Using sweet potato amylase extracts for the determination of starch in foodstuffs

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ABSTRACT

A study was carried out to assess the possibility of quantitative determination of starch in starchy foodstuffs using crude amylase extracts from Ugandan sweet potato cultivars. Amylolytic activity in 18 sweet potato cultivars grown at Namulonge was evaluated and there was a significant variation of activity among cultivars tested. Crude extracts from cultivars 271, 120, 137, and Sowola had the highest amylolytic activities and titrated respectively 61590, 56310, 55740, and 55200 units of amylase activity per 100 g of fresh roots. Determination of starch in foodstuffs using crude amylolytic extracts from above sweet potato cultivars was generally sensitive enough and accurate. As far as the starch concentration was lower than 2 mg per sample of foodstuffs, there was no significant difference between routine methods and the method using sweet potato amylase extracts. The method was found to be cost effective compared to other routine and standard methods using commercial and imported enzymes and chemicals.

Key Words: *Ipomoea batatas*, Post-harvest, Utilisation, Endogenous Enzymes

RÉSUMÉ

Une étude a été menée pour évaluer la possibilité de déterminer quantitativement de l'amidon dans des produits alimentaires en utilisant des extraits amylasiques des cultivars de patate douce Ougandaise. L'activité amylolytique des 18 cultivars de patate douce cultivés à Namulonge a été évaluée, et, la variation de l'activité entre cultivars testés était très significative. Les extraits crus des cultivars 271, 120, 137 et Sowola avaient l'activité amylolytique la plus élevée qui atteignait respectivement 61590, 56310, 55740, et 55200 unités d'amylases par 100 g de tubercule frais. La détermination de l'amidon dans les produits alimentaires en utilisant les extraits amylolytiques des tubercules de cultivars de patate douce ci-haut mentionnés a été généralement sensitive et précise. Tant que la concentration d'amidon dans l'échantillon de produit alimentaire était inférieure à 2 mg , la différence entre les méthodes de routine de détermination de l'amidon et celle impliquant l'usage d'extraits amylolytiques de la patate douce n'était pas significative. Cette dernière méthode a été trouvée bon marché en comparaison avec les autres méthodes standardes at de routine utilisant des produits chimiques et enzymes importés.

Mots-clés: *Ipomoea batatas*, Après-récolte, Utilisation, Enzymes Endogènes

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is an important crop in Uganda where it plays a major role in food system of the country (Bashaasha *et al*., 1995). Although Uganda is the largest sweet potato producing country in Africa and the fourth in the world (Woolfe, 1992), sweet potato processing and utilization are remarkably narrow. Farmers, entrepreneurs are unfamiliar with products, processes, and equipment to process sweet potato. Low per capita income, limited urbanisation means effective demand is weak. More often roots are steamed or roasted in the fresh form. Home or village-level processing of the crop is relatively uncommon. Except in northeast of the country with a long and hot dry season where serious attacks by weevils limit the length of time that roots can be stored in the ground and farmers harvest, chip and sun dry the roots as a way to preserve the crop (Hall et al., 1998). Unlike China, Uganda has not yet gone into industrial processing of sweet potato into animal feed, flour, starch or noodles and other biotechnological uses (Ge et al., 1992). Limited range of utilization hampers the potential benefits of the crop to farmers and other users. Therefore, possibilities of using sweet potato as a raw material for biochemical uses should be assessed.

Sweet potato is nutritious and provides energy and appreciable amounts of vitamins and minerals (Low et al., 1997; K'osambo *et al*., 1998). Approximately 80-90% of the dry matter is made up of carbohydrates, which consist mainly of starch, and sugars (Hagenimana *et al*., 1998). In addition to high starch content, sweet potato roots have a high content of amylases (Hagenimana *et al*., 1992). The native amylases have important and well-documented influences in sweet potato storage and processing (Walter *et al*., 1975). It is known that in baked sweet potato roots, endogenous amylases hydrolyse part of the starch into maltose and longer chain polysaccharides, resulting in a sweet taste (Picha, 1985).

