Formation of boiling-stable resistant cassava starch using the atmospheric argon-plasma treatment

Formação de amido resistente de mandioca estável a ebulição usando o tratamento com plasma de argônio atmosférico

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ABSTRACT

In the current study, structural, morphological properties and in vitro digestibility of argon-plasma treated starches were characterized. Granular cassava starch was treated by an atmospheric DBD Plasma device at 4-9 kV input for 0-40 min. Structural properties of treated sample were characterized using a FTIR measurement. The degree of cross-linking was reached at 8 kV and 10 min of treatment and it was 2-times higher than that of raw starch. Under scanning electron micrographs, treated granules were melted and linked to each other under plasma treatment. Besides, under enzymatic hydrolysis, a rough surface was found in both non-treated and treated granules. However, treated sample showed enzymatic erosion resistance than that of raw starch. Actually, the positive correlation between the degree of cross-link and resistant starch was found. Furthermore, the increase of heat-stable DCL leading to the formation of boiling-stable resistant starch.

Keywords: Argon-plasma. Cassava starch. Cross-linking. Resistant starch.

RESUMO

No presente estudo, propriedades estruturais, morfológicas e de digestibilidade *in vitro* de amidos tratados com plasma de argônio foram caracterizadas. O amido de mandioca granulado foi tratado por um dispositivo de Plasma DBD atmosférico na entrada de 4-9 kV por 0-40 min. As propriedades estruturais das amostras tratadas foram caracterizadas usando FTIR. O grau de reticulação foi alcançado em 8 kV e 10 min de tratamento e foi 2 vezes maior do que o do amido sem tratamento. Sob microscopia eletrônica de varredura, os grânulos tratados foram fundidos e ligados um ao outro sob tratamento com plasma. Além disso, sob hidrólise enzimática, foi encontrada uma superfície rugosa em ambos grânulos, tratados e não tratados. No entanto, a amostra tratada mostrou resistência enzimática quando comparada ao amido sem tratamento. Na verdade, a correlação positiva entre o grau de ligação cruzada e o amido resistente foi encontrada. Além disso, o aumento da DCL estável ao calor leva à formação de amido resistente ao aquecimento.

Palavras-chave: Plasma de argônio. Amido de mandioca. Cross-linking. Amido resistente.

INTRODUCTION

Cassava starch has been widely utilized in foods (James BeMiller; Roy Whistler, 2009). However, in industrial applications and nutrition, raw starch has some disadvantages. These shortcomings could be improved by various treatments that include chemical, physical or enzymatic routes (Steve, 2005). Resistant starch (RS) was a wide and diverse range of materials and it has four types exist (RS1–RS4) (Nugent, 2005). RS had functions of both probiotic and symbiotic (Brown, 1997; Wang, 1999). RS was a type of dietary fiber and RS has various benefits to human such as colonic health with a smaller impact on lipid and/or glucose metabolism. RS was fermented in the large intestine resulting in the production of short chain fatty acids that increased colonic blood flow, lower luminal pH, and prevented the development of abnormal colonic cell population (Topping & Clifton, 2001). RS ameliorated the symptoms of inflammatory bowel diseases such as ulcerative colitis. RS showed positive effects on lipid, glucose metabolism (including glycaemic index), energy expenditure and macronutrient oxidation (Nugent, 2005).

Theoretically, chemical, physical and enzymatic treatments could be used to prepared resistant starch. Plasma treatment, at an argon atmosphere, was a non-chemical technique for modification of starch properties. Under atmospheric pressure, the plasma was generated by dielectric barrier discharge (DBD). Plasma contained energetic particles such as electrons, neutrons, photons, atoms, molecules, ions, and free radicals that affected the changes in starch properties such as degree of cross-linking. The generation of cross-linkages (C-2 hydroxyl groups) followed the mechanism of previous study (Zou, Liu & Eliasson, 2004). These authors reported a novel for starch modification using glow discharge plasma. Deeyai et al. (2013) modified the cassava starch by the argon-plasma treatment to increase the degree of cross-linking. In those studies, starch was intensively crosslinked without the assistance of other chemicals.

