

# The chemical composition and *in vitro* digestibility of yambean starch

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The chemical composition and in vitro digestibility of starch extracted from yambean, Sphenostylis stenocarpa, were determined. The white, powdery starch was fibre-free and was characterized by low crude nitrogen, lipid and ash values but very high polysaccharide (nitrogen-free extract), 98.37%. Yambean starch (YBS) amylose was 34%. The reducing sugar yield from its acid digestion was  $103 \text{ mg } 100 \text{ mg}^{-1}$  sample. Only 20.8% of  $1\%$  gelatinized YBS was reduced to reducing sugars by 1% salivary amylose extract in 80 min when the achromic stage was attained using a qualitative iodine test. Except for Mg, Ca and Fe ions, the mineral content of yambean starch was very low. © 1998 Elsevier Science Ltd. All rights reserved

# INTRODUCTION

Legumes are not only good sources of protein but also excellent sources of carbohydrates (Jood et al., 1985). Endospermic pulses have starch as their storage polysaccharide, constituting roughly 15–65% (Norton et al., 1985). In its raw state, starch is crystalline and resistant to alpha amylase. Although gelatinization by cooking (or pelleting/extrusion) makes starches more susceptible to amylolytic breakdown, retrogradation modifications undergone by them during processing also render them resistant to alpha-amylase (Singh et al., 1980). Human salivary amylase is characterized by a sulfhydryl (SH) functional group as found in pancreatic amylase and functions optimally at pH 6.9 in the presence of chloride ions (Banks and Greenwood, 1977).

The *in vitro* (Gee and Johnson, 1985) digestibility study, simulating human luminal digestion, has clearly revealed that starch is never completely digested along the alimentary canal. However, it has also been shown that the proportion of dietary starch which normally escapes digestion by mammalian enzymes passes to the lower bowel where it undergoes bacterial fermentation (El-Harith et al., 1976). In all industrial food uses, whether or not starch is predegraded during processing, it is ultimately converted to glucose which is normally metabolized in the human body. In the present study the nutritional potential of yambean starch (YBS) as an industrial product was assessed.

# MATERIALS AND METHODS

#### Proximate analysis

Standard Association of Official Analytical Chemists methods (AOAC, 1980) were adopted for estimating ash, crude fibre, oil and nitrogen. The nitrogen-free extract (NFE), calculated by difference, represents the carbohydrate content.

## Mineral composition

Ground yambean starch was ashed by the nitricperchloric acid procedure of Thompson and Wagsta (1980) and analysed for Ca, Mg, K, Na, Mn, Cu and Zn, using an Atomic Absorption Spectrophotometer (AAS, Model 703). Total phosphorus was determined with an AAII Auto-analyser from Technicon Instrument Corporation, Tarrytown, New York, USA. Reaction between phosphorus and molybdovanadate forms a phosphomolybdovanadate complex measurable colorimetrically at 420 nm.

## Amylose determination

Amylose was estimated, employing the method of Sowbhagya and Bhattacharya (1979).

## Reducing sugar yield

YBS (0.5 g), slurried in 10 ml deionized water, was gelatinized in a boiling water bath for  $15-20$  min.

The cooked starch was cooled in an ice bath and chilled (10 ml) 72% perchloric acid was added to dissolve it. The starch extract was made up to 250 ml and 5 ml of it was digested with  $2.0 \text{ ml}$  of  $0.7 \text{ N}$  HCl at  $95^{\circ}$ C. The digest was cooled in the ice bath and allowed to attain ambient temperature. Phenolphthalein was added to the digest and titrated to an end-point, using 2 m barium carbonate. The colour of the neutral hydrolysate was discharged with 0.1 N oxalic acid. After diluting the digest to volume (100 ml) its total reducing sugar was measured colorimetrically by the Nelson method (Nelson, 1944). The acid-hydrolyzability of isolated starches at  $90-95^{\circ}$ C parallels the enzymatic digestibility (Friedmann et al., 1967). Therefore the amount of maltose produced from 100 mg starch was determined as reducing sugars and multiplied by 0.9 in a similar manner to glucose (Friedmann et al., 1967; Osman et al., 1970; Rickard and Behn, 1987).

