Tropical Journal of Pharmaceutical Research September 2014; 13 (9): 1393-1400
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i9.3

Original Research Article

Characterization of Digestion Resistance Sweet Potato Starch Phosphodiester

Guanglei Li[1]*, Benguo Liu[1], Guocong Zhang[2], Jie Zeng[1], Junliang Sun[1] and Hanjun Ma[1]

[1]School of Food Science, Henan Institute of Science and Technology, Xinxiang, 453003, [2]Shijiazhuang Academy of Agricultural and Forestry Sciences, Shijiazhuang, 050041, China

For correspondence:* ***Email:* *lgl70_hist@163.com;* ***Tel:*** *+86 373 3693693*

Received: 24 February 2014 *Revised accepted: 24 July 2014*

Abstract

Purpose: *To analyze the physicochemical properties and in vitro digestibility of sweet potato starch phosphodiester prepared using sodium trimetaphosphate.*
Methods: *The physicochemical properties of sweet potato starch phosphodiester were analyzed by using infrared spectrometry (IR), differential scanning calorimetry (DSC) and rapid visco-analyser (RVA). In addition, an in vitro digestibility method was applied to investigate starch digestion performances.*
Results: *FTIR spectrum showed new absorption peaks at 1033 cm^{-1} indicating that an esterification cross-linking reaction was found between sweet potato starch and sodium trimetaphosphate. Similar gelatinization temperature (70 ^{o}C), enthalpy change (10 J/g), and peak viscosity (600 cp) were obtained for sweet potato starch phosphodiester and the raw starch indicating that their gelatinization properties were identical. Compared with sweet potato starch, digestible starch content of sweet potato starch phosphodiester decreased sharply (from 63.4 to 15.8 %), while digestion resistance starch content increased significantly (from 14.5 to 58.7 %). Based on completion of starch hydrolysis, the glycaemic index (GI) of sweet potato starch phosphodiester was predicted to be 66.31.*
Conclusion: *Derived sweet potato starch phosphodiester presents higher digestibility and may be useful as a medium glycemic index (GI) food for diabetic patients.*

Keywords: *Sweet potato starch, Phosphodiester, Digestion resistance, Digestibility, Glycemic index*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Resistant starch cannot be digested and absorbed by the amylase in the human digestive tract, but it can be degraded through the glycolysis by microorganisms in the colon. Thus, it is effective in adjusting blood glucose, preventing cardiovascular and cerebrovascular diseases, as well as colon and rectum carcinoma [1-3]. Distarch phosphate products prepared using sodium trimetaphosphate as the raw material all met high edible safety standards. Chung et al. indicated that starch phosphodiester also possessed a certain digestion resistance [4,5].

Currently, the studies on the starch phosphodiester are mainly concentrated on the effect of preparation conditions on the substitution degree and viscosity of starch [6,7], while less attention has been drawn to the digestibility of starch phosphodiester.

The physicochemical properties of sweet potato starch phosphodiester have been studied by IR, DSC and RVA. An in vitro digestion kinetic method proposed by Goni [8,9] was used to simulate the human gastrointestinal system to further investigate the *in vitro* digestion performances of sweet potato starch phosphodiester. This research aimed to characterize sweet potato starch phosphodiester by digestion resistance quantification as this may further promote its application in the functional food and pharmaceutical industry.

EXPERIMENTAL

Materials

Sweet potato starch was provided by Agriculture Development Limited Company, Hezai, Henan, China. α-Amylase and glucoamylase were provided by Fuyuan Biological Science and Technology Limited Company, Zhengzhou, Henan, China. The other chemicals used are of analytical grade.

Preparation of starch phosphodiester

The preparation of starch phosphodiester was done according to a published method [10]. A quantity of sweet potato starch was mixed with a certain amount of STMP, sodium chloride, sodium hydroxide, urea, and water in sequence. The resulting solution was kept at a constant temperature of 40 °C and continuously stirred for the 2 h, thereafter, its pH was adjusted to approximately 6.5. After water-washing and four centrifugation stages, it was dried (again at 40 °C), crushed, and sieved (through a 180 µm sieve).

