STRUCTURAL, FUNCTIONAL PROPERTIES AND IN VITRO DIGESTIBILITY OF MAIZE STARCH UNDER HEAT-MOISTURE AND ATMOSPHERIC-COLD PLASMA TREATMENTS

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Received: 21 April 2018; Accepted for publication: 12 September 2018

ABSTRACT

Maize starch is one of important materials widely used in food applications and other industries. However, natural properties of raw starch can not be suitable for processed foods. So, the modification of starch is very important. In this study, heat-moisture and atmospheric cold argon-plasma treatments were applied in maize starch; then, structural, functional properties and digestibility of modified starch was investigated. Raw starch was heated at 20, 25 and 30\% of moisture content. Subsequently, the samples were then treated under argon-plasma environment at fixed parameters (137.5 V; 1.0 A for 10 min). The samples were investigated on degree of cross-linking, degree of relative crystallinity (DRC), degree of hydrolysis using alpha-amylase, in vitro digestibility, changes in the hydration properties such as water absorbance index, swelling factor and water solubility index. Results showed that degree of cross-linking, DRC, resistant starch of samples significantly increased under heat-moisture and plasma treatments; especially, sample of 20\% heat-moisture contains 3-folded to 10-folded increase comparing to raw starch base on with or without pre-boiling process. Furthermore, water absorbance index and swelling factor decreased but water solubility index increased under plasma treatment.

Keywords: degree of relative crystallinity, digestibility, heat-moisture treatment, maize starch, plasma.

Classification numbers: 1.2.5, 1.4.2, 1.4.4

1. INTRODUCTION

In field of human nutrition, starch plays an important role in the supply of energy for the metabolism. In recent researches, the slowly digestible starch (SDS) and resistant starch (RS) have a positive impact on human health. In contrary to rapid digestible starch (RDS), RS resists to enzymatic hydrolysis in digestive tract resulting in low level of glucose absorbance. RS, a
type of fiber, is not digested in small intestine so it moves to colon and be fermented by gastrointestinal microbiota. RS causes the increase of fecal volume, produces of short-chain fatty acids or butyrate, which was investigated to prevent colon cancer. Furthermore, RS reduces blood cholesterol and triacylglycerol, reduce the accumulation of fat [1]. RS1 represents physically enclosed and inaccessible starches and is found in partially milled grains, seeds, and legumes. RS2 is native granular starch normally found in unripe bananas and raw potatoes, and can be easily digested after gelatinization. RS3 is the starch fraction formed through retrogradation after gelatinization, and RS4 is the chemically modified starch [1].

Heat-moisture is a physical modification of starch at a limited moisture content (< 35 %, dry basis) and at a defined temperature which is higher than solid-liquid phase transition temperature (Tg) [1]. Theoretically, heat-moisture treatment (HMT) results in the formation of crystals, re-crystallization, and perfection of crystal region in starch that makes change of X-ray crystalline pattern. Besides, HMT causes the change of functional properties such as increase of gelatinized temperature, be more susceptible to enzymatic hydrolysis, changed of solubility and swelling factor. All of these are based on the origin of starch and parameter of HMT [2, 3].

In cold-plasma technique, electrons induce the ionization to maintain the plasma environment. Furthermore, it causes the stimulation and dissociation of atoms/molecules resulting in radicals and others. Besides, cold-plasma environment is safe and low-heat producing for normal application [4]. Under atmospheric cold-plasma treatment, starch was modified to create new cross-linkages (starch-OH+OH-starch → starch-O-starch) [5, 6] which was reported as a novel method for the formation of resistant starch [7].

In recent years, there are many scientific papers reporting the application of HMT or plasma technique to modify starch. However, there is no much information of the combination of these. Thus, our study object to describe the change of structural, functional properties and in vitro digestibility of maize starch using dual treatment of HMT and argon-plasma.

