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ORIGINAL PAPER

Production of cross-linked resistant starch from tapioca starch and effect of reaction conditions on the functional properties, morphology, X-ray pattern, FT-IR spectra and digestibility

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Abstract

The aim of this study was to investigate the effect of cross-linking temperature and pH on resistant starch (RS) formation as well as the functional, structural and morphology properties of tapioca starch. Tapioca starch was cross-linked using sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) at various temperature and pH. Increase in the reaction temperature and pH increased RS content and decreased swelling power (SP) of the samples. The sample cross-linked at 60 °C and pH 12 had the highest RS content (84.8%) and the lowest SP (3.0 g/g). The correlation coefficient between RS content and SP was 0.96. Cross-linking altered the pasting behavior significantly, while Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) patterns changed slightly. Cross-linking did not change the morphological structure and granular shape of the starch. In addition, cross-linked samples protect their original shapes during cooking and in vitro digestion probably since cross-linking restrict the water uptake and swelling due to the increase in the bonds within the molecule. Overall results proved that cross-linking at high temperature and high alkaline condition resulted in an increase in the RS content of tapioca starch.

Keywords Cross-linking · Tapioca starch · SEM · XRD · FT-IR · In vitro digestion

Introduction

Starch is a natural biopolymer, which plants synthesize to store the energy they obtain through photosynthesis. Starch, is the main component of many foodstuffs and is not only important in human nutrition, but it is also important due to its effect on the physical properties of foods (gel formation, consistency, water retention, etc.). Starch is used in food industry due to its unique properties [1, 2]. However, its applications are limited due to many reasons such as high retrogradation and syneresis, weak shear and heat resistance, high viscosity, poor process tolerance and thermal decomposition. Besides cooked starches forms a weak, cohesive and rubbery paste [3]. To overcome these limitations, starch is modified physically, chemically or enzymatically.

Starch can be chemically modified with several reactions such as acid hydrolysis, oxidation, etherification, esterification and cross-linking. Cross-linking (CL) of starch can be conducted in the presence of chemical agents that are responsible for the creation of ether or ester linkages between hydroxyl (–OH) groups. Some of these chemical agents are sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI) and phosphoryl chloride ($POCl_3$) [4, 5]. Cross-linking is responsible for the formation of new covalent bonds between inter- and intramolecular bridges causing the alteration of functional properties, mechanical strength, water resistance and thermal stability of starch [4].

It is known for a long time that chemical modification, such as esterification, etherification, and cross-linking of starch cause the resistance to the digestion [5]. It has also been reported that cross-linking of starches, using reagents such as STMP or an STMP–STPP mixture, caused

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resistance to digestion and therefore these resultant starches fit within the RS4 category [6].

Starch is classified in three main groups according to their digestibility period in the gastrointestinal tract; rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Starch that resist to the gastrointestinal digestion is defined as RS [7]. The recent studies are focused on the production of high amount of RS from various plants [8] as the consumption of resistant starch has positive effects due to its low glycemic index, decreasing effect of type II diabetes. In addition, it has the ability to lower the cholesterol metabolism and blood lipid and reduce the risk of colon cancer [9–11]. Besides its positive health effects, RS has less deteriorative effect on sensory properties of food samples compared to traditional fiber sources [12]. Resistant starch are divided into five groups: physically inaccessible starch (RS1), granular starch (RS2), retrograded or high amylose starch (RS3), chemically modified starch (RS4) and amylose–lipid complex (RS5) [5, 13].

Food grade starches are chemically modified using cross-linking techniques generally to protect the granular structure, restrict swelling and digestion [5]. The effect of cross-linking on the starch structure and property depends on different parameters such as botanical source of starch; type and concentration of cross-linking agents; degree of chemical substitution; pH, duration and temperature of the reaction [3, 6, 14–17]. Among the cross-linking reagents, STMP is widely used due to its low toxicity and effectiveness [18]. There is no limitation on the utilization of STMP cross-linked starch in foods. However, according to Code of Federal Regulations [19], the phosphorus (P) content of cross-linked starch should not exceed 0.04% when STMP is used for cross-linking. Instead, when the cross-linking is carried out using both STMP and STPP, the limit increase to 0.4% [19].

There are many studies in the literature concentrated on the physicochemical and nutritional properties of STMP:STPP cross-linked wheat, corn, rice and tapioca, oat, mung bean, banana and potato starches [5, 6, 18, 20, 21]. Several instruments can be used to investigate the structure of starch. X-ray diffraction is used to obtain knowledge about structure in terms of crystallinity. Fourier transform infrared spectroscopy (FT-IR) shows the alteration in bonds such as chain conformation, helicity and double helical structure [22]. Morphological structure can be investigated using scanning electron microscopy (SEM). Starch modification cause alteration in the structural properties of starch as well as functional properties. There are some studies in the literature about using XRD, FT-IR and SEM to observe the morphological structure of native and cross-linked starch [23–27]. However, limited studies are available in the literature that indicate the relationship between digestibility and the structural and morphological changes occurs after cross-linking.

