CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

TAPIOCA RESISTANT STARCH PRODUCTION AND ITS STRUCTURAL PROPERTIES UNDER ANNEALING AND PLASMA TREATMENTS

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Article history:
Received:
05.09.2016
Accepted:
28.12.2016

Keywords:
Argon-plasma;
Digestibility;
Cross-linking;
Crystallinity;
Starch

ABSTRACT

Structure of tapioca starch (raw) was reorganized under annealing treatment during 12-72 h. Then, raw and annealed starches were modified by Argon-plasma treatment (P sample) under atmospheric pressure at constant parameters (137.5 V, 1 A, 10 min). Treated samples were characterized degree of cross-linking (DCL), degree of relative crystallinity (DRC), and *in vitro* digestibility using Fourier Transform Infrared Spectroscopy, X-ray difractometry, respectively. Results showed that DCL increased during both annealing and plasma treatments. Besides, DRC of annealed starches was higher than that of raw, but it was slightly reduced under plasma treatment. Furthermore, A-type crystal pattern of samples was remained during treatments. Annealing resulted in a significant increase of resistant starch (RS) fraction and further plasma treatment continuously enhanced RS content from 30.5 % of raw starch to around 71.0 % of 72h-P (dual treated) sample. After boiling, RS fraction of the starch was (decreased by 4%) 67%. Thus, dual treatment (annealing and plasma) is a novel and potent method of both RS and boiled RS production.

1. Introduction

Starch, which is one of a major component in plant foods, is a main dietary source of carbohydrate in human nutrition. Tapioca root has a high content of starch with a wide range of applications. Tapioca starch is remarkable for food industry because of its odorless, paste clarity, and stickiness. However, raw tapioca starch has some disadvantages for industrial applications and in nutritional aspects such as insolubility in water, loss of viscosity, and especially high content of rapid digestible starch (RDS) under cooking process. These shortcomings of raw tapioca starch could be improved by various modification methods which include chemical or physical routes (Deeyai et al., 2013).

There are three fractions of starch: (a) rapidly digestible starch (RDS) consists mainly of amorphous and dispersed starch, found in high amounts in starchy food cooked by moist heat; (b) slowly digestible starch (SDS) is completely digested in the small intestine; and (c) resistant starch (RS) escapes digestion in the small intestine. RS fractions fermented by the colonic flora, resulting in short-chain fatty acids. RS has some beneficial effects such as a component of dietary fiber, prevention of colonic cancer, hypoglycemic effects, hypocholesterolaemic effects, a prebiotic,

reduction of gallstone formation and inhibition of fat accumulation (Sajilata, 2006)

Annealing treatment is a "physical modification of starch slurries in water at temperature below gelatinization". Annealed starch could be more difficult hydrolyzed by amylase enzymes than raw starch (Collado and Corke, 1999; Tester and Debon, 1999). Besides, plasma treatment is a physical treatment which is used to modify starch properties. In the plasma environment, energetic particles (electrons, atoms, molecules, ions and free radicals) attach to starch molecules and induce changes in properties. Previous publications reported the formation of cross-linking and resistant starch (RS) under argon-plasma treatment (Deeyai et al., 2013; Trinh et al., 2014). There are previous studies of starch modification focusing on structural properties and digestibility under annealing or plasma treatment (Tester and Debon, 1999; Trinh et al., 2014). However, there is a lack of reporting on dual treated (annealing and argon-plasma) starch for RS production. In this study, tapioca starch was treated by annealing and argon-plasma. Structure and *in vitro* digestibility changes of starches were investigated.

2. Materials and methods

2.1. Annealing treatment of starch

Starch was suspended in distilled water (1:3, $^{w}/_{w}$) and then incubated at 50^{o}C for 12-72 hours (Tester and Debon, 1999) Starch suspension was subsequently dried at 40^{o}C for 24 hours to reach the final moisture of around 11 %.