Actually, there was not so much published papers focus on resistant starch preparation using plasma treatment. The subject of this current study was a preparation of boiling-stable resistant cassava starch under atmospheric argon-plasma treatment. Besides, chemical, structural, morphological properties and in vitro digestibility of atmospheric argon-plasma treated starches were characterized.

MATERIAL AND METHODS

a) Atmospheric argon-plasma treatment

Cassava starch (5.0 g) was spread and blended regularly on a glass board, which was put on a cathode inside the plasma chamber. A DBD device, which was made by Dynamic Plasma Laboratory, Ho Chi Minh City University of Technology and Education and was designed following a previous paper (Deeyai et al., 2013), was used to generate the plasma medium throughout our experiments for 0, 5, 10, 20, 30 and 40 min. Plasma was generated in an argon gas-phase (flow rate of 5.0 ml/min) by applying a high potential difference between two electrodes. For each reaction time above, samples were treated at input power supply: (i) a constant electric current of 1.0 A; (ii) electric intension of 4, 5, 6, 7, 8 and 9 kV.
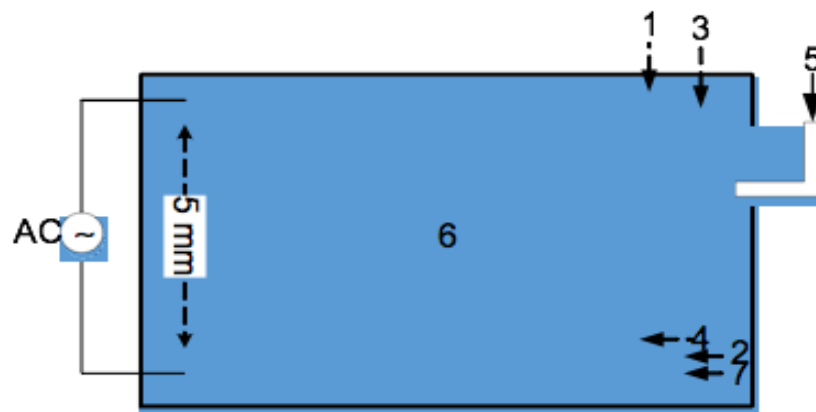
Figure 1. Construction of Dielectric Barrier Discharge Plasma device.

(*1. Cathode; 2. Glass board; 3. Dielectric; 4. Starch sample; 5. argon inlet; 6. Plasma environment; 7. Anode*)

b) Structural property

Structural property and the degree of cross-linking were determined by Fourier Transform Infrared Spectroscopy (FTIR) measurement. A FTIR-8400S device (Shimadzu, Japan) recorded FTIR spectra. The absorbance spectral was computed between 4000 and 400 cm^{-1} at room temperature and at a resolution of 2 cm^{-1}. All FTIR spectra were carried out in the transmittance mode and were converted to absorbance mode by using the equation: Absorbance=2-log_{10}%Tranmittance. The degree of cross-linking (DCL) is calculated from : (method A) the subtraction of the relative intensities of 1016 cm^{-1} and 1647 cm^{-1}; (method B) the subtraction of the relative intensities of 1016 cm^{-1} and 3282 cm^{-1} (Deeyai P. et al., 2013).