The aim of this study was to investigate (1) the effect of cross-linking reaction temperature and pH on RS formation; (2) the effect of cross-linking on the functional and structural properties properties of the cross-linked starch samples; (3) the effect of digestion on the morphology of the cross-linked starch. Therefore tapioca starch was cross-linked using STMP:STPP at several conditions obtained using response surface methodology (RSM). The samples were characterized in terms of functional properties such as swelling power and pasting. FT-IR and XRD analysis were also performed to investigate the structural changes occurs due to cross-linking. The sample having maximum RS content was also analyzed using SEM to investigate the morphological changes after cross-linking. The effect of digestion and cooking on the morphology of this sample was also examined using SEM.

Materials and methods

Materials

Tapioca starch was supplied from Smart Kimya Trade. Co. Inc. (İzmir, Turkey). Enzyme kits were obtained from Megazyme Inc. (Ireland). The chemicals used in the study were in analytical purity otherwise stated.

Methods

Experimental design for the cross-linked starch production

In this study, response surface methodology (RSM) was used to optimize the cross-linking conditions of tapioca starches to obtain maximum RS content. Design Expert (Stat-Ease, Minneapolis, MN, USA) was used to generate the experimental design. Temperature (T) and pH were chosen as two independent variables in the cross-linking process. It is indicated in the literature that higher temperature and alkaline pH ranges are required to produce cross-linked starches [6, 15]. Therefore, the ranges for temperature and pH were selected as 40, 50 and 60 °C and 10, 11, 12, respectively. Resistant starch (RS) and swelling power (SP) of the cross-linked samples were selected as the dependent variables in order to estimate the RS production efficiency.

Cross-linked (CL) tapioca starches were produced according to the method of Woo and Seib [6]. Starch samples (50 g) were dispersed in 70 ml of water including 12% STMP:STPP (99:1) and 10% sodium sulphate. The reaction was carried out under specific time and pH conditions (Table 1) by using magnetic stirrer, which was equipped with a temperature controller (Heidolph Mei Tec Heater, Germany). After 3 h, the pH

Table 1 Resistant starch (RS, %), phosphorus (P, %) and swelling power (SP, g/g) of the cross-linked tapioca starch samples

Sample ID	Reaction conditions		CL tapioca starches		
	T (°C)	pH	RS (%)	P (%)	SP (g/g)
NT	Control (native starch)		2.2 e	0.01 h	19.7 a
T40×10	40 (− 1)	10 (− 1)	2.3 e	0.18 g	8.0 b
T40×11	40 (− 1)	11 (0)	23 d	0.24 f	4.5 d
T40×12	40 (− 1)	12 (+1)	67 c	0.36 b	3.5 e
T50×10	50 (0)	10 (− 1)	3.5 e	0.18 g	5.9 c
T50×11	50 (0)	11 (0)	19.2 d	0.28 e	4.4 d
T50×12	50 (0)	12 (+1)	74.1 b	0.28 e	3.4 e
T60×10	60 (+1)	10 (− 1)	3.4 e	0.35 c	5.7 c
T60×11	60 (+1)	11 (0)	21.8 d	0.32 d	4.2 d
T60×12	60 (+1)	12 (+1)	84.8 a	0.42 a	3.0 f

Coded values are shown in parentheses. Means with different letters within each column are significantly different ($p < 0.05$)

of the dispersion was adjusted to 6.5 with 1 M of NaOH to end the reaction. The starches were recovered by centrifugation and washing with 70 ml water for two times. The sediment was dried at 45 °C overnight and dried starches were ground to pass through a 212 µm sieve. The moisture content of the samples were evaluated according to the AACCI Approved Method 44-15A [28].

Determination of phosphorus content

The phosphorus content of the native tapioca starch and the CL starch samples were measured according to the AOAC 986.24 Method [29, 30]. According to the method, 1 g of the starch sample was weighted into porcelain crucible and ignited in the muffle furnace for 16 h to remove organic molecules from the sample. After starch samples reach the room temperature, 10 ml HCl (%25, v/v) and 1 ml HNO_3 was added to each sample. The crucibles were placed onto hot plate to reach boiling temperature for a few minutes. Boiled solutions were decanted to 100 ml flask and diluted to 100 ml with distilled water. 1 ml of diluted solution was taken into a test tube and 1 ml of vanadate-molybdate and 3 ml of distilled water were added into tubes, respectively. The absorbance (400 nm) of the solution was measured after 10 min with UV spectrophotometer (Shimadzu UV1800, Germany). The calibration curve was constituted by using standard phosphate solutions (KH_2PO_4) containing 0.1–0.6 mg of phosphorus per ml. The remaining phosphorus matter was determined according the Eq. 1, where P is the phosphorus content, A is the absorbance.

$$P\ (\%) = \frac{A}{0.162} \tag{1}$$

Determination of RS content

The RS content of the samples were determined according to AOAC 991.43: Total dietary fiber determination method [29]. The principle of the method was the digestion of the samples using α-amylase, protease and amyloglucosidase enzymes. The digested starch samples were treated with ethanol for 1 h, then solution was filtered and the residue was washed with alcohol and acetone. Crucibles were dried at 105 °C overnight and the residue was weighted. RS content of the sample was expressed as % (g RS/100 g dry sample). The residue was collected for SEM analysis.