2. MATERIAL AND METHOD

2.1. Heat-moisture treatment

HMT was basically followed of the method by Kulp K. [8] and Olu-Owolabi [9]. Starch (10 g) was adjusted to exactly 20, 25 and 30 % (db) by distilled water (DW) and mixed carefully. Then, samples were covered and balanced at ambient temperature for 24 h. Subsequently, contained samples were heated at 100 °C for 16 h in air-forced dryer. After treatment, the samples were left at ambient temperature for 2 h and then were dried at 45 °C for 24 h to reach final moisture (~10 %). Raw (native) and HMT starch samples were labeled as M, M20, M25 and M30, respectively.

2.2. Cold argon-plasma treatment at atmospheric pressure

Starch (5 g) was treated using Dielectric Barrier Discharge (DBD) plasma device (Fig. 1), which was manufactured by University of Technology and Education [7]. The input parameters of plasma device were 1.0 A, 176 V and 50 Hz. The fixed parameters of sample treatment were flow-rate of argon (5.0 liter/min) for 10 min with mixing every 5 min.
2.3. Degree of alpha-amylase hydrolysis

Starch sample (0.2 g) was contained in 50 ml-flask with 0.1 M phosphate buffer solution (19 ml, pH 7.0). Then alpha-amylase (1.0 ml, 12 U/ml, Termamyl LS 120, Novozyme, Denmark) was added for the hydrolysis during 5 h at 37 °C. At interval hydrolysis time, sample (0.1 ml) was taken and put into a 2 ml-Eppendorf tube containing 5% NaOH (0.1 ml) and mixed well to stop the reaction [10]. Reducing sugar of sample was quantitative analysis by DNS method [11]. Degree of hydrolysis (DH, %) of sample was calculated by comparing to the reducing sugar of raw starch after completed hydrolysis by enzyme (24 h at 37 °C).

2.4. In vitro digestibility

Starch fractions under in vitro digestibility were measured following a previous method [12, 13]. Starch sample (30 mg) was added to a 2 ml-Eppendorf tube containing a glass bead and 0.1 M sodium acetate buffer solution (0.75 ml, pH 5.2). Sample was put on a shaking water-bath (240 rpm, 37 °C) for 10 min. Then enzyme solution (0.75 ml) was added. After 10 and 240 min of the hydrolysis, reaction was stopped by boiling (10 min). Sample was centrifuged (5000 ×g, 5 min) and glucose in supernatant was quantitatively detected using GOD-POD kit (BCS Co., Anyang, Korea). Color of reaction was measured by a spectrophotometer at the Abs 505 nm. RDS was calculated by the glucose content after 10 min of hydrolysis. SDS was the amount of glucose released from 10 to 240 min of reaction. RS was the fraction undigested after 240 min. Starch fractions of pre-boiled sample were prepared by boiling (30 min) starch sample in sodium acetate buffer before digestibility mentioned above.

2.5. Functional properties

Water absorbance index (WAI, g/g), swelling factor (SF, g/g) and water solubility index (WSI, % w/w) of sample was investigated using a published method [14]. Starch (W₁=50.0 mg, db) was added to a 2 ml-Eppendorf containing DW (1.0 ml) and mixed well. The Eppendorf tube was inserted in water-bath (90 °C, 10 min) with continuously shaking. Then the Eppendorf tube was cooled immediately on ice for 10 min and centrifuged (3000 ×g, 15 min). The supernatant was dried (105 °C) to a constant weight (Wᵣ). The sediment was balanced (Wₛ).

\[ WAI = Wᵣ/W₁; \ WSI = Wᵣ/W₁ × 100; \ SF = Wᵣ/(W₁ - Wₛ). \]

2.6. X-ray diffraction pattern and degree of relative crystallinity
X-ray diffraction pattern and degree of relative crystallinity (DRC) of samples were investigated by a powder X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany) at MANAR Center of HCMC National University. 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 5 to 45° (2 theta). The DRC was calculated using the equation:

\[
\text{DRC} = \frac{A_c}{A_c + A_a}
\]

where \( A_c \) is the area of crystalline portion and \( A_a \) is the area of amorphous portion [15, 16] with peak-fitting software (Origin version 8.5.1, Origin Lab, Northampton, Mass., U.S.A.).