Measurement of starch digestion resistance

The starch digestion resistance was measured according to a published method [11]. A certain amount of starch sample was mixed with buffer solution. After its pH was adjusted to 1.5, pepsin solution was added. The resulted solution was maintained at 40 °C for 1 h, cooled to room temperature, and adjusted to the pH between 6.0 and 7.0. Thermostable amylase was added, the resulting solution was kept at 90 °C for 30 min, cooled to room temperature, and adjusted to pH of 4.75. Glucoamylase solution was added to the above solution, the obtained solution was kept at 60 °C for 1 h, cooled to room temperature, centrifuged. The remaining sediment was mixed with 4 M KOH solution to ensure completely dissolution, this was then neutralised with an HCl solution. After glucoamylase solution was added, the resulting solution was kept at 60 °C for 1 h, cooled to room temperature, centrifuged. The collected supernatant was diluted to 100 mL with distilled water. Reducing sugar content was determined by 3, 5-dinitrosalicylic acid method. The obtained results were multiplied by 0.9, and this final yielding result was the resistant starch content. Starch digestion resistance (R) was computed as in Eq 1.

$$R = (RS/SD)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where RS is the content of resistant starch and SD is the total content of corn starch phosphodiester.

FT-IR

In order to further determine the structure of the wheat starches, FT-IR spectra were obtained using FT-IR (Nicolet 470; Perkin Elmer Inc., Waltham, MA, USA). The spectra were recorded in transmission mode from 4,000 to 500 cm^{-1} (mid-infrared region) at a resolution of 0.44 cm^{-1}.The sample was mixed with KBr (1:100, w/w) before acquisition and the background value from pure KBr was acquired before the sample was scanned.

DSC

The thermal properties of the starches were analyzed using a differential scanning calorimeter (DSC, TA instruments Waters LLC, New Castle,DE, USA) equipped with a thermal analysis data station. Aluminium pans (Perkin-Elmer) were used for the analysis. Starch samples (2 mg, dried starch basis, dsb) were precisely weighed in the sample pans, mixed with distilled water (4 mg), and sealed. The heating rate was 10 °C per min over the temperature range of 45 - 190 °C. Indium and zinc were used as the reference standards. Enthalpy change (△H), gelatinization onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc) were measured. The data were presented as means of three replicates for each starch sample.

RVA

The pasting properties of the starch were determined by using a Rapid Visco-Analyzer (RVA) (RVA-Series 4, Newport Scientific Pty Ltd, Warriewood, Australia). Each starch suspension (8 %, w/w; 28 g total weight) was equilibrated at 50 °C for 1 min and then heated at a rate of 6 °C/min to 95 °C and then maintained at that temperature for 5 min. The sample was then cooled to 50 °C at a rate of 6 °C/min. A rotating speed of the paddle (160 rpm) was used except the paddle speed was 960 rpm at the first 10 s.

Determination of starch digestion performance

Starch sample (200 mg) was placed in a test tube. By adding 15 mL of 0.2 mol/L sodium acetate buffer solution with a pH of 5.2, the solution was gelatinised by boiling in a water bath for 10 min. After cooling down, 10 mL of porcine pancreatic α-amylase and glucoamylase were added. Then the sample was agitated in a thermostatically controlled water bath at 37 °C: after hydrolysis for either 20 or 120 min, some 4 mL of ethanol enzyme inactivation agent was added after removing 0.5 mL of the hydrolyzate. The glucose content of the supernatant obtained by centrifugation was subjected to colorimetric determination using a glucose oxidase method at a wavelength of 510 nm [12]. The mass fractions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistance starch (RS) in the samples were computed as in Eqs 3 – 5, respectively.

$$RDS\ (\%) = [(G_{20}\text{-}FG)0.9]/TS \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$SDS\ (\%) = [(G_{120}\text{-}G_{20})0.9]/TS \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$RS\ (\%) = [TS - (RDS + SDS)]/TS \quad \ldots\ldots\ldots\ldots\ldots (5)$$

where G_{20} denotes the glucose content after conducting amylase hydrolysis for 20 min; G_{120} denotes glucose content after conducting amylase hydrolysis for 120 min, while FG refers to the free glucose content of starch before conducting enzymatic hydrolysis; TS represents the total starch content of the sample.