Determination of swelling power

Swelling power (SP) was measured according to the method of Crosbie [31]. Samples (0.5 g) were mixed with 10 ml of distilled water in a predried and weighed centrifuge tube. The tube was vortexed for 5 s and placed into hot water bath at 95 °C for 30 min. Vortexing was repeated for 5 s every 10 min. After 30 min, the tubes were centrifuged (Hettich Rotina 380R, Germany) at 2100*g* for 10 min. Final sediment was weighted and the swelling power was determined as the ratio of sediment to dry sample (g/g).

Pasting properties

The RVA pasting curve was obtained with the Standard 1 test profile (13 min) using Rapid Visco-Analyzer (RVA 4500, Perten, Sweden) [32]. Starch sample (3.5 g, 14%, db) was dispersed in 25 ml of distilled water. The slurry was held at 50 °C for 1 min, then heated to 95 °C at a rate of 12.2 °C/min. The temperature was kept constant at 95 °C for 2.6 min. Then it was cooled to 50 °C at a rate of 11.8 °C/min. After the analysis, the starch samples were freeze dried (Labconco, USA) for SEM analysis.

Scanning electron microscope (SEM)

To understand the effect of cross-linking on the morphology of the tapioca starch granules, scanning electron microscope (SEM) was used. The effect of in vitro digestion and cooking on the morphological structure of cross-linked (CL) tapioca starches were also investigated. The morphology of the residue collected from RS analysis was analyzed with SEM to examine the effect of in vitro digestion on the cross-linked starch morphology. This analysis was only performed with the CL sample having the highest RS content (T60×12, cross-linked at 60 °C and pH12) as there was no residue available for native tapioca starch after digestion. The morphology of the native tapioca starch and T60×12 samples collected from RVA analysis was analyzed with SEM to investigate the effect of cooking on the CL starch morphology. All of the samples were coated with gold for 4 min using a Sputter Quorum Coater prior to analysis [2]. The samples were viewed using a Zeiss Gemini SEM 300 (Germany) analyzer under 2 kV analyzing conditions.

Fourier transform infrared spectroscopy (FT-IR)

The structural features of the native tapioca starch and CL starch samples were examined with Fourier Transform Infrared Spectrophotometer (FT-IR, Thermo Nicolet Avatar 370). Measurements were performed in the wavelength range of 4000–400 cm^{-1} and each sample was screened 32 times [2].

X-ray diffraction (XRD)

The crystalline structure and structural properties of the native tapioca starch and CL starch samples were characterized using X-ray diffractometer (Bruker AXS D8 advance model, Germany). Measurements were performed under Cu-Kα radiation ($\lambda = 1.54056$ Å) and specific voltage 40 and current as 30 mA. The position of diffractometer was arranged as $2\Theta = 5°–40°$. Crystallinity of the starch samples were also calculated using the software of the diffractometer [12].

Statistical analysis

All experiments were conducted twice and the mean values were reported. RS and phosphorus contents, and swelling power of the samples were analyzed using one-way analysis of variance (ANOVA). When significant ($p < 0.05$) differences were found, Duncans' test was used to determine the differences among means. The effect of the independent variables (temperature and pH) on the RS content and swelling power of the samples were determined by regression equations created by Design Expert program. Response surface methodology (RSM) was used to optimize the reaction conditions (temperature and pH).

Results and discussion

Effect of reaction conditions on RS contents of CL starches

Resistant starch (RS) content of the CL starch samples are shown in Table 1. The RS content of the native tapioca starch sample was 2.2%. There was no significant difference between the RS content of the native tapioca starch and the CL tapioca starch samples produced at pH 10. RS content of CL tapioca starches produced at pH 11 were significantly higher than that of the native starch ($p < 0.05$), while no significant difference was observed within these samples regardless of the reaction temperature. The highest RS content for each reaction temperature was achieved at reaction pH of 12. At this reaction pH, RS content of the samples were 67.0, 74.1 and 84.8% when the reaction temperatures were 40, 50 and 60 °C, respectively. The CL sample produced at 60 °C and pH 12 (T60 × 12) had the highest RS content (84.8%) among the samples. The relationship with the reaction temperature and RS content of Cl starches are in agreement with the results of the literature [5, 15].

The increase in the reaction pH significantly increased the RS content of the samples at all reaction temperatures. The CL sample produced at 40 °C and pH 10 (T40 × 10) had a RS content of 2.3%, while the RS content significantly increased to 23.0% and 67.0%, when the reaction pH was 11 and 12, respectively. Similar results were also observed for 50 °C and 60 °C reaction temperatures. It was also indicated in the literature that higher alkaline pH ranges are required. Similar results were also observed when different starch sources were used [5, 15]. Woo and Seib [6] investigated the effect of reaction pH on the RS content of CL wheat starches and reported that the RS contents of the CL wheat starches increased as the reaction pH increased. In another study, the increase in pH resulted in an increase in the RS content of corn and wheat starches [15].

