics[a]

	α-amylase	PV1	PV2	PV2−PV1	PV2−PV1/PV1	SV1	SV2	SV2−SV1	SV2−SV1/SV1	starch
PV1	−0.68**									
PV2	−0.20	0.71**								
PV2−PV1	0.45**	−0.07	0.65**							
PV2−PV1/PV1	0.96**	−0.75**	−0.25	0.44**						
SV1	−0.38**	0.58**	0.52**	0.11	−0.38*					
SV2	−0.24	0.51**	0.61**	0.32*	−0.27	0.70**				
SV2−SV1	0.27	−0.25	−0.05	0.20	0.23	−0.63**	0.11			
SV2−SV1/SV1	0.28	−0.28	−0.11	0.15	0.23	−0.75**	−0.07	0.96**		
starch	−0.25	0.50**	0.47**	0.14	−0.21	0.46**	0.25	−0.37*	−0.40**	
sugar	0.22	−0.41**	−0.50**	−0.27	0.22	−0.32*	−0.34*	0.07	0.08	−0.55**

[a] $N = 44$. *, **, and *** refer to significance levels at $p < 0.05$ and < 0.01, respectively.

Table 3. Sweetpotato Classification Based on Sugar Content and Amylase Activity

low sugar content/low amylase activity		low sugar content/high amylase activity	high sugar content/high amylase activity	high sugar content/low amylase activity	
CN-946-25	93-006	OPS44	46-12A	CN94132	No. 65 CIP
CN-1489-89	VSP-6A	Inagahapon	V37-151	CN1425170	LOO2
BPISP2	V30-595		PNG L6	26 Pariados	NSPS
Miracle				Adams 3	UPLSP5
13b Tres colores				P5	89-2-10
Binicol				P16	88WS623
30 Inubi				No. 46 CIP	G-139-21
NTA 1023				12 Tres colores	UPLSP4
Catanduanes				Binoras 23	VSP-6B
UPLSP2				Inubi Zam.	VSP-7
G88				Taiwan	OP101-R89
25-11A				Bureau	OPS101

0.001), but if the difference is expressed in relation to PV1 as in (PV2−PV1)/PV1, the correlation becomes very high ($r = 0.96$, $p < 0.001$).

α-Amylase is an endoenzyme that breaks the α-1,4 glucosidic bonds on a nearly random basis, rapidly decreasing the size of the starch molecule and thereby reducing the viscosity of a starch solution or slurry. Relative viscosity measurements such as amylograph and falling number have been widely used to measure enzyme activity in wheat (Hoseney, 1990). The great differences in PV2 (peak viscosity after amylase inhibition) in sweetpotato make it inappropriate to try to estimate amylase activity from a single RVA measurement. The α-amylase activity increases the provision of sites for exo-acting *β*-amylases, but the additional effect of this on viscosity changes is relatively slight. The pasting viscosities in distilled water reflect the effect on starch of the endogenous amylase and would reflect the level of amylase present and its inherent resistance to hydrolysis. There were marked differences in PV1 among genotypes; however, it can be observed that the genotypes which exhibited highest PV2 are not the same genotypes which showed highest PV1, which may indicate not only the great differences in α-amylase activity but also some differences in resistance to starch hydrolysis. The mean PV1 was 156 RVU, ranging from 40 RVU in genotype 46-12A to 309 RVU in genotype 30-Inubi. There is considerable interest in resistant starch in sweetpotato as it affects its utilization both as animal feed and as food for humans. We note that relative differences in starch resistance to hydrolysis are greater for ungelatinized material than for gelatinized or cooked starch such as obtained during the visco-amylography. Because of the extremely high correlation of (PV2−PV1)/PV1 with biochemically measured α-amylase activity, we consider that for this diverse set of genotypes, differences in starch resistance to hydrolysis are not sufficient to meaningfully bias the results of this new method for α-amylase estimation.