Determination of starch digestion rate

A 200 mg starch sample was put into a test tube and 0.2 mol/L sodium acetate buffer solution of 15 mL, at a pH of 5.2, was added before gelatinization for 10 min in a boiling water bath, 10 mL of porcine pancreatic α-amylase and glucoamylase was added after a cooling period. Then the sample was agitated in a thermostatically controlled water bath at 37 °C and the corresponding times recorded. During hydrolosis, a reaction solution of 1 mL was removed and the removal time recorded until the reaction was stopped. A 3, 5-dinitro salicylic acid was applied to determine reducing sugar content and compute starch hydrolysis rate as in Eq 6 [13].

$$SHR\ (\%) = [(G_t \times 25 \times 0.9)/200]100 \quad \ldots\ldots\ldots\ldots (6)$$

Where SHR is the starch hydrolysis rate and G_t denotes the glucose content after conducting amylase hydrolysis for t min.

Calculation of starch glycemic index

The GI can be calculated using the hydrolysis curves for starch. The hydrolysis curves followed a first order reaction kinetic, i.e. the reaction rate was directly proportional to first power of the reactant concentration. Hydrolyzate concentration changed with time. The area under the curve was obtained as in Eq 7.

$$AUC = C\infty(t_f\text{-}t_0)\text{-}(C\infty/k)\{1\text{-}\exp[-k(t_f\text{-}t_0)]\} \quad \ldots\ldots (7)$$

where C∞ corresponds to the concentration (t180) at equilibrium; t_f is the final (end) time (180 min); t_0 is the initial time (0 min); k is a constant of the first order reaction kinetics which can be calculated from $C = C\infty(1 - e\text{-}kt)$. The hydrolysis index can be obtained by comparing the sample area under the hydrolysis curve with that for fresh white bread on its hydrolysis curve. Based on the experimental results of *in vivo* and *in vitro* GI values as analysed by Atkinson *et al*, starch hydrolysis index (HI) and GI presented good correlation (r = 0.894) [14]. Percent starch hydrolysis and GI showed better correlation at 90 min. Post-prandial glycaemic response of carbohydrate foods can therefore be predicted accurately.

Amylase hydrolysis was performed to obtain the percentage of starch hydrolysis at 90 min (H_{90}), while GI was predicted by regression as in Eq 8.

$$GI = (39.21 + 0.803)H_{90} \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Statistical analysis

Statistical analysis was carried out using DPS 7.05 software (Zhejiang University, Hangzhou, China). All measurements were repeated three times, and mean values used as data. Statistical comparisons were carried out using Dixon test. A p value of < 0.05 was considered to be significant.

RESULTS

Infrared scanning analysis

The IR spectra of sweet potato starch and its phosphodiester were shown in Figure 1. The sweet potato starch phosphodiester had similar infrared absorption characteristics to those of raw starch and mainly presented a small adsorption peak at 1033 cm^{-1}. The adsorption peak vibration corresponded to the stretching of C6-O-H in the starch glucose units.

On the basis of the chemical structural characteristics of sodium trimetaphosphate, phosphate compounds in fatty groups, and vibration rules for similar compounds, the fingerprint region presented a P-O-C stretching vibration absorption band. The wave number range was 1050 to 995 cm^{-1}. The infrared absorption peaks between 1400 to 1150 cm^{-1} when P = O were not observed. By an esterification cross-linking reaction of sweet potato starch and sodium trimetaphosphate, the increased strength of the adsorption peak at 1033 cm^{-1} was due to the fact that P-O-C groups were introduced into the glucose units. This phenomenon showed that the esterification cross-linking reaction between sweet potato starch and sodium trimetaphosphate introduced phosphate groups into the sweet potato starch with small adsorption peaks present. The extent of the substitution of sweet potato starch phosphodiester was low. Moreover, the infrared spectra showed that the characteristic absorption peaks of other groups underwent no apparent change. This indicated that the esterification cross-linking reaction merely added some new groups to the starch chain and did not damage its underlying chemical structure.

Thermal characteristics

The DSC curves of sweet potato starch, sweet potato starch phosphodiester, and sweet potato retrograded starch were shown in Figure 2 and Table 1.