Amylases play a major role in the digestion of starch and the combined action of α– and β–amylase is more effective than the action of the α– or β–amylase alone (Maeda *et al*., 1978). Etim and Etokikpan (1992) successfully used sweet potato flour in place of extraneous enzymes to increase saccharification in sorghum brewing. Hoover (1967) reported an enzyme activation technique for producing sweet potato flakes from starch and freshly harvested roots without adding extraneous saccharifying enzymes. Hagenimana *et al*. (1994) also found that sweet potato roots contained high amounts of

extractable amylolytic enzymes and used the outer tissue extracts to hydrolyze starch in sweet potato mashes.

Starch is an important industrial compound and its by-products have extensive uses in industries such as of food, pharmaceutics, and others. Consequently, it is of special interest to researchers in diverse disciplines. However, methods already established in the quantification of starch remain quite expensive for many of laboratories from developing countries and hence can not be, routinely, used in analysis of starch. This is especially true for those methods involving the use of purified and imported enzymes. In addition, these enzymes, namely amylases, are not readily available in Uganda and many other developing countries. They are usually imported from overseas countries.

However, there is a possibility of using sweet potato amylase extract in starch determinations and that would minimise the expenses. The crude amylase extracts would be readily available since plenty of sweet potatoes are under-utilized.

This study is aimed at establishing whether sweet potato amylase extracts could be utilized to quantify the amount of starch in some starchy foodstuffs without adding extraneous amylases.

MATERIALS AND METHODS

Plant material

Fresh sweet potato storage roots were kindly provided by Mr. Gard Turyamureeba from the sweet potato programme, Namulonge Agriculture and Animal Research Institute (NAARI), Uganda. Sweet potatoes were grown for six months under a clay-loam soil type at the farm (altitude of 1,150m, temperature 22.3±1ºC, rainfall 960 mm) of Namulonge Station during 1995-1996 season. Thirty centimeter-long apical sweet potato cuttings were planted on mounts (3 cuttings/mount) at a spacing of 0.8 X 0.8 m. No irrigation, fungicides or fertilizers were applied to the plots. Medium-sized sweet potato storage roots were maintained under ambient air conditions, washed, and amylases extracted four days after harvest.

Cassava and millet flours were obtained from Bwaise village, Kampala, Uganda. Maize and wheat flour was from UNGA Limited Manufacturers, Nairobi, Kenya.

Apart from sweet potato amylase extracts, others enzymes and chemicals were of analytical grade

Total amylase assay

Extraction. Three medium-sized sweet potato storage roots were thoroughly washed in water and sliced, 100 g were then homogenized in a Waring blender for three minutes with 300 ml of cold extraction buffer consisting of 20 mM Sodium phosphate (pH 6.0), and containing 0.3% Sodium chloride, 0.2% Calcium chloride, and 0.001% Mercaptoethanol, then filtered through four layers of cheesecloth. This extract was centrifuged at 13,000 x g for 10 minutes, and the supernatant removed and kept on ice for further use in amylase activity assays.

Total amylase activity. Total amylase activity was determined using the dinitrosalycylic acid (DNSA) assay of Bernfeld (1955). Suitably diluted enzyme preparations (0.5 ml) were pre-incubated for 10 min at 40ºC and then incubated with 0.5 ml soluble starch solution (1%) in Na-acetate buffer (pH 6.0) containing 5 mM $CaCl_2$ and 0.04 % NaCl for 10 min at 40ºC. The reaction was stopped by the addition of 1.0 ml of DNSA reagent (1 g of 3, 5 dinitrosalycylic acid with 20 ml of 2N NaOH and 30 g NaK-tartrate made up to 100 mL with distilled water). Reaction tubes were placed in a boiling water bath for 5 min, then cooled in an ice bath. After addition of 8 ml water, the optical density was measured at 540 nm using a spectrophotometer. A standard curve was prepared using maltose (0 to 2.0 mg/ml) and a linear regression analysis was used to determine the total reducing sugar present as mg maltose equivalents.

Chemical analyses

Dry matter determination. Three whole roots were randomly selected from each cultivar, washed, and chopped. Dry matter content was determined by drying triplicate 20-g samples at 70ºC for 72 h in a forced-air oven.

Total protein determination. Total proteins were quantified by the method of Lowry *et al*. (1951), with BSA as standard.