2.2. Plasma treatment of starch

Starch sample (5.0 g) was spreaded (and blended regularly) on a glass plate which was put on an electrode inside a DBD plasma divice (Figure 1). The DBD (Dielectric Barrier Discharge) plasma device was used to generate the plasma environtment throughout our experiments. Plasma was generated in an Argon gas-phase (flow rate of 5 ml/min) by applying a high potential difference between two electrodes. Starch was treated following the previous methods [1,5] under atmospheric pressure at constant parameters (137.5 V, 1 A, 10 min).

2.3. Fourier Transform Infrared Spectroscopy (FTIR) and ratio of ordered/amorphous structure

FTIR spectra were recorded using a FTIR-8400S (Shimadzu, Japan). The absorbance spectra were collected from 400 to 4000 cm^{-1} at room temperature, and at a resolution of 2 cm^{-1} (in at least triplicate) (Deeyai et al., 2013).

The ratio of ordered (α-helix)/amorphous structure was identified by the ratio of the height of the bands at 1039/1014 (Jeroen et al., 1995)

2.4. X-ray diffractometry

XRD was determined using a powder X-ray diffractometer (Model D8 Advance, Bruker, Germany). The operating conditions were 40 kV and 40 mA with Cu-Kα radiation of 0.15406 nm (Nickel filter; time constant, 4 s). Each scan was performed from 3 to 30◦ (2θ) (Trinh et al., 2014) DRC was calculated using the equation DRC = *Ac*/ (*Ac* + *Aa*), where *Ac* is the area of crystalline portion and *Aa* is the area of amorphous portion, according to the method of Nara and Komiya, 1983, with peak-fitting software (Originversion 7.5, OriginLab, Northampton, Mass., U.S.A.).

2.5. In vitro 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 ^{o}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 (pH5.2) was added and the tube was stored in a shaking incubator (37 ^{o}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. GOD-POD kit (BCS, Anyang, Korea) was used to determine the glucose content in the supernatant obtained by centrifugation (5 min, 5000×g). Starch *in vitro* digestibility was determined according to the method of Brumovsky and Thompson (2011).

3. Results and discussions

3.1.Fourier Transform Infrared Spectroscopy (FTIR) and ratio of ordered / amorphous structure

FTIR spectra of starches were shown on Figure 2. Between samples, there were not significant changes in shape of spectra and position of peaks. However, the height of peaks significantly changed by both annealing and plasma treatments indicating the stronger vibration of chemical groups. Besides, peak at 1039 cm^{-1} was sensitive to the amount of ordered (α-helix) starch and the wavelength of 1014 cm^{-1} was the characteristic of amorphous starch. Thus, α-helix/amorphous structure ratio (ORD) (Table 1) was identified by the proportion of the height of the wavelength at 1039 to 1014 cm^{-1} (Jeroen et al., 1995). In the presentstudy, this ratio was not significantly different between samples treated by annealing and plasma technique. The similar result was reported by Deeyai et al. 2013.

FTIR spectra (400-4000 cm^{-1}) of starches (Figure 2) in four main regions were as follows: the fingerprint region (600-1500 cm^{-1}), the double-bond region (1500-2000 cm^{-1}), the triple-bond region (2000-2500 cm^{-1}), and the X-H stretching region (2500-4000 cm^{-1}. It was not easy to (?) assign the exact band at the 600-1500 cm^{-1} because of highly overlapping and complex spectra. The skeletal modes of pyranose ring were observed at around 537 cm^{-1}. The peak at 764 cm^{-1} was the C-C stretching. The skeletal modes of α-1,4 glycosidic linkage (C-O-C) were found at around 930 cm^{-1} (Ramzan et al. 2002). The C-O-H bending occured at 1094 cm^{-1}. The absorption peak at 1163 cm^{-1} was due to the coupling mode of C-O and C-C stretching. The absorption band at 1241 cm^{-1} was attributed to the CH_2OH (side chain) related mode. The C=C and C=O stretching were observed in the 1500-2000 cm^{-1} region (Deeyai et al., 2013).

