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Studies on sweet potatoes—III. Distribution of unit chains of branched and unbranched molecules of starch

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Freshly prepared sweet potato (SP) starch was separated quantitatively into amylose and amylopectin by a concanavalin A precipitation method. Amylose was further fractionated into two subfractions containing essentially linear (Am) and moderately branched (Ax) molecules, by hot-butanol extraction. The molecular weight determined by GPC of Am was 3×10^6 Da, the values for Ap and Ax were 1.5×10^7 and 5×10^6 Da, respectively. The β-amylolysis limit values were 57, 70 and 92%, respectively, for Ap, Ax and Am. The native, as well as the derived β-limit dextrins of Ap and Ax, were debranched with isoamylase and their unit chain lengths, measured by GPC and SE-HPLC methods, showed Ap ($\overline{CL}$, 21.0) to have a trimodal distribution of unit chains with $\overline{DP}$ values of 52, 37 and 20, whereas Ax had tetramodal unit chains with $\overline{DP}$ values of 40, 30, 21 and 9. Am was more or less a linear molecule. *In vitro* SP starch was more digestible than legume and cereal starches. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Starchy foods have always been a dietary item. The susceptibility of starch granules to degradation by amylolytic enzymes generally depends not only on their source, but also on their molecular architecture. Starches from different plant sources exhibit a variety of characteristic functional properties. Sweet potato (*Ipomea batatas* L.) starch is used in food and non-food industries for various purposes (Chadha & Dakshinamurthy, 1965). It is known that the (crude) amylose obtained on starch fractionation by the classical methods gives rise to a range of amylose sub-fractions, which differ in their molecular size and β-amylolysis values (Takeda *et al.*, 1987). The presence of an anomalous material, called the intermediate fraction (Ax), has also been inferred in several starches (Madhusudhan & Tharanathan, 1996). The latter might have an impact on overall starch digestibility and glycemic index values. Continuing our studies on sweet potato starch, we now report on the structural elucidation of its branched and unbranched fractions. Preliminary data on its isolation and some physicochemical characterizations have recently been reported (Madhusudhan *et al.*, 1993).

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MATERIALS AND METHODS

Materials

Sweet potatoes were purchased in the local market. Starch was isolated and purified as described by Madhusudhan *et al.* (1993). Potato amylose and amylopectin, concanavalin A, enzymes such as β-amylase (E.C. No. 3.2.1.2, 10 units/mg solid), glucoamylase (E.C. No. 3.2.1.3 from *Rhizopus* species, 10 units/mg solid) and isoamylase (E.C. No. 3.2.1.68; 18 400 units/0.023 ml) were from Sigma Chemical Co., USA. Sepharose CL-2B, Biogel P-10 and standard dextrans (T-series) were from Pharmacia Fine Chemicals, Uppsala, Sweden. SE-HPLC columns, μ-Bondagel, E-linear and E-1000 were from Waters Associates, Milford, USA.

Methods

Sweet potato (SP) starch was fractionated into crude amylose and fairly pure amylopectin (AP) by a concanavalin A precipitation method (Yun & Matheson, 1990). The former, on hot-butanol extraction (~45°C for 1 h), yielded linear amylose (Am, butanol-insoluble) and a moderately branched intermediate (Ax, butanol-soluble) fraction (Takeda *et al.*, 1990). The purity and

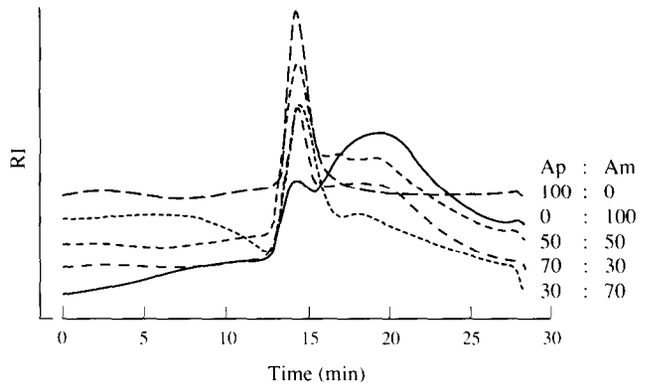

Fig. 1. SE-HPLC elution profiles of standard amylose and amylopectin of potato starch.

the average molecular weight of these fractions were examined by pre-calibrated GPC and SE-HPLC techniques (Kobayashi *et al.*, 1985; Brown & Volence, 1989). The latter was calibrated with standard amylose and amylopectin from potato (Sigma Co., USA) to find out their elution time characteristics (see Fig. 1). The λ_{max} of I_2–KI blue colour, % β-amylolysis, average chain length ($\overline{CL}$) and degree of polymerization ($\overline{DP}$) of starch and its fractions were determined as described previously (Madhusudhan & Tharanathan, 1996).

In vitro digestibility studies

Starch granules (100 mg), suspended in sodium acetate buffer (4 ml, 0.05 M, pH 4.6), were gelatinized in a boiling water bath for 30 min. The suspension was made up to 15 ml with acetate buffer and incubated at 60°C for 30 min (arbitrary time) with glucoamylase (40 units/mg starch) (Madhusudhan *et al.*, 1993). The released glucose was determined by the glucose oxidase method (Dahlquist, 1964).