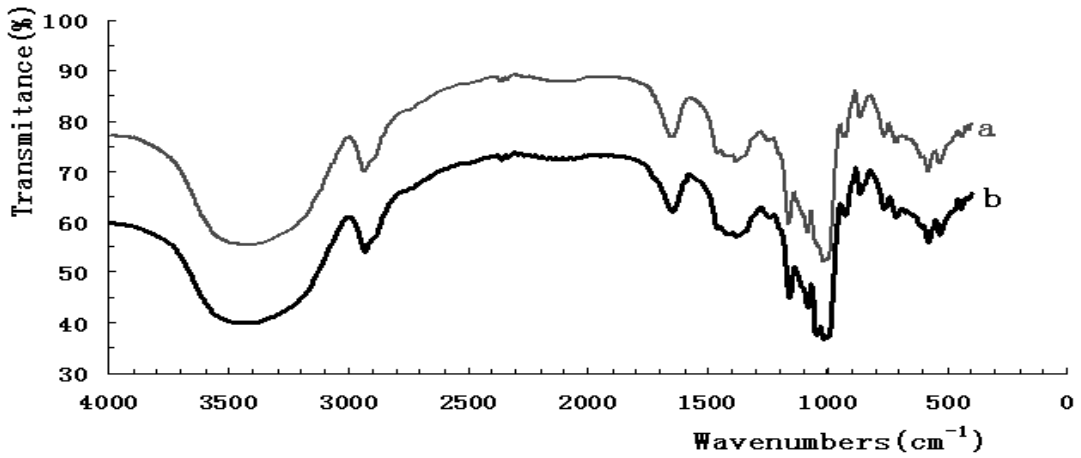

Fig 1: FTIR spectrum of sweet potato starch phosphodiester. ***Key:*** a = sweet potato starch; b = sweet potato starch phosphodiester

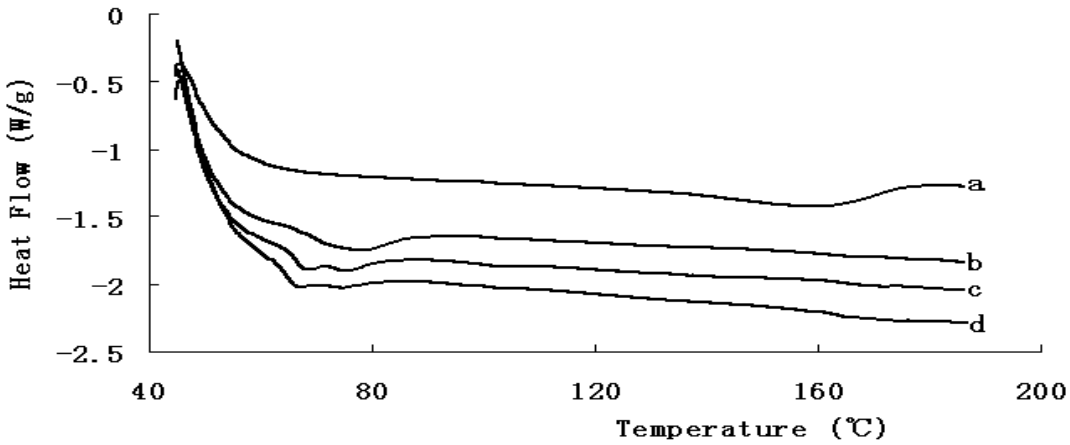

Fig 2: DSC scanning curves of the samples. ***Key:*** a = sweet potato retrograded starch(RS100%); b = sweet potato starch; c = sweet potato starch phosphodiester (RS23%); d = sweet potato starch phosphodiester (RS58%)

As shown in Figure 2 and Table 1, a small peak at about 75 °C was on the DSC curves of crude sweet potato starch, and a bigger peak was found at about 160 °C from DSC curves of sweet potato retrograded starch (RS100 %). DSC scanning curve of sweet potato starch phosphodiester was basically the same as that of raw starch, with the increase of starch digestion resistance, the gelatinization temperature and gelatinization enthalpy decreased.

Viscoelastic properties

RVA viscosity curves of sweet potato starch phosphodiester were shown in Figure 3.

As seen from Figure 3, peak viscosity and initial gelatinization temperature of sweet potato starch phosphodiester decreased with increase in digestion resistance. However, compared with sweet potato retrograded starch, sweet potato starch phosphodiester had a higher viscosity and was therefore more suitable for use in food as well as showing higher digestion resistance ability.