Depending on the type of C=O bond, carbonyl stretching occurred in the 1650-1830 cm^{-1}. C=C stretching was in around 1650 cm^{-1} but this band was often absent for symmetry or dipole moment reasons. The region above 2000 cm^{-1} was the vibration of metal carbonyls (Deeyai, et al. 2013).

The peak at 1635 and 3300 cm^{-1} reflected a tight bond and a weak absorption of water molecules. The degree of cross-linking (DCL) could be identified from the relative intensity of these two peaks with the C-O-H peak at around 993-1094 cm^{-1} (Deeyai et al., 2013).

In this study, during annealing treatment, DCL of treated starch continuously increases compared with raw. Moreover, further argon-plasma treatment significantly enhances DCL of raw and annealed starches to reach the maximum of 71h-P sample (both DCLa and DCLb values). Thus, both treatments caused the increase of DCL in samples (Table 1).

3.2. X-ray diffractometry (XRD) and degree of relative crystallinity (DRC)

XRD patterns of starch samples were presented in Figure 3. The diffractograms of the raw, annealing treated and plasma treated samples werenot significantly different. All samples showed strong diffraction at 2 theta of about 15.4 and 23.6^o and unresolved doublet at 16.6 and 18.3^o, which was close to the A-type structure (Table 1) (Bogracheva , 2001) DRC of starches was shown in Table 1. Annealing treatment did not change DRC of the starch. However, under plasma treatment, intensity of peaks was weakened, especially in the case of 16.6 and 18.3^o peaks (Figure 3), leading the disruption of crystalline structure. In a previous study, Deeyai P. (2013) stated that the change of DRC is rather small under plasma treatment and it is not easy to be detected by XRD (Deeyai et al., 2013).

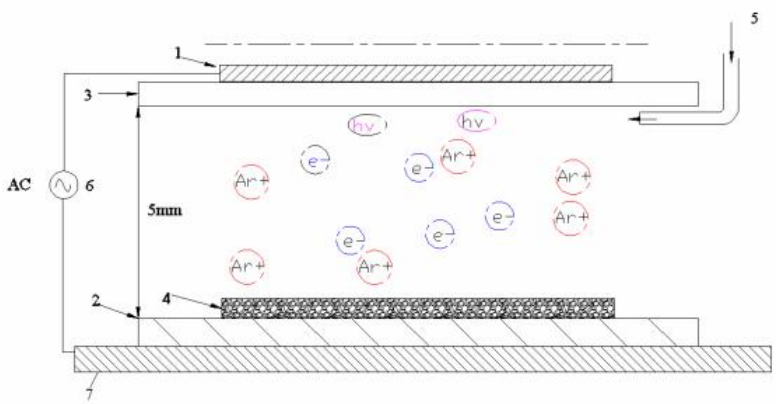

Figure 1. DBD plasma device: 1. Cathode, 2. Glass plate, 3. Dielectric material, 4. Starch sample, 5. Argon input, 6. Plasma environment, 7. Anode

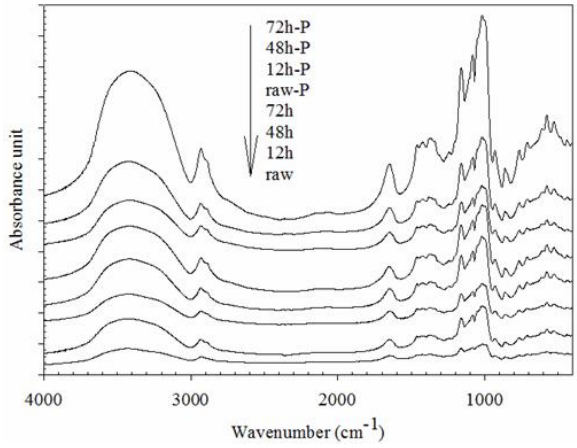

Figure 2. FTIR spectra of starches[*]; [*]12h, 48, 72h was annealed starches, which was incubated at 50 ^{o}C for 12-72 hours; P: plasma treated starch