Starch determination

Sample preparation. One-g sample of cassava, maize, millet and wheat flour was weighed into a test tube and washed by 5ml of methanol/chloroform/water (12:5:3) solution to remove interfering substances and soluble sugars. The mixture was left for 30 min and centrifuged at 2500 x g for 10 min. The supernatant was discarded and the insoluble residue was dried for 48 h at 70ºC, and used for further determinations of starch.

Starch determination using amyloglucosidase. Onto the dried pellet which the preparation was above described, 40 ml of 0.01 M sodium acetate (pH 4.5) were added and the samples were boiled for 2 min and the final volume made up to 100 ml with sodium acetate buffer. A standard of soluble starch from BDH was also run. After cooling, 0.5 ml was pipetted into another test tube and 0.5 ml of amyloglucosidase enzyme (100 U/ml) was added. The samples were incubated for 1 hour at 30ºC, and the reaction stopped by adding 1 ml of DNSA solution. The maltose equivalent content formed was determined as above described.

Starch determination using α–amylase + amyloglucosidase. Onto the dried pellet which the preparation was above described, 40 ml of 0.01 M sodium citrate (pH 5.1) were added and the samples were boiled for 2 min and the final volume made up to 100 ml with sodium citrate buffer. A standard of soluble starch from BDH was also run. After cooling, 0.5 ml was pipetted into another test tube and 0.5 ml of amyloglucosidase + α–amylase (100 U/ml) was added. The samples were incubated for 1 hour at 50ºC, and the reaction stopped by adding 1ml of DNSA solution. The maltose equivalent content formed was determined as above described.

Starch determination using perchloric acid/anthrone reagent. Onto the dried pellet which the preparation was above described, 40 ml of 35% perchloric acid were added and the samples were boiled for 2 min, cooled, and the final volume made up to 100 ml with perchloric acid. A standard of soluble starch from BDH was also run. 0.5 ml of suitably diluted starch solution was pipetted into test tubes and 5 ml of anthrone reagent was added. The tubes were then placed in boiling water for 12 min, cooled, and the absorbance read at 625 nm as described by Yemm and Willis (1954).

Starch determination using sweet potato amylase extracts. Onto the dried pellet, which the preparation was above described, 40 ml of 0.01 M sodium citrate (pH 6.0) was added and the samples were boiled for 2 min and the final volume made up to 100 ml with sodium citrate buffer (pH 6.0). A standard of soluble starch from BDH was also run. After cooling, 0.5 ml of suitably diluted starch solution was pipetted into another test tube and 0.5 ml of sweet potato amylase extracts was added. The samples were incubated for 1 hour at 40ºC, and the reaction stopped by adding 1ml of DNSA solution. The maltose equivalent content formed was determined by Bernfeld (1955) method as above described.

RESULTS AND DISCUSSION

Characteristics and total amylase activities of sweet potato roots

Characteristics and total amylase activities of sweet potato roots from 18 cultivars grown at Namulonge Station are shown in Table 1. There was a significant variation of amylase activity between sweet potato cultivars studied. Cultivars Sowola, 120, 137 and 271 had the highest amylolytic activity followed by cultivars New Kawogo, 277, and 320. Cultivars 192, 178, 52, Tanzania, 69, 282, and 218 had a medium amylolytic activity, while cultivars 316, 324 and 148, the lowest.

The dry matter content of cultivars studied was generally high, and varied from 28 to 39% (Table 1). Cultivar 69 had the highest dry matter of 39%, while cultivar 192 the lowest of 28%. Cultivar Sowola, 69, 218, 202, and 320 had a dry matter content of over 35%.

The root yield varied from 15 to 44 t/ha. Cultivar 52 had the highest yield of 44.5 t/ha, and cultivar 320 the lowest, yielding 15 t/ha. Storage roots from cultivar Sowola were the only ones, which had sprouts during the harvesting.

Determination of starch using crude amylolytic extracts from sweet potato

Storage root extracts from four sweet potato cultivars having the highest amylolytic activity were used for the determination of starch in comparison with three standard and routine methods of starch determination. The results are shown in Figure 1. Significant differences in precision were found for the seven methods when the samples contained more than 2 mg starch. However, for the starch concentration lower than 2 mg per sample, there was no significant difference between the seven methods. Our results indicate the potential of using sweet potato amylolytic extracts for the quantification of starch in a sample as far as it does not contain more than 2 mg starch (Figure 1).