To describe the effect of the reaction temperature (T) and pH to the RS content, the RS results were fitted by second order polynomial (quadratic) regression model. ANOVA analysis for the model is presented in Table 2. The F value (120.22), the p value (< 0.0012) and R^2 (0.9950) of the model indicated that the model was reliable. Significance analysis of the coefficients of each factor showed that only the reaction pH (coded as B) had an influence on the RS content of the tapioca starch samples ($p = 0.0002$). Reaction

Table 2 Analysis of variance (ANOVA) for the model

Source of variance	df	Resistant starch (%)		Swelling power (g/g)	
		F	*p*	*F*	*p*
Linear					
A (Temp)	1	3.65	0.1549	5.59	0.0992
B (pH)	1	547.68	0.0002*	54.63	0.0051*
Interaction					
AB	1	4.88	0.1142	2.82	0.1916
Quadratic					
A^2	1	0.29	0.6252	0.56	0.5088
B^2	1	44.59	0.0068	2.37	0.2212
Residual	3				
Model	5	120.22	0.0012*	13.19	0.0296*
R^2		0.9950		0.9565	

df degrees of freedom

*The significant factors ($p < 0.05$)

temperature did not have a significant effect ($p > 0.05$) on the RS content of the CL tapioca starch samples. Final estimative response model equation for the RS content of CL tapioca starch, in terms of the coded factors, was generated by Design Expert Software and given as follows (Eq. (2));

$$\mathrm{RS}\ (\%) = 20.37 + 2.95 \times A + 36.12 \times B + 4.17 \times A \times B + 1.45 \times A^2 + 17.85 \times B^2 \quad (2)$$

where A and B are the coded values of independent factors (T and pH, respectively).

Swelling power

Swelling power of native and CL tapioca starches are shown in Table 1. Native tapioca starch had the highest swelling power (19.7 g/g). On the other hand, swelling power values of the CL tapioca starch samples were significantly lower than that of the native one. Similar results were also reported in the literature. Wongsagonsup [21] produced cross-linked tapioca starch using different concentrations of STMP: STPP mixture and reported that increase in the cross-linking degree caused a decrease in swelling power. CL corn and rice starches had lower swelling power values compared to their native correspondings as stated by [5, 26], respectively.

At the same reaction temperature, the increase in the reaction pH caused significant decreases in the swelling power. For example, in case of the samples produced at 40 °C; the one produced at pH 10 had a swelling power of 8 g/g, whereas the sample produced at pH 11 and 12 had significantly lower swelling power (4.5 and 3.5 g/g, respectively). The increase in the temperature generally decreased the swelling power of the samples produced at the same reaction pH. For example, the sample produced at pH 10 and 40 °C (T40 × 10) had a swelling power of 8 g/g, the ones produced at 50 °C and 60 °C had significantly ($p < 0.05$) lower swelling power values (5.9 g/g and 5.7 g/g, respectively). On the other hand, the swelling power values of the CL samples produced at pH 11 were not significantly different regardless of temperature.

To describe the effect of the reaction temperature (T) and pH to the swelling power values, the swelling power results were fitted by second order polynomial (quadratic) regression model. ANOVA analyses for the model are presented in Table 2. The F value (13.19), the p value (<0.0296) and R^2 (0.9565) of the model indicated that the model was reliable. Similar to RS results, significance analysis of coefficients of each factor showed that only the reaction pH (coded as B) had an influence on the SP content of the tapioca starch samples ($p = 0.0051$). Reaction temperature did not have a significant effect ($p > 0.05$) on the SP content of the cross-linked tapioca starch samples. The final estimative response model equation for the SP content of CL tapioca starch, in terms of the coded factors, was generated by Design Expert Software and given as follows (Eq. (3));

$$\mathrm{SP}\ (\%) = 4.14 - 0.52 \times A - 1.62 \times B + 0.45 \times A \times B + 0.28 \times A^2 + 0.58 \times B^2 \quad (3)$$

where SP is the swelling power, A and B are the coded values of independent factors (T and pH, respectively).

It seems that there is a reverse relationship between RS content and swelling power of the CL samples. The increase in the RS content caused a decrease in the swelling power of the CL starch samples. For example the sample T60 × 12 (cross-linked at 60 °C and pH 12) having the highest RS level (84.8%), had the lowest SP value (3.0 g/g). When the samples that have significantly same RS content with the control sample (T40 × 10, T50 × 10, T60 × 10) are excluded (Table 1), a significant correlation coefficient was calculated between the RS content and swelling power ($R^2 = 0.96$). The reason of this relationship might be the increase in the bonds within the starch molecule during cross-linking [1].

Pasting viscosity

The pasting viscograms of the native and CL tapioca starches are shown in Fig. 1. Native tapioca starch sample had a regular pasting curve, whereas cross-linking reaction changed the pasting behavior significantly. The peak viscosity, shear thinning and final viscosity of the native tapioca starch was 7248, 5199 and 3383 cP, respectively. The sample cross-linked at 40 °C and pH 10 (T40 × 10), had peak viscosity, shear thinning and final viscosity as 5000, 418 and 8434 cP, respectively. The peak viscosity and shear thinning values of the native starch was higher than that of the T40 × 10 sample. Whereas, native tapioca starch had a lower final viscosity than T40 × 10 sample. On the other hand, the pasting temperatures shifted from 71.9 °C (native tapioca starch) to 75.1 °C (T40 × 10) and 79.8 °C (T50 × 10). There was a slight increase in the viscosity of the samples cross-linked at 50 °C and 60 °C at pH 10 (T50 × 10 and T60 × 10) as can be seen from Fig. 1. Nevertheless, this increase cannot be described as a real pasting property. The rest of the samples (T40 × 11, T40 × 12, T50 × 11, T50 × 12, T60 × 11, T60 × 12) did not show a pasting curve. In addition, in line with pasting viscograms, native tapioca starch and the T40 × 10 sample formed a gel like structure at the end of pasting, whereas the ones having lower viscosity values did not.