c) In vitro starch digestibility

The analytical method of Brumosky and Thomson (Brumovsky & Thompson, 2001), with a slight modification (Trinh et al., 2012), was used to determine *in vitro* starch digestibility. Two gram of Pancreatin (Sigma-Aldrich) was dissolved in 24 ml of ditilled water (D.W) and was stirred for 10 min. Enzyme suspension was centrifuged (10 min, 1500×g) and then 20 ml of supernatant was mixed with 3.6 ml of D.W and 0.4 ml of 300 L AMG (Amyloglucosidase, Novozymes). This solution was kept in waterbath (37 °C) for 15 min. Starch (30 mg) was put in a 2-ml microtube with a glass bead. Next, 0.75 ml of sodium acetate buffer (pH 5.2) was added and the tube was stored in a shaking incubator (37 °C, 10 min, 240 rpm). After adding 0.75 ml of the prepared enzyme solution, the microtube was shaken continuously. The enzymatic reaction was stopped after 10 or 240 min by boling for 10 min and then sample was left for cooling to ambient temperature. GOD-POD kit (BCS, Anyang, Korea) was used to determine the glucose content in the supernatant obtained by centrifugation (5 min, 5000×g). Based on the rate of hydrolysis, starch fractions were classified. To determine the

boiling-stability, starch samples were not gelatinized or were pre-gelatinized (boiled in 10 min) in sodium acetate buffer before *in vitro* digestibility measurement.

d) Morphology properties

Morphological property of starch was described based on SEM-1400 device (Jeol, Japan). Starch sample (0.4 g) was suspended in phosphate buffer (20 ml, 0.04 M, pH 7.0). Following the procedure of *in vitro* starch digestibility measurement above, a mixture of alpha amylase and pancreatin were added. Enzymatic hydrolysis was done for 10 min. Then, 95% ethanol (40 ml) was used to stop the reaction. Then, the mixture was washed two time with distilled water (20 ml) and was centrifuged (5000xg, 10 min) following by air-forced drying (40°C, 24 h).

e) Statistical analysis

All experiments were performed in triplicate. Average value and the standard deviation were reported. Analysis of variance (ANOVA) was conducted, and the mean separations were analyzed by Duncan's multiple-range test ($p < 0.05$). All statistical analyses were conducted using SPSS software (Ver. 17.0, SPSS, Chicago, Ill., U.S.A.). For regression analysis, trend-lines were conducted using Microsoft Excel 2016 software.