Sweetpotato can be classified by starch and sugar level (Morrison et al., 1993). Certain genotypes that had low sugar content and low amylase activity were identified (Table 3). These genotypes are recommended for further evaluation as they are considered staple types that have better potential for use as SPF and for use in specific bakery or pasta products either wholly or as a composite with wheat. Staple foods should generally be bland as they are eaten with other food items. Sugar not only affects the flavor but also gelatinization and retrogradation of wheat starch, which strongly affect the quality of baked products such as cakes and breads (Kohyama and Nishinari, 1991; Lund, 1984; D'Appolonia, 1972).

The other genotypes are sweet varieties that can be considered dessert-type and genotypes with high amylase activity that can be used as substrates or adjuncts in fermentation for the production of alcohol (Etim and EtokAkpan, 1992; Dhamija and Singh, 1979). Optimization of the utilization of endogenous amylases in sweetpotato alcohol fermentation (McArdle and Bouwkamp, 1986) and flow properties of sweetpotato puree and cereal-based weaning foods (Khin et al., 1995; Szyperski et al., 1986) have been the subject of previous research.

This classification of the genotypes is preliminary because it is based on a collection grown in a single environment. However, RVA amylography can be used for monitoring the amylase activity and its stability in SPF produced under different growing conditions and during storage. This small-scale test is simple, reliable, and reproducible and does not require high technical skill to perform, unlike an enzyme analysis. This may be used as a basis for recommending genotypes for specific end-use in a breeding or selection program.

Swelling Volume of SPF. The mean swelling volume of SPF in distilled water (SV1) was 15.7 mL/g, whereas the mean swelling volume in 0.05 mM $AgNO_3$ (SV2) was 18.1 mL/g (Table 1). SV1 was significantly

correlated with PV1 ($r = 0.58$, $p < 0.001$), and the pasting peak in $AgNO_3$ (PV2) was significantly correlated to SV2 ($r = 0.61$). In wheat, the swelling volume of the flour is significantly correlated to the pasting peak of the flour (Crosbie, 1991). This characteristic was correlated to the eating quality of Japanese udon noodles and is being used a rapid screening test for the Western Australian noodle breeding program.

The swelling volume was also evaluated because it, like peak viscosity, has been found to be significantly affected by amylase activity in wheat flour. However, SV1 was not highly correlated with α-amylase activity ($r = -0.38$, $p < 0.05$) (Table 2). SV2−SV1 and (SV2−SV1)/SV1 were not significantly correlated to α-amylase activity. SV2 was significantly correlated to total free sugar content ($r = -0.34$, $p < 0.05$). Thus, swelling volume does not seem to be a suitable test for evaluating amylase activity in SPF. This may be due to the great variation in free sugar content and fiber content, which could affect the gel height reading. In wheat flours, which did not have high levels of free sugar and which have a fairly constant amount of fiber, the swelling volume could be a more sensitive and useful test for evaluating amylase activity.

Conclusions. In sweetpotato breeding, germplasm evaluation involves selection for desirable agronomic traits and for eating quality. For sweetpotato evaluation, some basic biochemical tests such as moisture, fiber, protein, sugar, starch, and carotene content (Mendoza and Rodriguez, 1994) were proposed. Collins (1987) recommended α- and β-amylase activity as bases for selection for flavor in fresh root utilization; however, biochemical indices were not developed. Such indices should also be defined for SPF for its use in baking and pasta/noodles and for sweetpotato starch for food products as well as other industrial products that may be produced. Quality indices based on biochemical traits should be supplemented with physical properties that translate these to functional characteristics which may be critical for specific end-uses. However, exploitation of this approach requires establishment of relationships between measured quality traits and various aspects of actual product quality. The approach outlined in this paper enables rapid instrumental determination of α-amylase activity in SPF or other material such as fresh roots. This facilitates monitoring of α-amylase activity in various quality control situations.

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