Increasing in viscosity is related to the swelling of the starch granules. During cooking/pasting, starch granule absorbs water and the volume of the granule increase causing swelling. Swelling is accompanied by leaching of granule components, predominantly amylose and starch loses its granular structure and molecular order [3]. The samples produced at pH 10, which also showed at least a viscosity

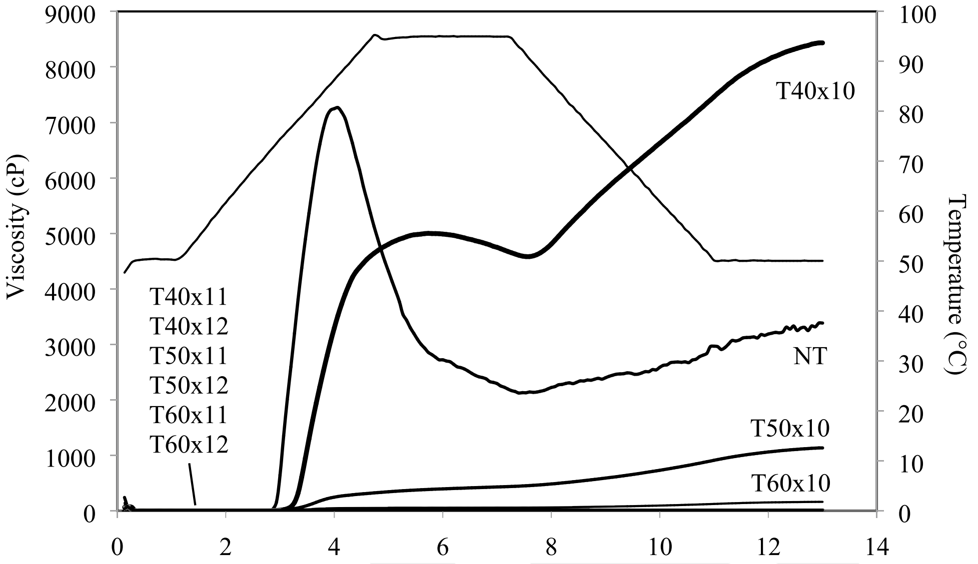

Fig. 1 Pasting viscosity graphs of native and cross-linked tapioca starch samples. T: Tapioca starch; NT: Native tapioca starch; 40, 50, 60: Reaction temperature (°C); 10, 11, 12: Reaction pH

during pasting, had a swelling power of comparable to native tapioca starch. The ones having swelling power lower than 4.5 g/g did not show a pasting curve. As stated before, when starch is cross-linked covalent bonds are formed and starch granule become more resistant to water-uptake during cooking, therefore swelling is limited [33]. Similar results in pasting properties were also observed in the study of Woo and Seib [6] and Chung et al. [33]; the pasting curves of the cross-linked starch samples did not rise above the baseline.

There is an interesting relation between pasting properties and RS content of the samples. All of the samples produced at pH 10 (regardless of the reaction temperature) showed at least a viscosity and had RS content lower than 3.5% (Table 1). The samples that did not show a pasting curve had RS contents between 19.2 and 84.8%.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images of native tapioca starch and the CL starch sample having the highest RS content (T60 × 12, cross-linked at pH 12 and 60 °C) were illustrated in Fig. 2a and b. SEM images showed that native tapioca starch granules were round in shape with trimmed ends and smooth surfaces (Fig. 2a), as also reported in the literature [18, 34]. Cross-linking did not induce a noticeable difference in the morphological structure and granule shape. The T60 × 12 sample had also similar granular shape and distribution with native tapioca starch. However, the surface of the granules seemed to be rougher compared to the native starch granule (Fig. 2b). Similar observations were also reported in the literature [27].

The changes in the morphology of native and CL tapioca starch after cooking (RVA pasting) are shown in Fig. 2c and d, respectively. Significant variations were observed between the starch samples. After cooking, the granular form of native tapioca starch completely disappeared, as expected. Increase in viscosity is due to the swelling of starch granules during heating as stated above [33]. On the other hand, RVA pasting did not cause a considerable change in the granular shape of the CL tapioca starch (T60 × 12). The shape of the granules after cooking was relatively similar to their counterparts (Fig. 2b). On the other hand, the form of the granules was doughnut shaped with deep hollows in the central core (Fig. 2d). Similar observations were also reported in the literature after various modification of starch [3, 35]. The formation of groove might be due to the drying method of starch after RVA pasting. In this research, the samples were freeze-dried after RVA pasting. Zhu et al. [36] produced cold swelling corn starch with two drying methods, oven drying and freeze drying. They also observed grooves in the freeze-dried starch samples and indicated that these changes were due to the internal fragmentation of the granules.