RESULTS AND DISCUSSION

a) Structural properties

FTIR spectroscopy was used to describe the organization and structural changes in starch (Figure 2). The region 800-1500 cm^{-1} showed highly overlapping and complex spectra; mainly caused by the vibration of the glucose molecule. The peak at 929 cm^{-1} was probably due to the glycosidic linkages in starch. In previous studies, this peak was seen at 924 or 930 cm^{-1}. These differences could be explained by the presence of α-1,6 linkages of amylopectin that shifted the band to higher wavenumber (Deeyai P. et al., 2013). The peak at 1016 cm^{-1} was due to the C-O-H bending mode, whereas the peak at 1163 cm^{-1} was attributed to C-O and C-C stretching. The band at 1242 cm^{-1} was attributed to the C-O-H deformation modes and CH_2OH (side chain) related mode (Deeyai et al., 2013; Ramazan et al., 2002). The vibration of the water molecule, absorbed in non-crystalline region of starch, was reflected at 1630 cm^{-1} peak. The region between 2800-3000 cm^{-1} and 3000-3600 cm^{-1} respectively was caused by the vibration of CH_2 deformation and O-H stretching. The peak at 3282 cm^{-1} indicated a weak bond of the water molecule (Deeyai et al., 2013; Ramazan et al., 2002). Besides, the degree of cross-linking was calculated from absorbance relative intensities of 1016 cm^{-1} - 1647 cm^{-1} (method A) and 1016 cm^{-1} - 3282 cm^{-1} (method B) (Deeyai et al., 2013; Ramazan et al., 2002). Thus, comparing to previous studies, there was no peak lost or new peak found in our study indicating there were no foundation of the new chemical group in starch molecule.
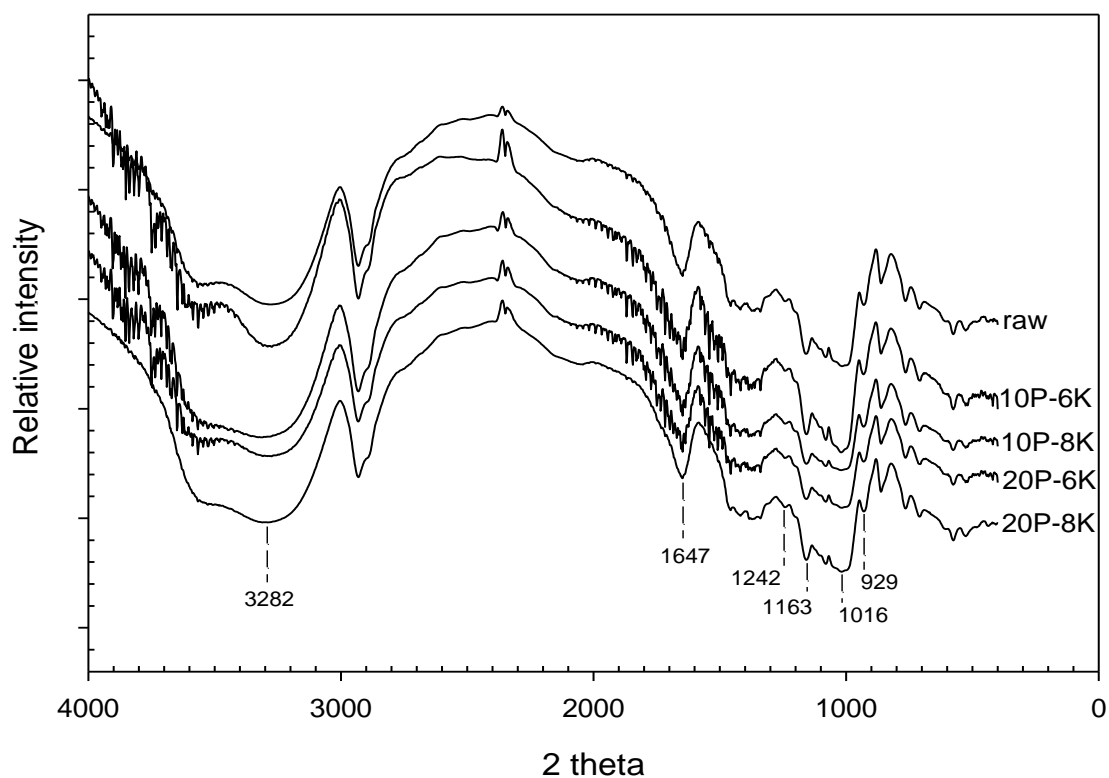
Figure 2. FITR spectra of raw and plasma treated starches

The relationship between treatment conditions (electric intension and treatment time) and DCL was shown in Figure 3. Obviously, both treatment time and input electric intension affected on DCL. The result from a two-way Anova calculation showed that both treatment time and input electric intension are the main factors affecting to DCL ($p<0.001$). Furthermore, the interaction of these factor is significant ($p<0.001$). Base on method A and B of calculation, the highest DCL was found on approximately 10 min and 8 kV. Out of these ranges, DCL value decreased. Basically, as argon was the discharge gas, the decomposition of water in starch sample would produced a few O-containing species. Unfortunately, the continuous argon flow in DBD device could gradually evaporated moisture out of samples. Besides, the formation of new crosslinkages resulted in the production of water molecules. These could be absorbed and acted as a sink of OH radical (*OH), which enhanced DCL value (Deeyai et al., 2010). Deeyai et al. (2013) stated low moisture content favoured to the increasing of DCL. Plasma just attacked on the surface or subsurface of starch and thus may reached saturation at the latter stage. At high water concentration, there was detrimetal effect on gas ionization and atomization. Thus, moisture content of sample changed leading to the change of DCL during treatment time. Furthermore, voltage applied and treatment time affected on the effect of cold plasma. Power applied for plasma generation resulted in the formation of active species, free radicals (*OH), concentration of ions. These are affected not only on the formation of crosslinkages but also the cleavage of glycosidic bonds, which cause the decreasing of DCL (Rohit et

al., 2016). Thus, maybe in our study, higher voltage could produced further glycosidic depolymerations.

