WHAT IS THE TRUE AMYLOSE CONTENT OF RICE STARCH ?

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Introduction

Starch, the major reserve polysaccharide, is of enormous nutritional importance as well as a raw material for various industries. It is used as a thickener, texturiser, stabiliser and fat-replacer in the food industry and in the manufacture of paints, adhesives, paper, textiles, pharmaceuticals and biodegradable plastics. The starch granule is composed of two glucose polymers: amylose, generally regarded as a linear chain of glucose units, and amylopectin, a highly branched and very large molecule.

Among the different methods of isolation of amylose and amylopectin, gel permeation chromatography (GPC) has been extensively used to separate starch into a high molecular weight fraction, found to be branched and considered to be amylopectin and a low molecular weight fraction generally designated as amylose[1-6]. The amylopectin was found to contribute to part of the iodine reaction with starch[5] and amylose although largely linear has been shown to have some degree of branching [7-15]. Some workers have considered "branched amylose" as a third polysaccharide component[15] intermediate between amylose and amylopectin[16-19].

Amylose content of rice starch

GPC of rice starch on Sepharose 2B[20] and Sepharose CL-2B[21] was also reported to yield amylopectin (FrI) and amylose (FrII). Several workers showed that the long-B chains of the amylopectin molecules react with iodine to give a blue colour. Hence the amylose content, as estimated by iodine reaction, was considered to be an overestimate[12,13,20,21]. Subsequently, the amylose content, as estimated by the classical iodine reaction was designated as `apparent amylose'[12] and more appropriately as `amylose-equivalent' (AE)[21]. These developments lead to a general agreement that the true amylose content of rice starch may be lower than had been generally suggested. From the respective iodine affinities of whole starch, pure amylose and pure amylopectin, the true amylose content was calculated to be in the range of 15-19% for non-waxy rices[12]. The molecular size of enzymatically debranched starch was determined using SEC-MALLS and the high molecular weight material can be considered as the true "amylose" [22].

More recently Ramesh and his associates[23] fractionated seven rice starches of graded amylose equivalent (AE) on Sepharose CL-2B column into a high molecular weight fraction (FRI) and a low-molecular weight fraction (FRII, further sub-divided into FR IIa, IIb and IIc). They observed that the polysaccharide-iodine complex λ_{max} of FRII was relatively lower (<630nm), except for the peak tubes (Fig.1). Which should not be the case if FRII were to be true amylose. Therefore, the FRI, IIa, IIb and IIc were debranched (using isoamylase) and fractionated on Biogel P-10. Surprisingly, all the four fractions in all the rice varieties gave a trimodal chain profile (Biogel fr1, fr2 and fr3), demonstrating that the low molecular weight fraction (FRII) also contained some molecules which had a similar branching structure to amylopectin (Fig.2). When whole starch was debranched and fractionated on Sepharose CL-2B, no material eluted at the void volume (FRI), confirming that FRI contained branched molecules alone.

Assuming, the pattern of branching in the molecules of FRIIa, IIb and IIc similar to that of FRI, the following ratio

(Long-B chains) / (Shorter chains) = (Biogel fr1) / (Biogel fr2 + fr3)

of FRI was applied to the branched molecules in FRIIa, IIb and IIc, from which the amount of long-B chains were calculated. By subtracting the long-B chain content from their total Biogel fr1 values the approximate amylose contents for non-waxy rices, in the range 7-11% (Table 1), were obtained.

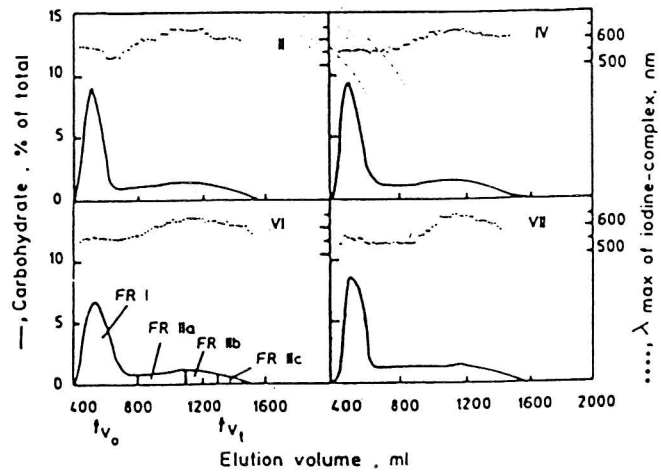

Figure 1. *Elution profile of Type II(Co 32), IV(Basmati 370), VI(Sukanandi) & VII(T 65) rice starches on Sepharose CL-2B. The polysaccharide-iodine complex λ_{max} and division of different fractions are shown*[23]*. (Reprinted with permission)*