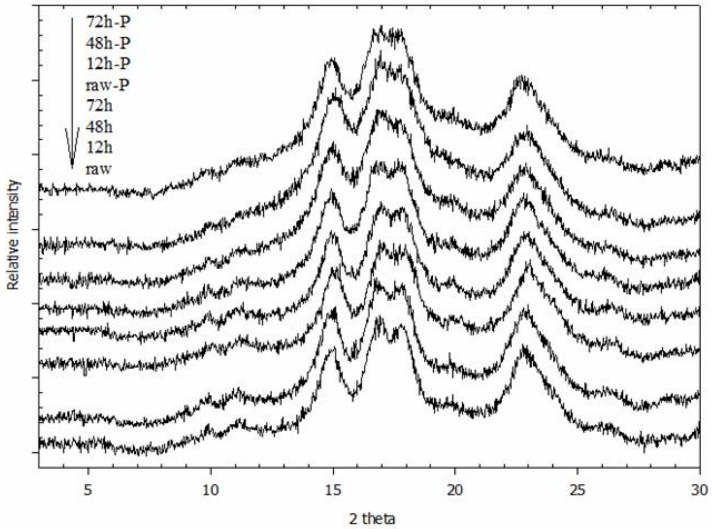

Figure 3. X-ray diffractograms of starches; [*]12h, 48, 72h was annealed starches, which was incubated at 50 ^{o}C for 12-72 hours; P: plasma treated starch

Table 1. Degree of cross-linking (DCL), α-helix/amorphous ratio (ORD), degree of relative crystallinity (DRC), and crystal pattern of starches*.

Sample	DCL		ORD	DRC	Crystal pattern
	997-1649 (DCLa)	997-3421 (DCLb)			
raw	0.08 ± 0.01^{h}	0.03 ± 0.00^{g}	0.89 ± 0.03^{a}	38.88 ± 0.6^{f}	A
12h	0.20 ± 0.05^{g}	0.07 ± 0.01^{f}	0.88 ± 0.02^{b}	45.67 ± 0.9^{c}	A
48h	0.27 ± 0.03^{e}	0.08 ± 0.01^{e}	0.86 ± 0.02^{c}	50.90 ± 0.7^{b}	A
72h	0.37 ± 0.06^{d}	0.17 ± 0.04^{b}	0.86 ± 0.00^{c}	57.21 ± 0.7^{a}	A
raw-P	0.37 ± 0.03^{d}	0.10 ± 0.03^{d}	0.88 ± 0.01^{b}	32.86 ± 0.8^{h}	A
12h-P	0.40 ± 0.06^{c}	0.16 ± 0.01^{c}	0.88 ± 0.02^{b}	34.62 ± 0.5^{g}	A
48h-P	0.54 ± 0.05^{b}	0.17 ± 0.02^{b}	0.85 ± 0.03^{c}	41.33 ± 0.7^{e}	A
72h-P	1.08 ± 0.06^{a}	0.36 ± 0.02^{a}	0.86 ± 0.02^{d}	43.64 ± 0.7^{d}	A

*superscipt in each column indicate the significant difference (P<0.05).

Table 2. In vitro digestibility* of non-boiled and boiled** starches.