Base on method A or B, the highest DCL, 1.17 or 1.00, was 2.0 or 1.7-times higher than that of raw starch. Absorbance intensity (Y-value) of 1016 cm^{-1} and 3282 cm^{-1} peaks showed positive correlation to DCL values (X-value) following on equations: Y =1109×X+0.345 (R^2=0.92) and Y =0.641×X–0.027 (R^2=0.93). These correlations indicated, in our study, intensity of these peaks are well described for DCL calculations.
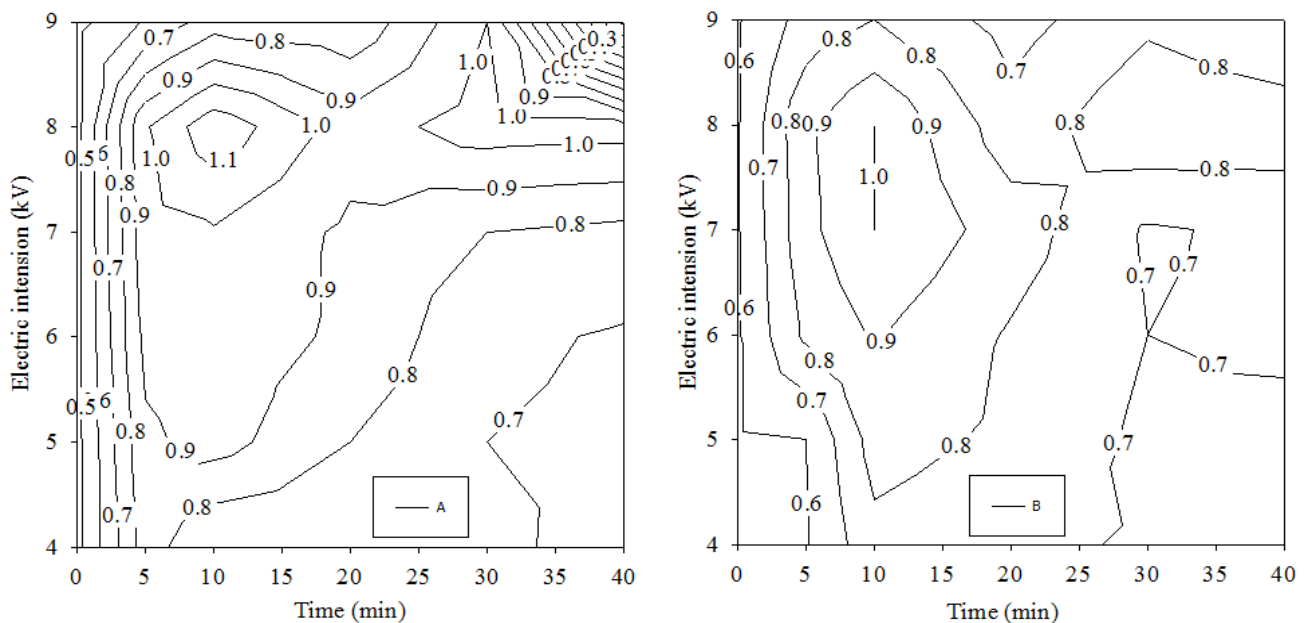

Figure 3. Effect of treatment time (min) and input electric intension (kV) on the degree of cross-linking of starches (Contour plot).

b) Morphological properties

SEM images of starches were shown in Figure 4. The SEM micrograph of raw starch showed typical granules of spheroid forms, 10-25 µm in size (Figure 4A). Under argon-plasma treatment granule size of samples did not significantly change. However, driven through gelatinization phase, the starch granules were melted and linked to the neighboring granules (Figure 4B) (Deeyai et al., 2010). Futhermore, under plasma treatment, these authors stated the rims of the starch granules were lost their sharpness, which did not find in the current study. Theoretically, five patterns of enzyme hydrolysis have been characterized: pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single holes in individual granules, and surface erosion (Evers, 1979). Scanning electron micrograph micrographs of samples showed that enzymatic erosion occurred mainly at the surface, causing the rough surface of the granules (Figure 4C and 4D, arrows). This observation was reported in a previous paper (Uthumporn, Zaidul & Karim, 2010). The surface of plasma treated-granules were rougher than that of raw sample. So, comparing to untreated starch, the formation of DCL in plasma treated-starch resulted in the higher resistance of enzymatic hydrolysis.

