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Properties of Starches in Chinese Yam, *Dioscorea oppsita* Thunb. Irradiated with Proton Beam

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Abstract - The survival rate and thermal, crystal, and physicochemical properties of starches in chinese yam tubers irradiated to proton beam were determined. Survival rate was decreased with increased proton beam irradiation. Amylose content of *D. opposita* starches from different proton beam ranged from 13.2% to 17.8%. *D. opposita* starch at 5 Gy showed the highest ΔH_{gel} values (12.0 J/g) while *D. opposita* starch at 25 Gy showed the lowest values (10.1 J/g). Several parameters such as PKV (peak viscosity), HPV (Hot peak viscosity) and CPV (Cooling peak viscosity) decreased with the increase in irradiation dose. The degrees of crystallinity of the *D. opposita* starches at 5, 10, 15, 20 and 25 Gy were 37.2, 38.3, 38.9, 39.2 and 39.9%, respectively. It might be deduced that proton beam irradiation causes changes of starch, especially at high dose irradiation.

Key words - *Dioscorea opposita*, Chinese yam, Proton beam, Starch, DSC, RVA

Introduction

Mutation breeding is a useful method for crop improvement. The type of mutagenic treatment is an important factor to obtain successful results in mutation breeding. Radiation tool of food crops is one of the most common techniques for induction of crop mutations. Physical mutagens, such as gamma rays and X-rays, have mainly been used to induce mutations, and many varieties have been released (Yamaguchi *et al*., 2009). The mutants are useful for developing new crop cultivars as well as for improving starch properties, especially in starchy crops such as rice, wheat, barley and potatoes (Yu and Wang, 2007). Recently, proton beam including ion beams have attracted attention in the fields of plant breeding (Shikazono *et al*., 2003). A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally, as opposed to low linear energy transfer (LET) radiation such as gamma rays and X-rays (Yang and Tobias, 1979; Tanaka, 1999). Mutation induction with ion beams, using various plants, has been attempted since the 2000s in Korea, the 1990s in Japan (Tanaka, 1997). Until now, it has been demonstrated in Arabidopsis that ion beams induce mutations with high frequency and show a broad mutation and novel mutants have been obtained (Hase *et al*., 2000; Shikazono *et al*., 2003; Tanaka *et al*., 1997). Thus, the characteristics of ion beams have been gradually clarified, and ion beam irradiation has evolved as a new mutation method. However, in terms of the mutagen for mutation breeding, the characteristics of ion beams, especially in comparison to gamma rays and X-rays, and the criterion of optimum irradiation dose for practical use have not been sufficiently clarified in Chinese yam. Recently, we investigated the effects of proton beams on changes of gel properties of Chinese yam including basic information on the physicochemical processing characteristics of Chinese yam flours.

Materials and Methods

Chinese yam (*Dioscorea opposita* Thunb. cv. Anwon) was exposed to proton beam (LET 1.7 keV/μm) accelerated to 45 MeV with a dose of 5, 10, 15, 20, and 25 Gy. The LET values of the beam were calculated at the surface of the tubers. After

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irradiation, fifty tubers (vM_1) from each irradiation treatment were sown into plastic pots to investigate the effects of proton beams on survival. After 30 day incubation, they were moved and grown into a greenhouse. Each tuber was used to determine survival rate (LD_{50}) and then harvested tubers (vM_2) derived from irradiated with proton beam were estimated to analyze properties of starch. Freeze-dried tubers were ground and sieved with a 100 mesh sifter. After sieving, powders were extracted with 100 and 85% MeOH. Starches were also extracted with SDS five times. Finally the starches were washed three times with 85% MeOH and distilled water, respectively. Total starch was performed with the Megazyme kit according to AACC 76-13 (AACC, 2003). The absorption curves of starch and iodine complexes were measured by a UV/VIS spectrophotometer (Model Evolution 300, Thermo Electron Corporation, USA) at 700 to 500 nm. A solution containing 2 mg iodine and 20 mg potassium iodate was added to 1 mg NaOH-gelatinized and HCl-neutralized starch, and made up to 25 ml. The wavelength at maximum absorption (λmax) and blue value (BV), absorbance at 680 nm, were determined (Fujimoto *et al.*, 1972). According to the method of Kainuma (1977), amperometric iodine titration of defatted starch was carried out at 1A and 50 mV. The pasting properties of the starches (3 g, 14% moisture basis) in water (25 ml) were determined using the Rapid Visco Analyzer (RVA, Newport Scientific Pty. Ltd., Narrabeen, Australia). Gelatinization of rice starch was performed on a Differential Scanning Calorimeter (DSC-SP, Rheometric Scientific, New Castle, DE, USA) and the instrument was calibrated with indium. Starch samples and distilled water (1:3, w/w) were hermetically sealed in aluminum pans, held overnight, and heated from 30 to 120℃ with 10℃/min heating speed. An empty aluminum pan was used as reference. The collected data for tuber yield were analyzed by using SAS package for Duncan's multiple range tests.