3. RESULT AND DISCUSSION

3.1. Degree of alpha-amylase hydrolysis

Under the attack of alpha-amylase, 1,4-alpha-D-glucosidic linkages of starch were cleaved randomly to release smaller fractions (dextrins). Degree of alpha-amylase hydrolysis (DH, %) was affected by the origin of starch and parameters of the reaction [17]. Figure 3 showed the DH of samples. Raw starch (M) was quickly hydrolyzed after 20 min and reached the maximum of DH (83.5 %) after 300 min of reaction. HMT treated starches (M) showed lower DH compared to the raw starch. The DH value of these samples were ascendingly ranked as M20<M25<30<M. Thus, the lower moisture content of HMT treated starch resulted in higher resistance to the attack of alpha-amylase. As reported by Celia et al. [18], maize starch being heat-moisture treated at low moisture content (18 %) had lower DH compared to HMT starch at higher condition (27 %); and both showed lower DH than the untreated sample. Besides, the decrease of DH under HMT was observed in waxy maize, normal maize or high-amylose maize starches at the moisture content of 30 % (100 °C, 16 h) [19]. Hoover and Manuel [20] reported that the decrease of DH caused by the formation of amylose-lipid complex and/or the increase of linkages between chains in amorphous regions.

![Figure 3. Degree of alpha-amylase hydrolysis.](image)

(M: raw starch; M20, M25 and M30: HMT starch at 20, 25 and 30% moisture content; P: plasma treated starches).

Raw starch and HMT starch under plasma treatment (P) led to more resistance to alpha-amylase hydrolysis comparing to untreated samples. After 300 min of hydrolysis, DH value of M20P, M25P and M30P was 47.2, 50.1 and 53.8, respectively. Khanh Son Trinh et al. [7]
described the resistance of maize starch under atmospheric cold-plasma treatment from alpha-amylase. In argon-plasma environment, cross-linkages were formed between C-2 hydroxyl groups [21]. These linkages were the reason of the resistance of samples. Thus, both HMT and plasma treatments could make starch become more stable under enzymatic hydrolysis. Obviously, the combination of these factors would be more advantaged than a single treatment.

3.2. Fractions of starch under in vitro digestibility

Fractions of starch under in vitro digestibility were shown in Figure 4. For without pre-boiling samples (Fig. 4A), M had RDS and RS fractions (%), correspondingly, highest and lowest than the others. It indicates that raw starch is easily digested and the level of blood glucose is rapidly increased after intake comparing to the others. Theoretically, HMT could elevate RS₃ fraction [1, 22]. Under HMT, RDS and RS respectively reduced and elevated; especially the M20 sample. Continuously, further plasma treatment supported the higher increase of RS fraction of HMT starches. In our study, dual treatments (HMT+plasma) resulted in the incredible increase of RS (2.45-3.11-fold) comparing to its raw sample.

For pre-boiling samples (Fig. 4B), bRS and bSDS (pre-boiled RS and SDS, respectively) were converted to bRDS base on the gelatinization which lose the granular property and semi-crystalline structure of starch. Thus, under boiling, the crystalline and alpha-helix regions were changed to amorphous state that is easier for hydrolysis by digestive enzymes. In this study, raw starch (M) had highest bRDS (91.3 %) and lowest bRS (4.4 %). Among the HMT starches, bRS of the M25 sample was the highest. Furthermore, further plasma treatment by HMT reflected a significantly increase of bRS; bRS of M25P was the highest (44.7 %). Clearly, RS₄ was produced during plasma treatment based on the formation of cross-linkages between C-2 hydroxyl groups of starch [1, 21]. Surprisingly, dual treatment of maize starch resulted in the elevation of boiling-stable RS₄ (8.36-10.16-fold) comparing to that of raw starch.