c) In vitro digestibility

In vitro digestibility of non-boiled starches and boiled starches were shown in Table 1. In both pre-treatments, raw starches contained highest RDS and lowest RS content than others. Contrary, 10M-8K sample exhibited highest RS and lowest RDS. Under boiling, due to the gelatinization, RDS increased and RS of samples significantly decreased. The difference of RS content between non-boiled and boiled starches (ΔRS, %) indicated that RS of 10M-8K sample was lowest lost compared to raw and other samples. The relationship between RS (Y-value) content and DCL (X-value, base on method A of DCL calculation) of samples was calculated as equations: Y (non-boiled sample) $=47.48X+3.968$ ($R^2=0.966$) and Y (boiled sample)$=67.33X-25.79$ ($R^2=0.965$). Thus, under plasma treatment, the formation of cross-linkages resulted in high RS content. Besides, these results indicated that the cross-linkage are boiling-stable one.
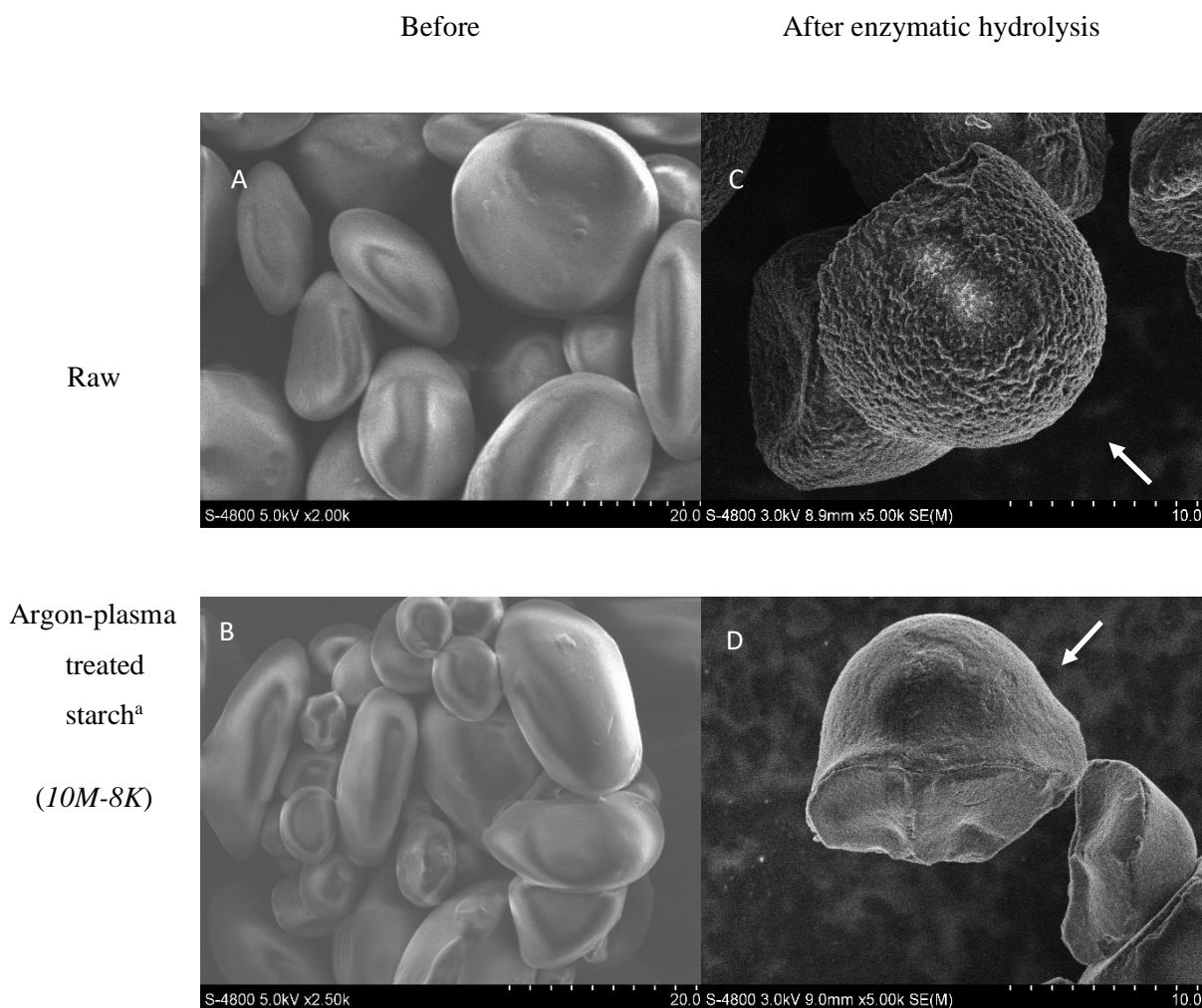