To observe the effect of in vitro digestion to the granule morphology, CL tapioca starch samples were also examined after digestion. As stated before, this analysis could not be performed with native tapioca as there was no residue available after digestion. The SEM images after digestion of the CL tapioca starch produced at 60 °C and pH 12 (T60 × 12) are shown in Fig. 3a and b with magnifications of 250× and 500×, respectively. It could be easily detected from the SEM images that CL starch granules protected their typical granule appearance. Besides, there are grooves in the center core of the granules, similar to the samples freeze-dried after RVA pasting (Fig. 2d). There are some researches investigating the effect of digestion to the structure of cross-linked starch granule. Commercially available RS4 starch samples (wheat and potato) were subjected to in vitro digestion and freeze-dried afterwards

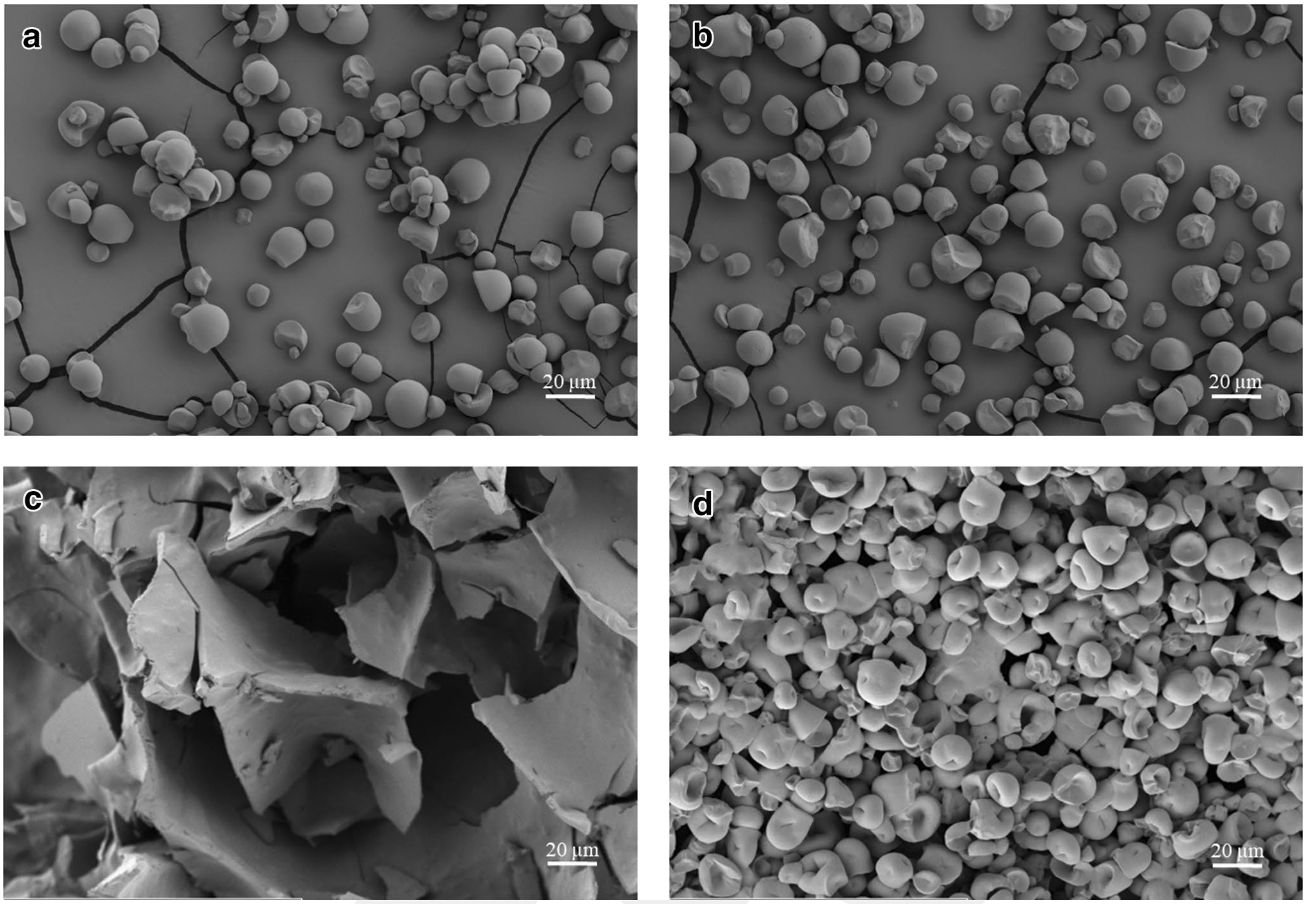

Fig. 2 SEM images of **a** native tapica starch; **b** cross-linked tapioca starch; **c** native tapioca starch lyophilized after RVA pasting; **d** cross-linked tapioca starch (60 °C, pH 12) lyophilized after RVA pasting. Magnification ×500