Over 2 mg starch per sample, the precision was higher for the perchloric acid method followed by the mixture of alpha-amylase and amyloglucosidase, amyloglucosidase alone, extract from the sweet potato cultivar Sowola and others sweet potato extracts. At the concentration of 3.2 mg starch per sample, perchloric acid method tended to over-estimate the amount of starch present. Notably, the perchloric acid method required protective clothing and equipment for special ventilation to remove acid fumes and reduce possible explosion hazards, and this represented high level of ecological

concerns. However, on the other hands, it was highly efficient in processing large numbers of samples.

It is interesting to note the high amylolytic activity of the sweet potato cultivar Sowola and its good performance in the breakdown of starch into low molecule end products during the starch determination. Cultivar Sowola has been reported to early sprout in the field (Mwanga *et al*., 1995).

During the process of germination, the breakdown of insoluble starch material to soluble maltodextrins in plants is thought to be accomplished by endoamylases (α–amylase: EC 3.2.1.1) (Manners, 1985). Our results on high amylolytic activity from the cultivar Sowola agree with Hagenimana *et al*. (1994b), who reported a general increase in total amylolytic activity during sweet potato root sprouting, and found that α–amylase protein in the sweet potato roots increased during sprouting and they suggested that it was synthesised de *novo* during the sprouting. α–Amylase plays a major role in the digestion of starch and Maeda *et al*. (1978) found that the combined action of α– and β–amylase was more effective than the action of α– or β–amylase alone. The ubiquitous distribution of β–amylase throughout the sweet potato roots reported by Hagenimana *et al*. (1992) and the synthesis of α–amylase in sprouting roots made the cultivar Sowola more suitable for the hydrolysis of starch and its subsequent determination.

Determination of starch in cassava, maize, millet, and wheat flour using seven methods is shown in Table 2. Overall, the enzyme methods yielded low starch values than the perchloric acid method. However, the perchloric acid method tended to overestimate the starch content in different foodstuffs. Since it is not 100% specific for starch, the accuracy of the starch values depends upon the relative proportions of starch and other interfering polymers, like pectins, in foodstuffs (Rose *et al*., 1991).

We run SAS General Linear Models procedures (SAS, 1988) for comparing starch contents obtained from different samples using the seven methods, and found that methods using sweet potato extracts were not statistically different to that the one using amyloglucosidase. Method using the extract from the cultivar Sowola was slightly statistically different from the method using α–amylase +amyloglucosidase and highly different from the method of perchloric acid ($P>0.001$).

Cost for the determination of starch using different methods

About 1.7 million Uganda Shillings was required for analysing starch of four foodstuffs samples when the method of a-amylase + amyloglucosidase was used. The cost was only 1.2 millions with amyloglucosidase alone, and 0.3 million with perchloric acid. The cheapest of all the methods were with extracts from sweet potatoes, which required only 0.16 million Uganda Shillings.

CONCLUSION

There is a significant variation of amylase activity that depends on cultivar; extract from cultivar Sowola showed high and consistent amylolytic activity during the starch determination;

Sweet potato amylolytic extracts could be used at a low cost for the estimation or determination of starch in foodstuffs in place of imported amylolytic enzymes;

More research and development are still required to refine the extractability, stability, and homogeneity of sweet potato amylolytic extracts.

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REFERENCE

Bashaasha, B., Mwanga, R.O.M., Ocitti p'Obwoya, C., and Ewell, P.T. 1995. *Sweet potato in the farming and food systems of Uganda: A farm survey report.* International Potato Center (CIP) and National Agriculture Research Organisation (NARO), Lima, Peru.

Bernfeld, P. 1955. Amylases, α– and β–, p 149. In: *Methods in Enzymology. Vol.1,* Colowick, S.P. and Kaplan, N.O. (Eds**.**)**.** Academic Press, New York.

Etim, M.N. and Etokakpan, O.U. 1992. Sorghum brewing using sweet potato flour to increase saccharification. *World Journal for Microbiology and Biotechnology 8:* 509-511.