Debranching with isoamylase

The fraction (50 mg, Am, Ax and Ap) was dissolved in aqueous DMSO (85%, 1 ml) and the material was precipitated with methanol (3 vols) to facilitate later an easy dissolution in water (9 ml) by heating in a boiling water bath for 30 min. The solution was cooled, acetate buffer (1 ml, 0.1 M, pH 3.5) and crystalline isoamylase (9000 units) was added, and the mixture incubated in a shaker bath at 40°C for 48 h to the complete reaction. The enzyme was heat-inactivated and the digest centrifuged (8000 rpm for 15 min). The clear supernatant was subjected to GPC on Biogel P-10 and SE-HPLC analyses.

The various other methods were as reported before (Madhusudhan & Tharanathan, 1995*a*).

RESULTS AND DISCUSSION

Figure 1 shows a standard HPLC of potato starch. In Table 1 the percentage yield values and some characteristics of sweet potato starch fractions are shown. The Am content of SP starch was 18.8%, a value similar to that reported by Takeda *et al.* (1986*a*), but it was considerably lower than that of the Garnet variety of SP starch (22.5%) (Shen & Sterling, 1981). The intermediate fraction, Ax, was, however, present in comparable yields (~4.0%). In comparison, the content of Ax is slightly more in legume and cereal starches (~6.5%) (Madhusudhan & Tharanathan, 1996). The presence of Ax was discernible in SE-HPLC analysis by its elution at 16.26 min along with a major peak of Am eluting at 22.21 min (Fig. 2). Ap was eluted as a single symmetrical peak at 14.32 min, indicating that the fraction is free from other low molecular weight contaminants. From Table 1 it is clear that Am has the highest λ_{max} value (653 nm), whereas the value for Ax (598 nm) was between those of Am and Ap (557 nm). The trend in their molecular weight values was similar, Ap and Am showed distinctly measurable differences. Ap had a molecular weight of 1.5×10^7 Da, which corresponds to a chain length mass of ~92 000 anhydroglucose residues, and suggests a higher order of molecular architecture for the SP-Ap fraction. In comparison, the Ap of potato starch has a molecular weight of 7.75×10^6 Da, corresponding to ~48 000 anhydroglucose residues (Thorn & Mohazzeb, 1990).

Table 1. Physico-chemical properties of sweet potato starch fractions

Fractions	Ap	Ax	Am	β-LD-Ap	β-LD-Ax
Yield (%)	77.0	4.2	18.8	—	—
Average molecular weight (Da)	1.5×10^7	5.0×10^6	3.0×10^6	—	—
No. of anhydroglucose residues	~92 600	~30 800	~18 500	—	—
λ_{max} (nm)	557	598	653	—	—
β-Amylolysis limit (%)	57.0	70.0	91.6	—	—
$\overline{CL}$	21.0	142	365	10.4	81.0
$\overline{DP}$*					
1	52.0	40.0	—	27.6	22.1
2	37.0	30.0	—	17.0	15.8
3	20.2	20.5	—	2.0	3.1
4	—	9.0	—	—	—

λ_{max}, absorption maxima (in nm).
$\overline{CL}$, average unit chain length value.
*$\overline{DP}$, average degree of polymerization, the numbers indicate the peak position.β-LD, the β-limit dextrin.

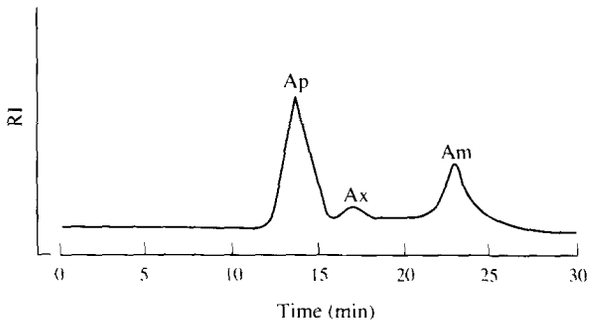

Fig. 2. SE-HPLC elution profile of isolated amylopectin (Ap), intermediate fraction (Ax) and amylose (Am) of sweet potato starch.

The low β-amylolysis limit value of 57% for the SP-Ap accords with the values generally reported for Aps from several other starches, and suggests extensive branching in the molecule. Debranching of both SP-Ap and its derived β-limit dextrin (β-LD) gave elution profiles as shown in Fig. 3. The absence of the V_0 peak is a clear indication of an ideal fractionation of starch and also complete debranching of the branched molecules. Isoamylase debranching gave a $\overline{CL}$ value of 21.2 for SP-Ap in agreement with that determined by an alternative method (Takeda *et al.*, 1986*b*). This value was slightly lower than those of potato (23.7) (Thorn & Mohazzeb, 1990) and lily (23.6) (Suzuki *et al.*, 1985) starch Aps, but similar to those of tapioca (21.1) and another variety of SP-(21–22) Aps (Takeda *et al.*, 1986*b*). From the $\overline{DP}$ values (see Table 1) it was evident that the SP-Ap has three types of chains, viz. very long B-, long B- and long A-chains, whereas its debranched β-LD showed considerable amounts of maltose and maltotriose together with unit chains of short B- and A-chains. The former is released from very short A-chain stubs.