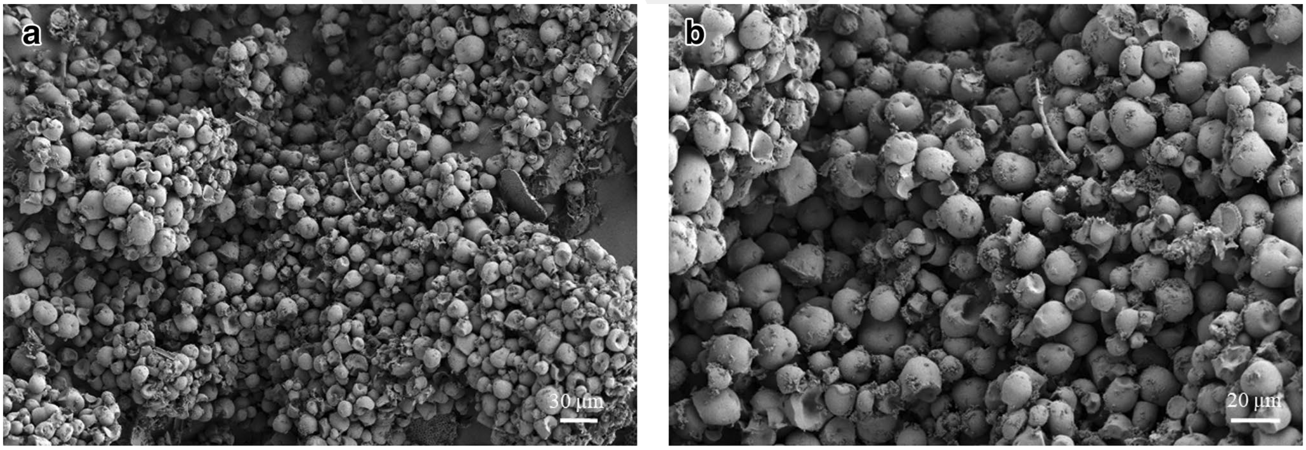

Fig. 3 SEM images of the sample T60 × 12 (cross-linked at 60 °C and pH 12) after enzymatic digestion. Magnification **a** ×250 **b** ×500

[25]. Similar to our study, the cross-linked wheat and potato granules also kept their integrity after digestion. Moreover, there were similar grooves on the surfaces of the granules exposed to digestion. As stated above, the formation of groove might be due to the drying.

When starch is heated in excess water, granules swell due to the breaking down of hydrogen bonds [3, 37, 38].

Water absorption and swelling of the starch granule leads amylose–amylopectin phase separation, and then amylose leaches to the inter-granular space, which causes irreversible disintegration of the granules [38]. As can be seen from the SEM images of the cross-linked tapioca starch, collected after RVA pasting and in vitro digestion (Figs. 2d and 3, respectively), granules have protected their specific shape. Neither RVA pasting nor in vitro digestion did not cause a change in shape arise from swelling of starch granules. As stated before, cross-linking restricts the water uptake and swelling of the granules probably due to the increase in the bonds within the starch molecule during cross-linking. This restricted swelling behavior of the cross-linked starch granules was also examined during RVA analysis. While native starch sample had a regular pasting behavior, CL starch did not exhibit any pasting profile (Fig. 1).

Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis was used to define the additional structural organization of cross-linked starch samples. FT-IR a spectrum of the cross-linked tapioca starches and native corresponds are shown in Fig. 4. The small peak seen at 3640 cm^{-1} wavelength indicates the free water entry on the surface of starch sample. Existence of free water was also reported in the literature [39]. The presence of stretching vibrations at about 3600 cm^{-1} wavelength confirms the existence of O–H bond (Fig. 4). The IR band at 1248 cm^{-1} is arising from the bending vibrations of O–H and the absorption bands near the 1395 cm^{-1} wavelength match up with the C–H bending vibrations. The absorption band seen at 2930 cm^{-1} contributes to the C–H stretching vibration. Similar peaks were also observed in the literature [1, 27, 40]. The peak at 1647 cm^{-1} is related to the bound water found in the starch granule [1, 41]. As can be seen from Fig. 4, apparent difference was not observed between the native and cross-linked tapioca starch samples in terms of O–H, C–H and C–O vibrations bands and parallel absorption peaks were observed for the starches. This might be due to the fact that, cross-linking does not change the general starch chemical structure directly. Similar observations were reported by Ashwar et al. [1] and Gao et al. [27] in terms of cross-linked rice and corn starch, respectively.

The intensity of the O–H stretching vibration band between 3640 and 3000 cm^{-1} seemed to be decreasing as a result of cross-linking STMP/STPP. In addition, a small peak was seen between 1244 and 1180 cm^{-1} range in cross-linked samples. This peak demonstrated the presence of P–O bonds in the sample [1, 27, 39]. As the cross-linking reaction occurs at OH groups [42], cross-linking seemed to cause the reduction of the intensity of the O–H stretching band and the formation of new P–O bonds.