### Rate of amylolysis of pre-cooked YBS

The method of Salimath and Tharanathan (1982) was adopted. Human saliva (1 ml) was freshly drawn at about 8 a.m. after tooth brushing and mouth washing with warm water. The saliva was diluted to 100 ml with 0.2% brine, as applied by Osman et al. (1970), to establish an optimum condition for alpha-amylase activity. One percent starch suspension, made in 0.2 m  $KH<sub>2</sub>PO<sub>4</sub> buffer (pH 6.9) was cooled in a boiling water$ bath for about 20 min. The gelatized starch was rapidly cooled under running water to  $37^{\circ}$ C after which it was incubated with the dilute salivary alpha-amylase in a ratio 4:1 (vol/vol) of starch to enzyme. A water to enzyme mixture uas similarly set up as a control. Aliquots (1 ml) from the reaction mixture and control were separately withdrawn at time intervals between 0 and 80 min. Each aliquot was immediately mixed with 5 ml of 70% alcohol to arrest further enzymatic activity. Distilled water was added to the 10 ml mark in a standard centrifuge tube and the digest was centrifuged at  $1610\$ g for 20 min. The resulting centrifugates were estimated at room temperature  $(29^{\circ}C)$  for reducing sugars (as maltose) by the Nelson method (1944). The reducing sugar produced was calculated from a standard curve and its time course plotted on another graph.

## RESULTS AND DISCUSSION

#### Proximate composition of YBS

The proximate analysis values of fractionated YBS compared to yambean flour (YBF) are shown in Table 1. All components, except nitrogen-free extract, are apparently lower in the YBS than YBF. Low nitrogen, ash and fibre contents of a starch, especially from leguminous source, are indices of good quality (Lii and Chang, 1981; MacGregor and Ballance, 1980).

Table 1. Proximate Compositions of YBF and starch isolate

	Composition in $\%$	
Parameters measured	YBF	YBS
Crude protein	23.80	$0.42 \pm 0.1$
Crude fibre	5.35	
Ash	3.40	$0.25 \pm 0.1$
Ether extract	3.10	$0.96 \pm 0.1$
N-Free extract	64.35	$98.37 \pm 1.6$

Values are means  $\pm$  SE of triplicate determinations.

YBS was fibre-free but its ash content was comparatively high relative to the values reported for pindax and pinto starches by Dreher et al. (1983). YBS nitrogen content was similar to the values of  $0.06-0.07\%$  reported by Naivikul and D'Appolonia (1979) and Dreher et al. (1984) for pinto, navy, lentil, faba and mung-bean starches. The fractionation process, which led to the removal of remarkable amounts of the seed's nitrogen, ash and fibre, automatically enhanced the quality of the bean's starch. About 1% fat is bound to YBS granules. The fat-amylose complex formation (Cone *et al.*, 1990) may substantially inhibit granule swelling (Osman et al., 1970; Nierle and El-Baya Detmol, 1990)

#### Mineral retention of YBS

Table 2 shows the mineral compositions of YBS and yambean flour (YBF). The total concentrations of Mg (58.3%), Ca (66.7%) and Fe (93.4%) retained in the fractionated starch are bound to its granules and are therefore unleachable by processing. Such minerals retain their nutritional potential when the starch is involved in food fabrications such as noodle-manufacture. Low concentrations of other minerals, on the other hand, imply their loss by leaching during processing. For instance the YBS phosphorus reported in this work, compared to the value of 0.1 mg  $g^{-1}$  reported by Lii and Chang (1981) for red bean starch, is similarly low. Unless a YBS-based food is supplemented extraneously, its exclusive use, may cause an imbalance of the Ca:P ratio for good teeth or bone building especially in growing children.

Table 2. Mineral Compositions of YBF and YBS

Measurement	YBF	<b>YBS</b>
$P g 100 g^{-1}$	$0.25 \pm 0.04$	$0.01 \pm 0.00$
Ca g $100 g^{-1}$	$0.03 \pm 0.00$	$0.02 \pm 0.00$
$Mg g 100 g^{-1}$	$0.17 \pm 0.01$	$0.01 \pm 0.00$
K g $100 g^{-1}$	$1.27 \pm 0.02$	$0.02 \pm 0.00$
Na g $100 g^{-1}$	$0.11 \pm 0.01$	$0.01 \pm 0.00$
Fe $\mu$ g g <sup>-1</sup>	$91.2 \pm 3.68$	$88.20 \pm 4.50$
Mn $\mu$ g g <sup>-1</sup>	$32.4 \pm 1.53$	Traces
$\text{Zn}\,\mu\text{g}\,\text{g}^{-1}$	$28.8 \pm 1.07$	Traces
Cu $\mu$ g g <sup>-1</sup>	$18.8 \pm 0.71$	Traces

Values are means  $\pm$  SE of triplicate determinations.