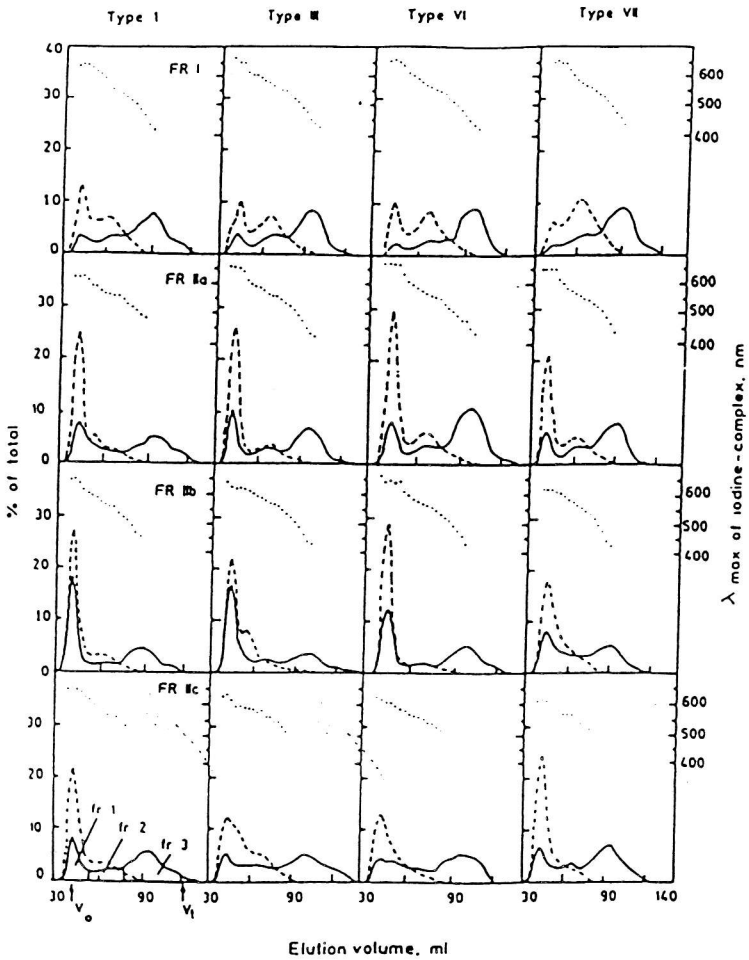

Figure 2. *Biogel P-10 chromatograms of the debranched Sepharose CL-2B fractions FRI, IIa, IIb and IIc of different rice starches. (—) Carbohydrate; (---) AE; (...) λ_{max}. The division of Biogel fr1, fr2 and fr3 representing Long-B (± amylose), intermediate-B and A- and short-B chains respectively are shown [23] (Reprinted with permission).*

We have recently confirmed the above by separating amylose from enzymatically debranched rice starches using SEC-MALLS-RI (Size exclusion chromatography coupled to multi angle laser light scattering and differential refractometer)[24], following the method of Ong and her associates[22]. Starch from four varieties of rice were debranched (using isoamylase) and fractionated on SEC-MALLS-RI. The following columns were used in series for fractionation : one guard column (Phenomenex; 6x40mm) followed by 5 fractionation columns (one TSK GEL G3000 PWXL; 7.8x300mm, two Asahipak GS-320H; 7.8x250mm, one TSK G2500 PWXL; 7.8x300mm and one TSK G-oligo PWXL)[22]. In the chromatogram, the fraction I (FrI) was assumed to be true amylose (Fig.3). Similar

Table 1. *Approximate amount of long-B chains (LB) and amylose (Am) in rice* [23]