Ge, L.W., Xiuqin, W., Huiyi, C., and Rong, D. 1992. Sweet potato in China, pp. 41-50. In: Scott, G., Wiersema, S., and Ferguson, P.I. (eds*.). Product development for root and tuber crops. Vol.I-Asia.* Proceedings of the International Workshop, held April 22-May 1, 1991, at Visayas State College of Agriculture (VISCA), Baybay, Leyte, Philippines, sponsored by the International Potato Center (CIP), the Centro Internacional de Agricultura Tropical (CIAT), and the International Institute for Tropical Agriculture (IITA). CIP, Lima, Peru. 384p.

Hagenimana, V., Karuri, E.G., and Oyunga, M.A. 1998. Oil content in fried processed sweet potato products. *Journal of Food Processing and Preservation 22:* 123-137.

Hagenimana, V., Simard, R.E., and Vézina, L.P. 1992. Distribution of amylases within sweet potato (*Ipomoea batatas* L.) root tissues. *Journal of Agricultural and Food Chemistry 40:* 1777-1783.

Hagenimana, V., Simard, R.E., and Vézina, L.P. 1994. Sweet potato α– and β–amylases: Characterisation and kinetic studies with endogenous inhibitors. *Journal of Food Science 59:* 373-377.

Hagenimana, V., Simard, R.E., and Vézina, L.P. 1994b. Amylolytic activity in germinating sweet potato (Ipomoea batatas L.) roots**.** *Journal of the American Society for Horticultural Science 119*: 313-320.

Hall, A., Bockett, G., and Nahdy, S. 1998. *Sweet potato post-harvest systems in Uganda: Strategies, constraints, and potentials*. Social Science Department Working Paper No. 1998-7. International Potato Center (CIP), Lima, Peru.

Hoover, M.W. 1967. An enzyme activation process for producing sweet potato flakes. *Food Technology 21:* 322-325.

K'osambo, L.M., Carey, E., Misra, A.K., Wilkes, J., and Hagenimana, V. 1998. Influence of age, farming site, and boiling on pro-vitamin A content in sweet potato (*Ipomoea batatas* (L.) Lam.) storage roots. *Journal of Food Composition and Analysis 11*: 305-321.

Low, J., Kinyae, P., Gichuki, S., Oyunga, M.A., Hagenimana, V., and Kabira, J. 1997. *Combating vitamin A deficiency through the use of sweet potato. Results from phase I of an action research project in South Nyanza, Kenya.* Lima, Peru

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin Phenol reagent. *Journal of Biological Chemistry 193:* 265-275.

Maeda, I., Kiribuchi, S., and Nakamura, M. 1978. Digestion of barley starch granules by the combined action of α– and β–amylases purified from barley and barley malt. *Agricultural Biology and Chemistry 42:* 259-267.

Manners, D.J. 1985. Starch, p. 149-203. In*: Biochemistry of Storage Carbohydrates in Green Plants,* P.M. Dey and R.A. Dixon (Eds)**.** Academic Press, London.

Mwanga, R.O.M., Odong, B., Smit, N., Ocittip'Obwoya, C., and Gibson, R.W. 1995. Submission to the Variety Release Committee for release of sweet potato varieties. The *Ugandan Ministry of Agriculture, Animal Industries & Fisheries, Application for inclusion of a crop variety in the National Cultivar List No F00004-9*.

Picha, D.H. 1985. HPLC determination of sugars in raw and baked sweet potatoes. *Journal Food Science 50:* 1189-1190.

Rose, R., Rose, C.L., Omi, S.K., Forry, K.R., Durall, D.M., and Bigg, W.L. 1991. Starch determination by perchloric acid vs enzymes. Evaluating the accuracy and precision of six colorimetric methods. *Journal of Agricultural Food Chemistry 39:* 2-11.

SAS. 1988. SAS Institute Inc., Cary, NC, USA.

Walter, W.M., Jr., Purcell, A.E., and Nelson, A.M. 1975. Effects of amylolytic enzymes on "moistness" and carbohydrate changes of baked sweet potato cultivars. *Journal of Food Science 40:* 793-796.

Woolfe, J.A. 1992. *Sweet potato: An untapped food resource.* Cambridge University Press, Cambridge.