Starch digestion performance

In vitro digestion performance of corn starch, sweet potato starch, and sweet potato starch phosphodiester is listed in Table 2.

Table 2: *In vitro* digestibility of the samples

Sample	RDS (%)	SDS (%)	RS (%)
corn starch	86.4±1.9	11.5±1.5	1.9±1.0
sweet potato starch	63.4±1.6	22.1±1.1	14.5±1.4
sweet potato starch phosphodiester （RS58%）	15.8±0.9	25.5±1.2	58.7±1.4

Table 1: DSC parameters for sweet potato starch phosphodiester

Sample	Enthalpy change ΔH (J/g)	Gelatinization onset temperature To (°C)	Peak temperature Tp (°C)	Conclusion temperature Tc (°C)
Sweet potato starch	12.25	65.67	75.86	87.50
Sweet potato starch phosphodiester (RS23%)	10.82	64.55	68.07	83.59
Sweet potato starch phosphodiester (RS58%)	8.195	62.19	66.48	79.80
Sweet potato retrograded starch (RS100%)	21.12	136.80	161.44	177.29

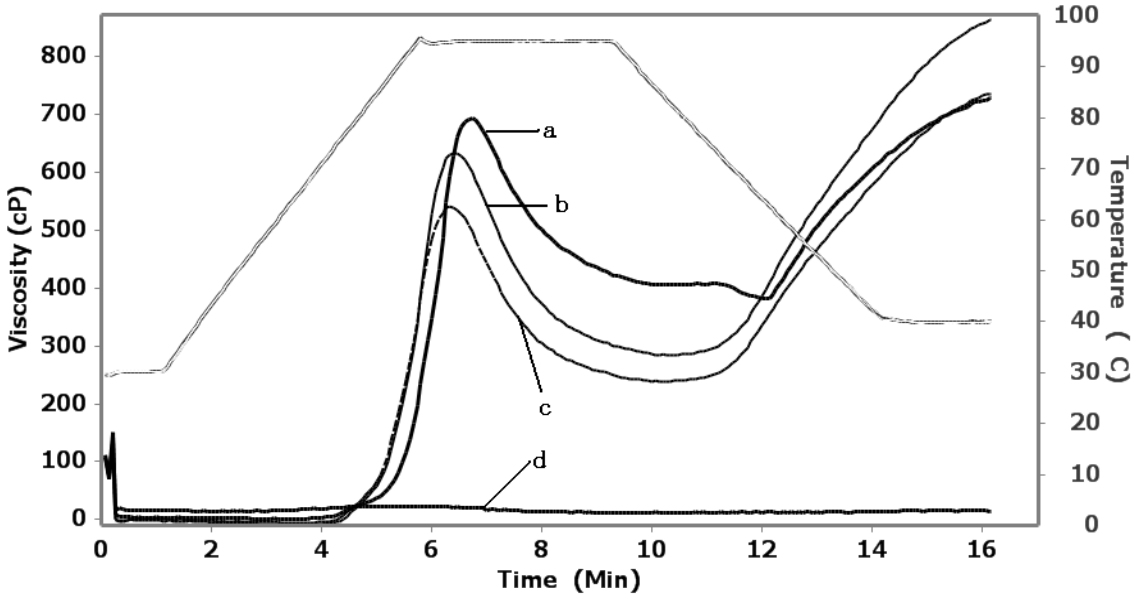

Fig 3: RVA curves of the samples. ***Key:*** a = sweet potato starch; b = sweet potato starch phosphodiester (RS23%); c = sweet potato starch phosphodiester (RS58%); d = sweet potato retrograded starch (RS100%)

As shown in Table 2, RDS content of corn starch was highest (86.4 %) while RS content was approximately 2 %. After modification by phosphodiester, the RS content of sweet potato starch increased to 58.7 %. Also, RDS content decreased to 15.8 %, which suggests that the nutritional quality of sweet potato starch phosphodiester was been significantly improved.