Table 3. Reducing sugar and granule composition of YBS

Measurement	Value
Reducing sugar (mg $100 \,\mathrm{mg}^{-1}$ of starch)	$103 \pm 5.6$
Amylose $(\% )$	$34.4 \pm 0.4$
Amylopectin $(\% )$ by difference	$65.6 \pm 0.8$

Values are means  $\pm$  SE of triplicate determinations.

# Granule composition and reducing sugar yield of YBS isolate

The aylose and amylopectin composition and glucose yield are shown in Table 3. YBS amylose content (34.4%) is remarkably high. This value, although not as high as 47% reported for pigeon-pea and mung-bean starches (Singh *et al.*, 1989) and  $42.3\%$  in field bean starch (Lii) and Chang, 1981), is similar to the 35.1% in black bean starch (Lai and Varriano Marston, 1979). These reports indicate that the amylose contents in leguminous plant starches are generally fairly high. High amylose content in YBS may cause retrogradation as well as digestibility problems if foods involving its use are stored too long after preparation or are allowed to undergo pyrolysis during preparation. High reducing power indicates a high degree of degradation via acid hydrolysis.

#### Salivary amylolysis of YBS

The time-course of in vitro alpha-amylase breakdown of yambean, as shown in Fig. 1, is curvilinear. This result contrasts the report of Gee and Johnson (1985) showing a linear relationship for three bean starches subjected to alpha-amylolysis. The present report indicates that YBS is resistant to salivary amylase breakdown as only



Fig. 1. The time course of 1.0% YBS hydrolysed with salivary alpha-amylase.

20.8% (of 1.0% of the pre-gelatinized starch) could undergo breakdown to reducing sugars after 80 min. This result corroborates the earlier claim (EL-Harith et al., 1976) that legume starches are almost entirely refractory to gastro-intestinal enzymes in normal human subjects. Resistance of legume starch to alpha-amylase breakdown implies low maltose release. Amylase from saliva or pancreas can only cause hydrolysis at every other 1,4-alpha-glucoside bond except the outermost bonds and those next to the branches (Mcgilvery and Goldsmith, 1979). The result is hydrolysis of the polysaccharide into a mixture of linear (maltose and malto-triose) and a range of branched alpha-limit dextrins the smallest being the tetrasaccharide 6<sup>3</sup>-alphaglucosyl malto-triose (Walker and Whelan, 1960; Mcgilvery and Goldsmith, 1979). At a stage, however. amylose-released malto-triose may be hydrolysed to maltose and glucose (Walker and Whelan, 1960). Low salivary amylase concentration, in relation to substrate and time allowed for enzyme substrate collision, may also contribute to low reducing sugar yield. Salivary amylase has been shown to contain 400 mg of pure enzyme litre<sup>-1</sup> (Meyer *et al.*, 1948).

A very low in vitro enzymatic digestion implies possible under-utilization of dietary starches of legume origin when consumed as such or incorporated into food formulations. In spite of the advantage of efficiency of in vivo over in vitro digestion (Dreher et al., 1984), no food ever yields a complete hydrolysis of its available starch content (Gee and Johnson, 1985). Generally the loss of digestible starch to the large intestine has been suggested to be a physiologically normal phenomenon (Levitt, 1983).

The starch examined in this work was of commercial quality based on its chemical composition. In addition to the local consumption of yambean, its large-scale production may be a great advantage for producing starch that may be widely exploited industrially.