Figure 4. Fractions (%) of starches under in vitro digestion of sample without (A) or with (B) pre-boiling before measurement. (M: raw starch; M20, M25 and M30: HMT starches at 20, 25 and 30 % moisture content; P: plasma treated starches).

3.3. Functional properties

Functional properties such as water absorbance index, swelling factor and water solubility index of samples were shown in Figure 5. Under HMT, WAI and SF were lower than that of the
raw starch (M); however, WSI was not significantly changed. The higher moisture content is, the lower WAI and SF are. Subsequently, WAI of starch was strongly reduced from 8 to 10 % of raw and HMT starches to < 3 % of plasma-treated starch. SF showed the similar behavior comparing to WAI. Contrary, WSI remarkably increased from 2% of the raw and HMT starches to 55 – 75 % of the plasma-treated starch.

Swelling factor of starch granule took place in the free amorphous region and was limited in the region closing to the crystallites. The swelling caused by amylopectin molecules. This property was reduced in the granule because of the formation of amylose-lipid complex. Theoretically, there are three main reasons for the reduction of SF: (a) the perfection of crystalline region, (b) the interaction between components in amorphous region, and (c) the formation of amylose-lipid complex. The two-last reasons resulted in the amylose leaching of sample [2]. Furthermore, the reduction of SF caused by the conversion of amorphous to alpha-helix structure or the interaction between crystalline and amorphous regions. Besides, the descending of WAI based on the perfection of amorphous structure and the formation of H-bonds between amorphous and crystalline regions [23]. At 90 °C, amylose molecule, which leaches out of the granule, contains ascending molecular weight and number of side-chain [24]. Previous studies reported that HMT leading to the reduction of SF and WSI causing by the re-arrangement of starch molecules [25]. The degradation of starch molecules that leads to the decrease of molecular weight caused by plasma treatment, could be reflected in the elevation of WSI [24, 26].

3.4. X-ray diffraction pattern and relative crystallinity

X-ray diffraction is a popular measurement for identifying the pattern of crystal and degree of relative crystallinity (Fig. 6). All samples displayed the A-type X-ray pattern with specific peaks at 15, 20, and 23° (2 theta) and two overlapped peaks at 17 and 18° [27]. The 20° peak indicated the amylose-lipid complex [28, 29, 30]. Generally, A-type pattern of cereal could not be changed under heat-moisture treatment [1]. Kawabata et al. [31] gave the evidence for the formation of amylose-lipid complex at 13° (2 theta). In our study, except for the raw (M) and dual-treated starches (MP, heat-moisture + plasma treatment), the others showed a peak at 13°, especially the samples M30 and M30P. Stute R. [32] stated that amylose-lipid complex (13 and 20°) prevented the re-arrangement of amylose molecules so it made these molecules more easily to be leached, resulting in an increasing of WSI [30].
Comparing to raw starch (M), the HMT samples contained higher DRC value based on the re-arrangement of starch molecules [3]. Under HMT, M20 showed the highest DRC (24.0 %). This value subsequently increased under plasma treatment. Sample M20P contained the highest DRC (36.9 %). The production of cross-linkages between C-2 hydroxyl groups resulted in the more perfection of alpha-helix structure. Thus, it caused the increase of DRC [21, 26]. Besides, the relationship between DRC and RS of samples was investigated (Fig. 7). Clearly, crystalline structure played an important role in RS fraction, especially non-boiling RS.

4. CONCLUSION

Heat-moisture treatment resulted in the increase of crystallinity leading to the reduction of rapidly digestible starch, water absorbance index and swelling factor. Subsequent treatment of argon-plasma caused the continuous increase of crystallinity, cross-linking level but reduced molecular weight leading to the decrease of water absorbance index and swelling factor. Both the formation of cross-linkages and depolymerization occurred during plasma treatment, so they were responsible for the small product that contain more compact of structure than its raw starch. This change explains the resistance to enzymatic hydrolysis of plasma-treated starch and other functional properties. Thus, the dually-treated products are of more boiling stability and higher solubility comparing to their raw starch.
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