In vitro digestion rate

With reference to fresh white bread, the *in vitro* digestion rates of corn starch, sweet potato starch, and sweet potato starch phosphodiester were determined (Figure 4). The *in vitro* digestion rate was defined as the percentage of starch hydrolysis at various times. In Figure 4, the hydrolysis rates of corn starch and sweet potato starch rapidly increased during the first 30 min and stabilised. The corn starch and sweet potato starch were likely to be hydrolyzed by amylase to therefore produce glucose. During the first 120 min, the hydrolysis rate of sweet potato starch phosphodiester increased slowly, and then decreased at a slower rate. After introducing phosphate groups into sweet potato starch, the digestion ability of starch was greatly reduced due to the combination of amylase and starch being inhibited.

Hydrolysis index of sweet potato starch phosphodiester

The GI for various starches was obtained using the percentage of starch hydrolysis at 90 min. The results are listed in Table 3.

Table 3: Hydrolysis rate (H_{90}) and predicted Glycemic index (GI)

Sample	H_{90}	GI
Corn starch	94.92±1.8	115.43
Sweet potato starch	79.11±1.5	102.74
Sweet potato starch phosphodiester (RS58%)	33.75±1.4	66.31

With white bread as the standard, (GI of 100) and a GI for sucrose of 92 it was observed that glucose level in blood increased slightly faster than that with sucrose. The experimental results indicate that both GI of both corn starch and sweet potato starch are greater than 100, which indicates that both corn starch and sweet potato starch were high GI foods. The GI of sweet potato starch phosphodiester (66.31) was between 56 and 69. Therefore, sweet potato starch phosphodiester can be classified as a medium GI food and can therefore be used by diabetic patients as an additive to other foods.

DISCUSSION

Starch is generally classified into three types, namely, RDS, SDS, and RS. Among these, RDS is likely to produce high blood sugar responses and insulin resistance after eating. It is apt to cause chronic diseases or metabolic syndromes. SDS is able to maintain a low blood sugar response since it can continuously digest and release glucose. For RS, it can generate the necessary short-chain fatty acids in the large

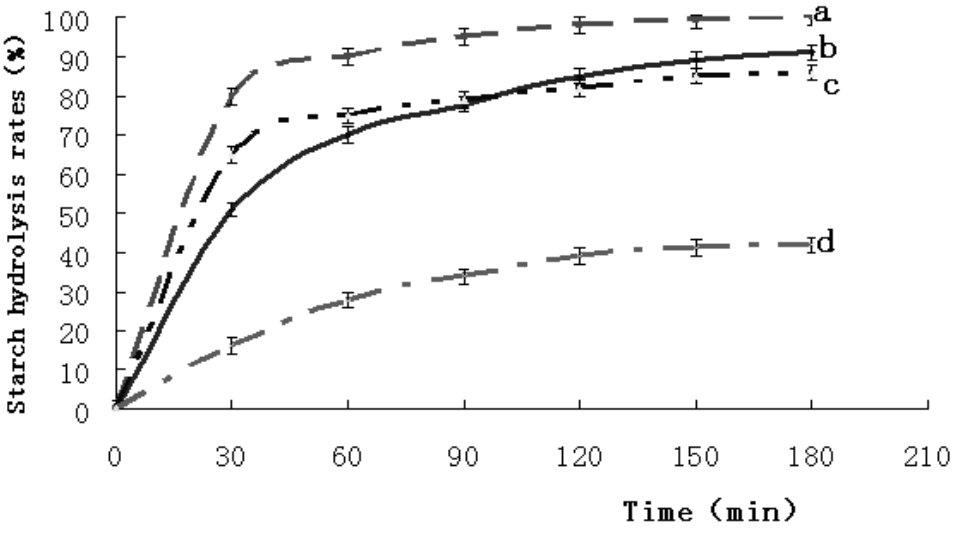

Fig 4: *In vitro* starch hydrolysis rate of the samples. ***Key:*** a = corn starch; b-fresh white bread; c = sweet potato starch; d = sweet potato starch phosphodiester (RS58%)

intestine after microbial fermentation. These short-chain fatty acids promote intestinal health. Robertson *et al* found that a diet with high resistant starch could notably reduce postprandial blood glucose and insulin reaction, and improve insulin sensitivity, which is deemed beneficial in delaying the postprandial blood glucose rise of Type 2 diabetic patients and in the management and control of their condition [3].