Sepharose CL-2B Fraction	Chains in Biogel frl	Approximate amount of LB & Am (% of total carbohydrate in rice)						
		Rice quality type						
		I	II	III	IV	VI	VII	VIII
FRI	LB	10.5	10.0	8.6	6.7	6.6	5.8	2.4
	Am	-	-	-	-	-	-	-
FRIIa	LB	1.9	1.9	1.9	1.5	1.3	1.3	0.3
	Am	2.8	2.4	2.7	3.6	1.8	2.3	–
FRIIb	LB	1.7	1.6	1.6	1.0	0.8	1.0	0.1
	Am	7.6	6.6	7.7	5.7	4.6	4.2	0.2
FRIIc	LB	0.5	0.5	0.8	0.4	0.4	0.3	0.1
	Am	0.5	0.5	0.6	0.3	0.3	0.7	0.5
Total	LB	14.6	14.0	13.1	9.6	9.1	8.4	3.3
	Am	10.9	9.3	11.0	9.6	6.7	7.2	0.7

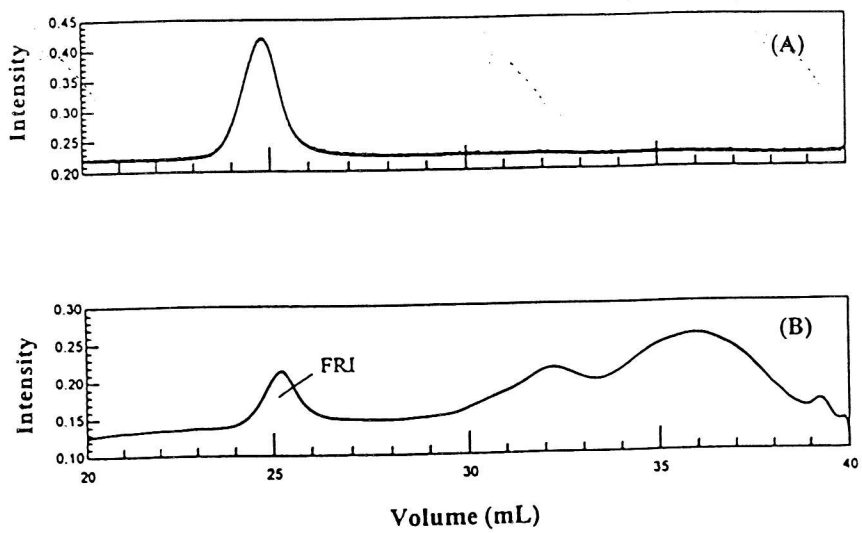

Figure 3. *Representative elution profile of isoamylase debranched rice starch (Jhona-20) fractinated on SEC-MALLS-RI system. (A) laser-light-scattering profile (B) RI profile.*

elution profiles has been reported for different starches, referring the FrI as amylose and the subsequent fractions as the various chain populations of amylopectin[22]. It is possible that the amylose contained a mild degree of branching which was removed by the enzymatic treatment. The molecules must however, have mostly a long linear portion. In

the chromatogram a baseline was established and the area under the amylose fraction was calculated from the sum of the area of several Gaussian components fitted to the data using a multiple Gaussian fit (Microcal Origin 3.0, USA). Interestingly, the amylose content, ranged from 7-11%, agreed well with the calculated amylose contents[23] (Table 2). The amylose values observed in the recent studies[23,24] are not only lower than obtained by the classical iodine assay applied to whole starch but also lower than the values reported from an iodine assay for chemically separated ``amylose''[12]. The molecular weight and polydispersity (M_w/M_n) values of the amyloses (Table 2) are in agreement with the values reported for purified rice starch amyloses[25].

Table 2. *Amylose contents of rices and their molecular weights*

Rice		Amylose					
		Ramesh et al., [23]		SEC-MALLS [24]			
Quality Type	Variety	Iodine method (%)	Calculated (%)	(%)	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w / \bar{M}_n$
I	T(N)1	28.2	10.9	-	-	-	-
	Jaya	29.4	-	11.6	3.8×10^5	5.2×10^5	1.4
III	Jhona-20	29.9	11.0	10.9	2.7×10^5	3.5×10^5	1.3
VI	Sukanandi	24.3	6.7	6.8	2.6×10^5	3.6×10^5	1.4
VII	Changlei	17.6	-	6.9	1.2×10^4	1.3×10^4	1.2
	T65	20.3	7.2	-	-	-	-

Conclusion

The above recent reports have indicated that the rice starch contains branched molecules or amylopectin of varied sizes, from very big to very small, and the content of amylose is much less than generally stated in literature. A primary aim of future work should be to investigate the true amylose content of starches from other botanical origin by using these approaches to understand whether the low true amylose content is a feature of other starches or is unique of rice starch.

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