Yemm, E. and Willis, A. 1954. The estimation of carbohydrates in plant extract by anthrone. *Biochemistry Journal 57:* 508-514.

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FIGURE 1. Determination of starch using different methods

TABLE 1

Cultivar	Skin colour	Flesh colour	Root yield (t/ha)	Dry matter (%)[a]	Soluble protein (g/100g fresh weight)[a]	Total amylase activity (Munits[b]/100g fresh weight)[a]
New Kawogo	Red	Cream	20.1	32.4 ± 0.9	6.5 ± 1.3	46.2 ± 1.2
Tanzania	Cream	Yellow	31.1	33.4 ± 0.8	4.4 ± 0.8	30.3 ± 1.9
52	Cream	Yellow	44.5	30.2 ± 1.0	3.3 ± 0.3	32.0 ± 0.7
Sowola	Brown	Cream	29.1	35.8 ± 0.0	6.5 ± 0.4	55.7 ± 2.9
69	Cream	Yellow	31.5	38.7 ± 0.9	8.6 ± 1.6	31.1 ± 0.6
178	Red	White	32.9	29.9 ± 0.9	3.2 ± 0.4	31.8 ± 0.4
277	Cream	Cream	30.0	32.3 ± 1.4	1.6 ± 0.4	41.8 ± 0.5
316	Cream	Orange	28.7	29.3 ± 0.1	2.3 ± 0.1	25.5 ± 0.6
324	Cream	Cream	38.3	34.8 ± 0.8	6.5 ± 0.2	29.5 ± 0.8
271	Cream	Cream	32.7	33.0 ± 0.3	3.5 ± 0.1	61.6 ± 2.3
218	Cream	Yellow	28.7	36.0 ± 0.3	3.9 ± 0.1	30.2 ± 0.8
202	Cream	White	38.3	35.5 ± 0.8	1.5 ± 0.0	31.2 ± 0.4
192	Brown	Cream	32.7	28.1 ± 1.3	5.9 ± 0.3	30.6 ± 0.7
148	Yellow	Yellow	29.9	31.2 ± 0.2	5.9 ± 0.0	27.6 ± 0.4
282	Red	Yellow	38.3	30.1 ± 0.2	2.6 ± 0.3	32.9 ± 0.3
137	Yellow	White	16.8	34.2 ± 0.8	4.2 ± 0.2	55.2 ± 1.9
120	Yellow	Cream	15.1	30.2 ± 1.2	4.3 ± 0.6	56.3 ± 2.6
320	Yellow	Cream	15.0	37.5 ± 1.3	2.0 ± 0.0	44.2 ± 1.0

[a] mean ± SD

[b] One unit of activity releases 1µmole of reducing sugars (as maltose equivalents) per min at 40°C.

TABLE 2.

Methods	Samples				
	Soluble Starch (100 g)	Cassava Flour (100 g)	Millet Flour (100 g)	Wheat Flour (100 g)	Maize Flour (100 g)
Amyloglucosidase	87.3 ± 1.9	57.0 ± 1.7	31.3 ± 0.5	30.0 ± 0.0	50.5 ± 0.6
Alpha-Amylase + Amyloglucosidase	90.8 ± 0.5	66.0 ± 2.5	34.5 ± 1.0	34.5 ± 3.7	55.3 ± 1.7
Perchloric Acid	96.5 ± 0.6	73.5 ± 0.6	45.0 ± 2.2	44.5 ± 1.3	72.0 ± 2.3
Extract Cultivar 137	81.0 ± 2.5	54.0 ± 0.0	23.5 ± 0.6	24.8 ± 1.0	39.0 ± 0.8
Extract Cultivar 271	77.0 ± 0.8	54.5 ± 0.6	25.0 ± 0.0	26.0 ± 0.0	38.8 ± 1.0
Extract Cultivar 120	76.0 ± 1.2	51.8 ± 1.3	22.5 ± 0.6	37.8 ± 3.1	41.0 ± 0.0
Extract Cultivar Sowola	78.3 ± 1.3	54.8 ± 0.5	31.5 ± 1.0	30.8 ± 0.5	43.8 ± 0.5
Adjusted Means	85.3 ± 3.1	59.7 ± 1.3	30.9 ± 1.3	33.5 ± 2.0	49.5 ± 1.5