X-ray diffraction (XRD)

The X-ray diffractograms of native and cross-linked tapioca starches were illustrated in Fig. 5. According to the crystal structure, starch is divided into three groups as A, B and C type starches [43]. The starches having A type crystal form are generally cereal origin and form their own structure by giving significant peaks in X-ray diffraction pattern at 2Θ = 15°, 17°, 18° and 23° [44]. In our study, the native tapioca starch had peaks at 15.2°, 17.3°, 18.09° and 23.15° (2Θ) diffraction angle (Fig. 5). As seen easily from the diffractograms cross-linked

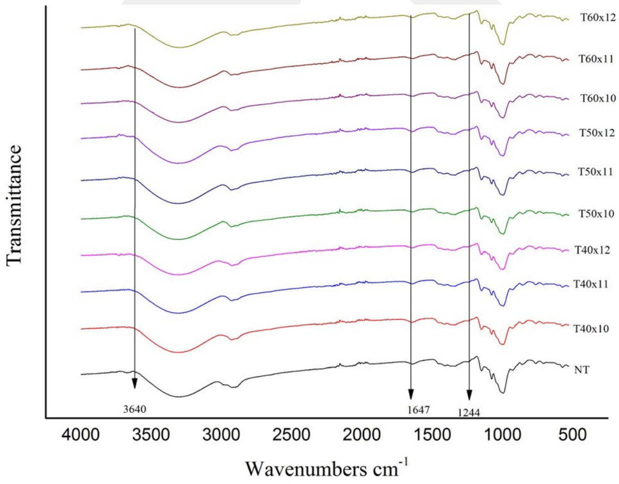

Fig. 4 Fourier transforms infrared spectroscopy (FT-IR) spectrums of native and cross-linked tapioca starch samples. T: Tapioca starch; NT: Native tapioca starch; 40, 50, 60: Reaction temperature (°C); 10, 11, 12; Reaction pH

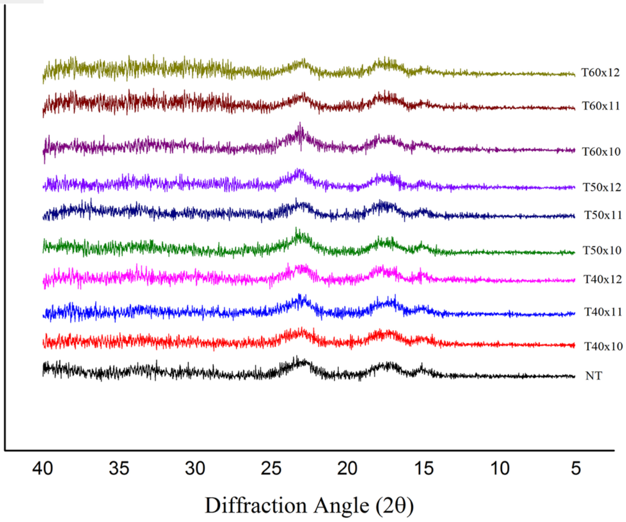

Fig. 5 X-ray diffraction angle (XRD) pattern of native and cross-linked tapioca starch samples. T: Tapioca starch; NT: Native tapioca starch; 40, 50, 60: Reaction temperature (°C); 10, 11, 12; Reaction pH

tapioca starch samples had similar peaks with the native tapioca starch at the specific A type crystalline diffraction angle. It seems that cross-linking procedure did not directly affect the XRD pattern of starch samples. Similar observations were also reported in the literature [1, 5]. Koo et al. [5] reported that the XRD patterns of the cross-linked starches did not change if < 12% of STMP:STPP was used for cross-linking.

On the other hand, the crystallinity of the cross-linked starches slightly changed compared to the native tapioca starch. As cross-linking reaction occurs in the amorphous region of the starch granules the crystallinity of starch samples may change. While native starch samples had 49.4% crystallinity, the starch sample cross-linked at 60 °C and pH 12 (T60 × 12) had a 46.9% crystallinity. The slightly decreasing in the crystallinity of cross-linked linked starch was demonstrated in the literature for rice starch samples [1].

Conclusions

The present study proved that cross-linking at high temperature and high alkaline condition resulted in an increase in the RS content of tapioca starch. On the other hand, high RS containing starches had low swelling power and did not show any swelling ability during RVA analysis. Resistance to enzymatic digestion and swelling behavior (swelling power and pasting) of cross-linked starch can be associated with starch structure, as cross-linking cause formation of new intermolecular bonds. Cross-linking did not alter the morphology of the starch granule, however, the surface of the granules were rougher compared to the native starch. While the native starch lost its granular shape and integrity during cooking and in vitro digestion, the sample having the highest RS content (T60 × 12) protect its granular shape and integrity. There is a growing interest in food industry to enhance the RS content of foods due to its beneficial physiological effects, similar to dietary fiber. Since the high RS containing cross-linked tapioca starch produced in this study did not swell during gelatinization, it has a good potential to be used as a dietary fiber source in heat-treated products for which an increase in consistency is not preferred. On the other hand due to its low swelling capacity, it can be used without formulation change encountered when dietary fibers are used in bakery products. Further studies are needed to produce starch preparation with high RS content with improved functional properties.

Author contributions KK: Conceptualization, Methodology, Validation, Writing—Review & Editing, Supervision, Project administration, Visualization. AK: Conceptualization, Software, Validation, Formal analysis, Writing—Original Draft, Writing—Review & Editing, Visualization.

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