Results and Discussion

The effect of proton beam irradiation on survival rates in Chinese yam plant was estimated (Fig. 1). Survival rate in Chinese yam tubers irradiated with proton beam ranged 14.5 to 96.5 % depending on the dose of radiation. When proton beam intensity was increased, survival rate was slightly decreased until 15 Gy, however, it decreased rapidly at 20 and 25 Gy. It was assumed that 50% lethal dose was ranged from above 15 Gy to below 20 Gy with proton beams. Physicochemical properties of flours were also determined from the tubers irradiated with proton beam (Table 2). Maximum absorption (λmax) degree for tuber flours irradiated with different doses was slightly increased with elevated proton beam doses. Blue value was increased as the dose of proton beam irradiation increased. Starch content was not changed by proton beam irradiation as compared to the control. Unlike a change of starch content, amylose content showed that highest proton beam irradiation leaded to significant increase. In cereal crops, amylose content was reduced with increasing gamma ray (Bao *et al.*, 2005).

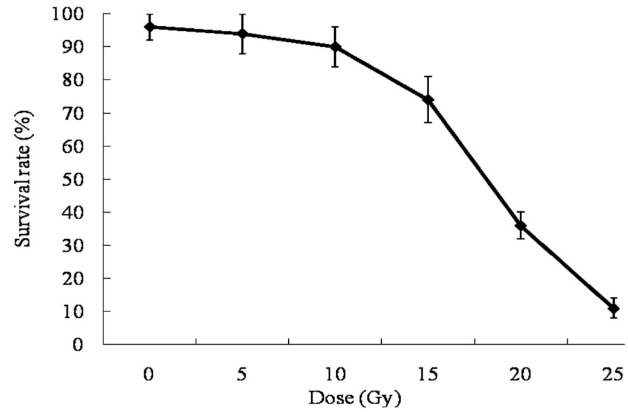

Fig. 1. Effect of proton ion beam doses on survival rate of Chinese yam tubers. Survival rate is defined as the number of seedlings from the irradiated tubers divided by the number of seedlings from non-irradiated tubers. Vertical bars indicate SE (n=3).

Table 2 shows gelatinization properties of starch determined by DSC. DSC parameters recorded onset temperature (T*o*), peak temperature (T*p*), conclusion temperature (T*c*), gelatinization range (R, T*c*-T*o*), enthalpy of gelatinization (ΔH), and peak height index (PHI). The gelatinization temperature and enthalpies associated with gelatinization endotherms was caused by increasing proton beam irradiation. Onset temperature in tuber starches with 5 to 25 Gy was ranged 73.2 to 74.6℃. Onset temperature was lower in starches extracted from proton beam irradiation than in the control. Otherwise, peak temperature shows opposite results against increasing proton

Table 1. Effects of proton beams on wavelength at maximum absorption (λmax), blue value of proton beams on starch and amylose contents in Chinese yam flours.

Dose (Gy)	λmax (nm)	Blue value (at 680 nm)	Starch content (%)	Amylose content (%)
0	591	0.22	84.7	13.2d
5	603	0.27	84.6	13.6cd
10	611	0.29	85.7	14.1c
15	613	0.35	85.4	15.2bc
20	613	0.37	85.5	17.0b
25	618	0.38	85.6	17.8a

Different letters within each column indicate significant differences ($p < 0.05$).

Table 2. Effects of proton beam irradiation on thermal properties of Chinese yam flours determined by Differential Scanning Calorimeter (DSC).

