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ISOLATION AND CHARACTERIZATION OF ISOFORMS OF SOLUBLE STARCH SYNTHASE FROM CASSAVA *Manihot esculenta* CRANTZ Cultivar Kasetsart 50 TUBER.

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In this study, isoforms of soluble starch synthase (SSS) from cassava tubers were identified and characterized. Soluble starch synthase showed highest activity during the ninth month of development. Soluble starch synthase was extracted from parenchyma of cassava tubers and purified by precipitation at 20-60% saturated ammonium sulfate, followed by phenyl sepharose and Q-sepharose column chromatographies. The chromatographic profiles showed three peaks of starch synthase activity. The three isoforms named as SSS1, SSS2, SSS3 were purified up to 425, 513 and 141 folds with 20.9, 5.0 and 5.4 % recovery, respectively. They showed optimum pH at 9.5, 7 and 9.5, and optimum temperatures at 37, 35 and 37 °C, respectively. Branched carbohydrates seemed to be better substrates for chain elongation.

Introduction: Cassava starch is one of the major raw materials used in many industries. Starch composed of the glucose homopolymers: amylose and amylopectin. Both polymers are synthesized from ADPGlc in various plastids by the actions of three families of enzymes. Starch synthases add glucosyl units to the non reducing end of a glucan chain through α(1-4) linkages, thus elongating the linear chain. Starch branching enzymes introduce the α(1-6) branch linkages. Starch debranching enzymes which hydrolyze α(1-6) branch linkages, are required for normal starch biosynthesis although it is not yet know whether they play a direct or indirect role in the process. Understanding starch biosynthesis is complicated by the fact that plant tissues possess multiple forms of each biosynthetic enzyme and that complex interactions exist between the isoforms. In this study, isolation and characterization of soluble starch synthases from cassava tubers from cultivar KU50 were performed.

Materials and Methods:
Cassava tubers cultivar Rayong 1 (R1) and Kasetsart 50 (KU50) at 3, 6, 9 and 12 months were obtained from Rayong Field Crops Center at Rayong province, Thailand. Cassava tubers were peeled and the cortex was removed. The parenchyma was chopped and homogenized in ice-cold 50 mM Tris-acetate buffer pH 8.5 containing 10 mM EDTA and 2 mM DTT. The buffer was added with final concentration of 1 mM PMSF and 20% glycerol to prevent some protease activity and stabilize the enzyme. Soluble starch synthase was precipitated with 20-60% ammonium sulfate saturation. The pellet was collected by centrifugation at 12,000 rpm for 60 minute at 4 °C and dissolved in 50 mM Tris-acetate buffer pH 8.5 containing 1 M $(NH_4)_2SO_4$, 10 mM EDTA and 2 mM DTT. The enzyme was further purified by phenyl sepharose and Q-sepharose column chromatographies. Soluble starch synthase activity was assayed by 2 methods. The radioactive (Assay A) method, measured incorporation of ^{14}C-ADP-glucose into α-D-glucan synthesized by stimulation with rabbit liver glycogen. In HPLC method (Assay B), the amount of ADP produced in the reaction catalyzed by soluble starch synthase was quantified.

Result and Discussion:
Soluble starch synthase (SSS) activity was monitored in tubers of cassava cultivars Rayong 1 and KU 50 at 3, 6, 9 and 12 months. The SSS activity increased gradually from 3 – 12 months in Rayong 1 whereas in KU 50 SSS increased to the maximum at 9 months.

Table 1. Purification table of soluble starch synthase from cassava tubers.

Fraction	Total protein (mg)	Total activity (Unit)	Specific activity (unit/mg)	Yield (%)	Folds
Crude enzyme	2276	2967	1.3	100.0	1.0
Ammonium sulfate	767	2401	3.1	80.9	2.4
Phenyl -sepharose					
SSS1	22	794	36.1	26.7	8.5
SSS2	17	150	8.9	5.0	7
SSS3	15	227	15.1	7.6	11.6
Q-sepharose					
SSS1	1.1	620	563.6	20.9	425
SSS2	0.2	147	668.1	5.0	513
SSS3	0.9	159	184.6	5.4	141

SSS was purified by 20-60% ammonium sulfate precipitation, followed by phenyl sepharose and Q-sepharose column chromatographies. Phenyl sepharose separated SSS into 3 isoforms by stepwise elution with 1, 0.5, 0.2, 0.1 and 0 M ammonium sulfate, respectively (Table 1). Each isoform was further cleaned up with Hitrap Q-sepharose column. The enzyme preparation obtained were purified up to 425, 513 and 141 folds with 20.9, 5.0 and 5.4 % recovery for isoforms 1, 2 and 3, respectively.

Purified SSS 1, 2 and 3 displayed maximum activity at pH 9.5, 7.0 and 9.5, respectively. Optimum temperatures of SSS were determined to be 37 °C for isoforms 1 and 3 while isoform 2 showed higher activity at 35 °C (Fig 1).

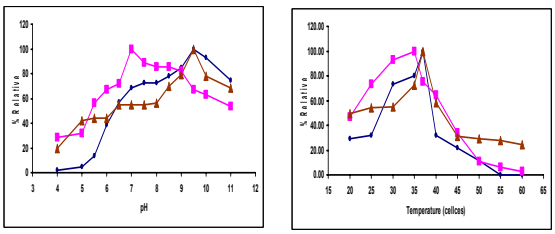

Fig 1. Optimum pH and temperature of soluble starch synthase ● isoform 1 ▲ isoform 2 ■ isoform 3

Cassava soluble starch synthase was assayed using various primers. The primers can be defined to 3 groups. First, the homopolymers of glucosyl units,. i.e. rabbit liver glycogen, oyster glycogen, amylopectin and amylose. The second group is starch from different sources: cassava, rice, potato and corn. And the last group is malto-oligosaccharides which 5-6 glucose units. The starch synthase activity assayed with rabbit liver glycogen as primer was defined as 100% activity.

Soluble starch synthase showed highest activity when oyster glycogen, rice starch and potato amylopectin were used as primers for isoforms 1, 2 and 3, respectively (Fig 2). The enzymes can also add glucose units from ADP-glucose to malto-oligosaccharides.

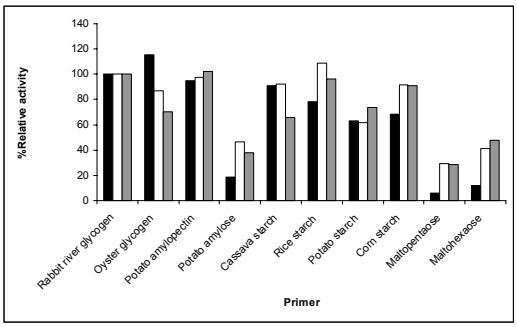

Fig. 2 Effect of primer on soluble starch synthase activity. ■ isoform 1 □ isoform 2 ▒ isoform 3

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