The high β-amylolysis limit value ($\sim$92%) of SP-Am is indicative of its more or less linear α-1,4-D-glucan structure. The $\overline{CL}$ value of SP-Am (365) is much higher than those of a few other tuber starches, but lower than those of potato (670), (Takeda *et al.*, 1984), nagaimo (525) and lily (475) (Takeda *et al.*, 1986*b*) starch amyloses. Such an inference is contrary to the finding of sparsely branched Am in legume starches (Madhusudhan & Tharanathan, 1995*a*, 1996). Whereas some cereal starches are composed essentially of linear Am molecules, in amyloses of wheat, triticale and rye, a limited degree of branching (β-amylolysis limit of 77–82%) has been inferred (Lii & Lineback, 1977). From our earlier studies it is known that the quantitative and qualitative nature of amylose *per se* has a significant role to play in the overall digestibility of starch (Madhusudhan & Tharanathan, 1995*c*). The higher the Am content and more branched the Am molecule, the lower the starch digestibility, as in the case of several legume starches. Cereal and some tuber starches, in general, are easily digestible because of their low Am content and linearity in structure (Madhusudhan & Tharanathan, 1995*b*).

More of the B-chains, as in Ap and to certain extent in Ax, is indicative of dense branching in the molecule since, by definition, B-chains are those to which one or more A-chains are attached. In fact, an increased A- to B-chain ratio, as reported in the Ap of triticale starch (2.1) (Lii & Lineback, 1977), is due to the presence of innumerable unit chains in multiple branching. At a molecular level, the latter is manifested by altering the ease of *in vitro* digestibility of starches.

The Ax molecule of SP starch has a structure between those of Am and Ap. Its β-amylolysis limit value ($\sim$70%) indicates the molecule to be moderately branched as shown by further isoamylase debranching data (see Fig. 3). A tetramodal distribution of unit chains, comprising long A-, long B- and short A-chains (see Table 1) was evident from the GPC profile. Its β-LD showed, in addition to large amounts of maltose, small peaks of short A- and short B-chains, probably due to incomplete debranching.

Comparatively, the sweet potato starch was better digestible with glucoamylase than some of the legume and cereal starches (Table 2). The poor digestibility of the latter, particularly the legume starches, has been ascribed to their high amylose content ($\sim$42%), which is considerably branched and is of a relatively high molecular weight, as well as due to the presence of very highly branched amylopectin and the intermediate fraction (Madhusudhan & Tharanathan, 1996). On the other hand, the high digestibility of cereal (and some tuber) starches could be due to their low amylose values (therefore more of amylopectin) and comparatively less

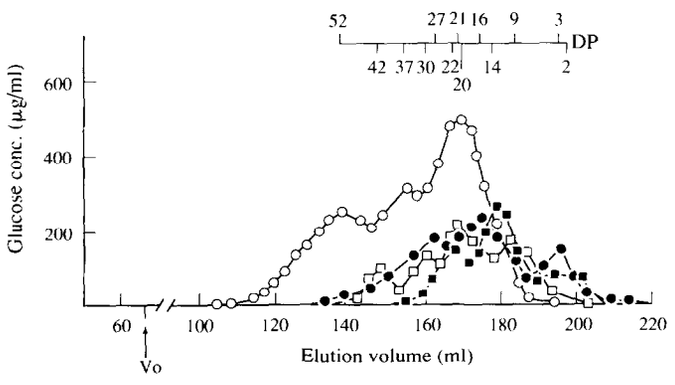

Fig. 3. GPC on Biogel P-10 of debranched fractions of sweet potato starch: – o –, Ap; – ● –, β-LD; – □ –, Ax; – ■ –, β-LD.

Table 2. *In vitro* digestibility* of sweet potato starch and various other starches

Starch	Glucose released**(%)	Starch hydrolysed (%) Glc×0.9
Sweet potato	96.0	86.4
Chick pea	50.0	45.0
Green gram	54.5	49.1
Finger millet	78.0	70.2
Rice	87.0	78.3

*With glucoamylase at 60°C for 30 min.
**Determined by the glucose oxidase method.

branching and low molecular weight of the constituent fractions.

In conclusion, it may be argued that the structural details, at a molecular level, pertaining to wide differences in the digestibility values of starches *per se*, are multifactorial, in that the distribution of unit chains, especially for Am and Ax (if any) molecules, has a significant but crucial role to play. The poor digestibility, especially of some legumes, is the primary cause of flatulence. Not only are the non-reducing oligosaccharides such as raffinose, stachyose and verbascose present in high concentration in legumes implicated in flatulence, but even the molecular architectures of the constituent molecules of starch are responsible, as also reported by El Faki *et al.* (1983, 1984).

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