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## REFERENCES

- AOAC (1980) Official Methods of Analysis, 13th edn Association of Official Analytical Chemists, Washington D.C.
- Banks, W. and Greenwood, C. T. (1977) Mathematical models for the action of alpha-amylase on amylose. Carbohydrate Research 57, 301-315.
- Cone, J. W., Utrecht and Wolters, M. G. E. Zeist (The Netherlands). (1990) Some properties and degradability of isolated starch granules. Starke 42(8), S298.
- Dreher, M. L., Dreher, C. J. and Berry, J. W. (1984) Starch digestibility of foods: a nutritional perspective. CRC Critical Review in Food Science Nutrition 20, 47-71.
- Dreher, M. T., Padmanaban, G. and Frazier, B. A. (1983) Characteristics of pindak bean starch. Journal of Agriculture and Food Chemistry  $31.844 - 846$ .
- EL-Harith, E. A., Dickenson, J. W. T. and Waker, R. (1976) Potato starch and caecal hypertrophy in the rat. Food, Cosmetics, Toxicology 14, 115-121.
- Friedmann, T. E., Witt, N. E., Neighbors, B. W. and Weber, C. W. (1967) Deterination of available carbohydrates in plant and animal foods. Journal of Nutrition 91(2), 67.
- Gee, J. M. and Johnson., I. R. (1985) Rates of starch hydrolysis and changes in viscosity digestion in a range of common foods subjected to simulated digestion in vitro. Journal of the Science Food Agriculture 36,  $614-620$ .
- Jood, S., Mehta, I., Singh, R. and Bhat, C. M. (1985) Effect of processing of fiatus-producing factors in legumes. Journal of Agriculture and Food Chemistry  $33, 268-271$ .
- Lai, C. C. and Varriano-Marston, E. (1979) Studies on the characteristics of black bean starch. Journal of Food Science 44, 528-530.
- Levitt, M. D. (1983) Malabsorption of starch—a normal phenomenon. Gastroenterology 85, 769-770.
- Lii, C. Y. and Chang, S. M. (1981) Characterization of red bean (Phaseolus radiatus var- Aurea) starch and its noodle quality. Journal of Food Science 46, 78-81.
- Macgregor, A. W. and Ballance, D. L. (1980) Hydrolysis of large and small starch granules from normal and waxy barley cultivars by alpha-amylases from barley malt. Cereal Chemistry 57, 397-402.
- Mcgilvery, R. W. and Goldsmith, G. (1979) Hydrolases: digestion. In Biochemistry, A Functional Approach. W. B. Saunders Co., Philadephia.
- Meyer, K. H., Fischer, E. H., Staub, A. and Bernfeld, A. (1948) Amylases, alpha and beta. In Enzymes of carbohydrate metabolism. Helvetica Chimie Acta 31, 2158.
- Naivikul, O. and D'Appolonia, B. L. (1979) Carbohydrates of legume flours compared with wheat flour II starch. Cereal Chemistry  $56, 24-8.$
- Nelson, N. (1944) A photometric adaptation of the somogyl

method for the determination of glucose. Journal of Biological Chemistry  $153$ ,  $275-380$ .

- Nierle, W. and EL-Baya Detmold, A. W. (1990) The effect of fatty acids, monoglycerides and monoglyceride ethers on pasting temperature and viscosity of wheat starch. Starch/ Starke 42, 268-270.
- Norton, G., Bliss, F. A. and Bressani, R. (1985) Biochemical and nutritional attributes of grain legumes, In Grain Legumes Crops. eds R. J. Summerfield and E. H. Roberts, pp. 73-214. Collins, London.
- Osman, H. P., Theurer, B., Hale, W. H. and Mehen, S. M. (1970) Influence of grain processing in in vitro enzymatic starch digestion of barley and sorghum grain. Journal of Nutrition 100, 1133-1140.
- Rickard, J. E. and Behn, K. R. (1987) Evaluation of acid and enzyme hydrolytic methods for the determination of cassava starch. Journal of the Science of Food Agriculture 41, 373-379.
- Salimath, P. V. and Tharanathan, R. N. (1982) Carbonhydrates of field bean (Dolichos latlab). Cereal Chemistry 45, 430±435.
- Singh, U., Jambunathan, R. and Narayanan, A. (1980) Biochemical changes in developing seeds of pigeon pea (Cajanus Cajan L.). Phytochemistry 19, 1291-1295.
- Singh, U., Voraputhapron, W., RAO, P. V. and Jambunathan, R. (1989) Physicochemical characteristics of pigeon pea and mung bean starches and their noodle quality. Journal of Food Science 54, 1293-1297.
- Sowbhagya, C. M. and Bhattacharya, K. R. (1979) Simplified determination of amylase in milled rice. Starch/Starke 31, 159±165.
- Thompson, K. C. and Wagstaff, K. (1980) Simplified method for the determination of cadmium, chromium, copper, lead and zinc in sewage sludge using atoic absorption spectrophotoetry. Analyst 105, 883-893.
- Walker, G. I. and Whelan, W. J. (1960) The mechanism of carbohydrase action. 7. Stages in the salivary alpha-amylolysis of amylose, amylopectin and glycogen. Journal of Bio $chemistry. 76, 257–263.$