Dose (Gy)	Gelatinization parameters					
	T*o*(℃)	T*p*(℃)	T*c*(℃)	ΔH_{gel} (J/g)	PHI	*R*
0	75.1a	84.9d	92.4b	12.4	0.72	17.3a
5	74.6b	85.5c	92.3b	12.0	0.78	15.4b
10	74.3b	85.8c	92.5b	11.6	0.91	12.8c
15	73.7c	86.7b	92.4b	11.3	1.06	10.7d
20	73.5c	87.5a	93.0a	10.5	1.08	9.7e
25	73.2d	87.9a	92.2b	10.1	1.33	7.6f

Different letters within each column indicate significant differences ($p < 0.05$). T*o*, onset temperature; T*p*, peak temperature; T*c*, conclusion temperature; R, gelatinization range (T*c*-T*o*); Δ*H*, enthalpy of gelatinization (based on starch dry weight); PHI, peak height index Δ*H* gel/(T*p*-T*o*).

Table 3. Effects of proton beams on pasting properties of Chinese yam flours determined by Rapid Visco Analyser.

Dose (Gy)	Pasting time (min.)	Pasting temp. (℃)	Viscosity (RVU)				
			PKV	HPV	CPV	Breakdown	Setback
0	4.9	94.4a	3,811a	3,018a	4,193a	793a	382a
5	4.8	94.1a	3,769a	3,011a	3,975a	758b	206c
10	4.6	94.2a	3,617b	2,973b	3,934a	644d	317b
15	4.5	93.4b	3,503c	2,894b	3,261b	609e	-242d
20	4.5	91.7c	3,419d	2,753c	3,201b	666c	-218c
25	4.4	90.6d	3,374e	2,705d	3,118c	669c	-256e

Data are means of three determinations. Different letters within each column indicate significant differences ($p < 0.05$).

beam irradiation. The more proton beam irradiation was increased the more peak temperature in tuber starches was also increased. The enthalpies of gelatinization in starches reflect the loss of molecular order (Cooke and Gidley, 1992) and gelatinization temperature is also considered a parameter of crystallite perfection (Tester and Morrison, 1990). The enthalpies of gelatinization in tuber starches were slightly reduced by increasing proton beam doses as compared to the control. Gelatinization range was significantly reduced by elevated proton beam doses. Gelatinization temperature range in tuber starch irradiated with 25 Gy was 9.7℃ lower than that of control. With the analysis of RVA, seven major parameters of starch pasting properties, peak viscosity (PKV), hot pasting viscosity (HPV), cool pasting viscosity (CPV), setback (CPV minus PKV), breakdown (PKV minus HPV), peak time, and pasting time were significant decreased with the increasing

Table 4. Changes of X-ray diffraction data of Chinese yam starches as affected by different proton beam dose.

Dose (Gy)	Crystallinity (%)	Diffraction peaks at 2θ values			
		6°	17°	18°	23°
0	34.4a	6.2	17.3	18.1	23.0
5	37.2b	6.4	16.5	18.7	23.0
10	38.3c	6.0	16.3	18.5	23.1
15	38.9c	6.3	16.4	18.4	23.3
20	39.2d	6.3	16.3	18.8	23.1
25	39.9e	6.1	16.5	18.3	23.4

dose (Table 3).

The pasting time in tuber starch irradiated with proton beam was 0.5 min shorter than that of the control. Pasting temperature ranged 90.6 to 94.1℃. Pasting temperature of tuber starch was decreased with increasing proton beam. Peak viscosity (PKV), hot pasting viscosity (HPV), cool pasting viscosity (CPV), setback (CPV minus PKV), breakdown (PKV minus HPV), peak time, and pasting time were correlated negatively with increasing proton beam dose. These changes in pasting properties were also referred to the breakage of starch granules caused by proton beam irradiation (Yu and Yang, 2007). The crystallinity of tuber starch was significantly affected by increasing proton beam dose (Table 4). The crystallinity degree of tuber starch irradiated with proton beam ranged from 37.2 to 39.9%. Crystallinity of irradiated starches was 2.8 to 5.5% higher than that of the control. Increased crystallinity of starches determined by X-ray diffraction techniques has been reported in wheat (MacArthur and D'Appolonia, 1984) and rice (Wootton *et al*., 1988) starches. It was also reported that the decrease of ΔH in irradiated potato starch induced a decrease in crystallinity (Ciesla and Eliasson, 2002). Consequently, the proton beam application causes a reduction viscosity of Chinese yam starch. It can be concluded from the present study that proton beam irradiation causes physicochemical changes of starch properties in Chinese yam.

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