Figure 4. Scanning electron micrograph of raw and plasma treated starch under enzymatic hydrolysis

[a]M: treatment time (min); K: electric intension (kV)

Table 1. *In vitro* digestibility of starches[a].

Pre-treatment	Sample[1]	RDS (%)	SDS (%)	RS (%)	ΔRS (%)
non-boiled	raw	49.5 ± 0.3[a]	23.0 ± 0.7[a]	27.4 ± 0.4[e]	-
	10M-6K	32.8 ± 1.2[d]	15.4 ± 0.9[d]	51.8 ± 1.1[b]	-
	10M-8K	26.6 ± 0.2[e]	12.5 ± 0.7[e]	61.0 ± 0.5[a]	-
	20M-6K	40.2 ± 1.0[b]	17.0 ± 0.3[b]	42.9 ± 1.2[d]	-
	20M-8K	36.3 ± 1.2[c]	16.5 ± 0.1[c]	47.1 ± 1.3[c]	-
boiled	raw	57.4 ± 0.1[a]	35.2 ± 0.3[a]	7.5 ± 0.0[e]	-19.9[e]
	10M-6K	38.9 ± 0.4[d]	13.8 ± 0.0[d]	41.9 ± 0.5[b]	-9.9[b]
	10M-8K	34.7 ± 0.3[e]	13.0 ± 0.2[e]	52.3 ± 0.2[a]	-8.7[a]
	20M-6K	48.3 ± 0.2[b]	22.5 ± 0.5[b]	29.2± 0.4[d]	-13.7[d]
	20M-8K	45.1 ± 0.3[c]	19.6 ± 0.3[c]	35.4 ± 0.1[c]	-11.7[c]

RDS: rapidly disgestible starch; SDS: slowly digestible starch; RS: resistant starch; ΔRS: the difference of RS content between non-boiled and boiled-starches; M: treatment time (min); K: electric intension (kV).

[a]The values with different superscripts in a same column (non-boiled and boiled samples were seperately calculation) are significantly different ($P < 0.05$)

CONCLUSIONS

Under appropriate conditions of argon-plasma treatment (10 min, 8 kV), the highest degree of cross-linking was found. Both treatment time and electric intension effect on the formation of cross-linkages in treated sample. Plasma treated and raw starches exhibited the difference in morphological properties under enzymatic hydrolysis. Treated starch became highly resistant to enzymatic hydrolysis leading to the increasing of resistant starch content. Furthermore, new cross-linkages were heat-stable reflecting on high RS content under boiling treatment. The current study introduced an innovative, simple and non-chemical technique for boiling-stable resistant starch formation.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

FUNDING

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