The infrared spectrum analysis showed that sweet potato starch had an absorption peak at 1033 cm^{-1} after esterification by sodium trimetaphosphate. The phenomenon indicated that phosphodiester was formed. While DSC determined that sweet potato starch phosphodiester was basically the same as that its raw starch: with the increase of starch digestion resistance, the gelatinization temperature and gelatinization enthalpy decreased. The RVA gelatinization curves showed that with increased digestion resistance, both peak viscosity and initial gelatinization temperature of sweet potato starch phosphodiester decreased. By determining the digestion properties of starch, the RSD content of sweet potato starch phosphodiester was shown to have been lower than that of corn starch and sweet potato starch. While the RS content was higher than that of corn starch and sweet potato starch. The nutritional quality of sweet potato starch after esterification by sodium trimetaphosphate was improved significantly.

Therefore, the sweet potato starch phosphodiester presented not only high digestion resistance but also similar gelatinization properties its raw starch counterpart. As one of the functional foodstuffs, the obtained sweet potato starch phosphodiester can be used as a medium-GI food for diabetic patients.

CONCLUSION

The nutritional quality of sweet potato starch shows improved significantly after esterification by sodium trimetaphosphate. Furthermore, the material presents not only high digestion resistance but also similar gelatinization properties to its raw starch counterpart. Thus, sweet potato starch phosphodiester has good application prospects in the functional foods and pharmaceutical industry.

ACKNOWLEDGEMENT

This study was funded by Program for Innovative Research Team (Science and Technology) of University of Henan Province (13IRTSTHN006), China, and Major Breeding Project of Henan Institute of Science and Technology (2012ZD03), China.

REFERENCES

1. *Chung C, Sanguansri L, Augustin MA. Resistant starch modification: effects on starch properties and functionality as co-encapsulant in sodium caseinate-based fish oil microcapsules. J Food Sci 2010; 75（9): E636-E642.*
2. *Zhang J, Wang ZW. Optimization of reaction conditions for resistant Canna edulis Ker starch phosphorylation and its structural characterization. Industrial Crops and Products 2009; 30(1): 105-113.*
3. *Robertson MD, Bickerton AS, Dermis AL, Vidal H, Frayn K.N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 2005; 82 (3): 559-567.*
4. *Rong H, Can-Peng L, Deyi C, Gaihong Z, Weihua C. Preparation of phosphorylated starch by dry-heating in the presence of pyrophosphate and its calcium-phosphate solubilizing ability. J Food Sci Tech 2013; 50 (3): 561-566.*
5. *Chung HJ, Deulre M, Kim JY, Lim, ST. Effect of minor addition of xanthan on cross-linking of rice starches by dry heating with phosphate salts. J App. Polym Sci 2007; 105（4）: 2280-2286.*
6. *Lars P, Falk L, Klaus F. Synthesis and properties of novel hydrogels from cCross-linked starch phosphates. Macromol Symp 2006; 244(1): 180-193.*
7. *Fukuoko M, Gen I, Konoo S, Watanabe, H. Gelatinization properties of cross-linked phosphate di-ester type tapioca starch. J Jpn Soc Food Sci 2004; 51(11): 637-640.*
8. *Goni. I, Alejandra A. A starch hydrolysis procedure to estimate glycemic index. Nutri Res 1997; 17 (17): 427 -437.*
9. *Jenkins DJA, Wolever TMS, Taylor RH. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34: 362-366.*
10. *Mahmoud ZS, Salah ML, Said SE. Optimizing the conditions for starch dry phosphorylation with sodium mono- and dihydrogen orthophosphate under heat and vacuum. Starch/Stake 2000; 52(4): 95-100.*
11. *Goni I, Garcia-dizl. Anlysis of resistant starch: a method for foods and food product. Food Chemistry 1996; 56(4): 445-449.*
12. *Ornanong S, Kittipongpatana N K . Physicochemical, in vitro digestibility and functional properties of carboxymethyl rice starch cross-linked with*

epichlorohydrin. Food Chemistry 2013; 141(2): 1438-1444.

13. *Zabidi M A, Aziz NAA. In vitro starch hydrolysis and estimated glycaemic index of bread substituted with different percentage of chempedak (Artocarpus integer) seed flour. Food Chem 2009; 117 (1): 64-68.*
14. *Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care 2008; 31(12): 2281-2283.*