Sample	Non-boiled			Boiled		
	RDS (%)	SDS (%)	RS (%)	bRDS (%)	bSDS (%)	bRS (%)
raw	46.1 ± 1.4^{a}	23.3 ± 1.5^{g}	30.6 ± 0.2^{h}	48.9 ± 0.2^{a}	44.6 ± 0.5^{a}	6.5 ± 0.3^{h}
12h	17.0 ± 0.1^{f}	39.6 ± 0.4^{a}	43.3 ± 0.5^{f}	32.5 ± 0.3^{b}	30.9 ± 0.6^{d}	36.6 ± 0.8^{f}
48h	19.7 ± 1.5^{c}	34.1 ± 1.0^{d}	46.3 ± 0.8^{e}	31.2 ± 0.5^{b}	28.9 ± 0.7^{e}	40.0 ± 0.9^{e}
72h	18.1 ± 0.5^{e}	25.7 ± 0.4^{f}	56.2 ± 0.3^{b}	30.7 ± 0.9^{b}	27.9 ± 0.4^{g}	41.5 ± 0.7^{d}
raw-P	20.2 ± 0.2^{b}	38.1 ± 1.4^{b}	42.8 ± 0.2^{g}	26.3 ± 0.8^{b}	37.5 ± 1.1^{b}	36.1 ± 0.4^{g}
12h-P	14.3 ± 0.3^{h}	37.7 ± 0.7^{c}	47.8 ± 0.4^{d}	21.3 ± 0.8^{c}	35.6 ± 0.9^{c}	43.1 ± 0.5^{c}
48h-P	18.3 ± 0.4^{d}	27.2 ± 0.8^{e}	54.5 ± 0.6^{c}	24.8 ± 0.1^{b}	28.6 ± 0.5^{f}	46.4 ± 0.6^{b}
72h-P	15.0 ± 0.6^{g}	14.2 ± 0.7^{h}	71.0 ± 0.3^{a}	13.3 ± 0.1^{c}	19.8 ± 0.4^{h}	66.9 ± 0.3^{a}

*superscipt in each column indicate the significant difference (P<0.05); **after stop reaction, starch sample was boiled for 15 min before digestibility measurement.

3.3. In vitro digestibility

In vitro digestibility was presented in Table 2. During annealing treatment, RDS reduced while SDS and RS levels increased. Furthermore, sample 72h-P contained 56.2% of RS, which was 25.6% higher than that of the raw. Some previous authors reported the crystalline "perfection" and the increaseof crystallinity was found in annealed starch (Trinh et al., 2014). Hyun-Jung Chung et.al. (2009) reported that annealing decreased SDS whereas the treatment increased RDS and RS levels in granular starches. By contrast, annealing decreased RDS and increased SDS, RS levels in gelatinized starches (Hyun-Jung Chung et al., 2009). In that study, the authors

concluded that a superior crystalline structure caused the resistance to enzymatic hydrolysis. Under plasma treatment, RDS was decreased while RS was increased significantly. The sample 72h-P reached RS content of 71.0 %, which was 14.8 and 40.4 % higher than that of 72h and raw samples, respectively.

Such an increase in RDS content and reduce in RS content were generally observed in boiled starches because the boiling destroyed the semi-crystalline structure of raw starch granules (Eliasson, and Gudmundsson, 2006) indicates the boiling-stable of this starch fraction. In the present study, boiled-RS of 72h-P was more than 60 % higher than that of raw. Hence, the dual treatment (annealing and plasma treatment) resulted in boiling-stable RS formation.

In this study, the relationship between RS-DCL and bRS-DCL were presented in these equations:

(a) RS= 35.961×DCLa + 34184 (R^2= 0.8429);
(b) bRS= 47.85×DCLa + 19.84 (R^2= 0.7633);
(c) RS= 110.36×DCLb + 33.336 (R^2= 0.9002);
(d) bRS= 14.63×DCLb + 19.456 (R^2= 0.7583).

Obviously, both RS and bRS levels correlate to the degree of cross-linking (DCL).

4. Conclusions

Under annealing and plasma conditions, structural properties of starch were changed, especially in the increase of DCL and DRC. These changes resulted in the increase of resistant starch fraction in the treated samples. Positive effects of dual treatment surpassed those induced by single treatment.Our results also showed that the physically dual modification was a useful and efficient route for RS and boiling-stable RS formation.

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