Original Research Article

Preparation and *In vitro* Digestibility of Corn Starch Phosphodiester

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Abstract

Purpose: To optimize the process conditions and analyze in vitro digestibility of corn starch phosphodiester prepared by sodium trimetaphosphate (STMP).
Methods: By using response surface method, the effects of STMP concentration, pH, esterification temperature, and urea addition on digestion resistance of corn starch phosphodiester were investigated and optimal conditions were determined. Corn starch phosphodiester was identified by Fourier transform infrared spectroscopy (FT-IR) and an in vitro digestibility method was applied to investigate starch digestion performances of corn starch phosphodiester.
Results: The optimum conditions for the preparation of corn starch phosphodiester were as follows: STMP, 3.3 %; pH 9.07; esterification temperature, 42 °C; and urea, 2.3 %. Under these conditions, corn starch phosphodiester with a digestion resistance of 66.02 % ± 1.63 % was obtained. Compared with corn starch, the digestible starch content of corn starch phosphodiester decreased sharply (from 86.6 to 10.9 %), while digestion-resistance starch content increased significantly from 1.9 to 66.0 % (p < 0.05), and its digestibility was reduced. FTIR spectrum showed new absorption peaks at 1028 cm-1 indicating that an esterification cross-linking reaction occurred between corn starch and sodium trimetaphosphate.
Conclusion: The corn starch phosphodiester obtained has a lower digestbility and therefore, can potentially be used as a medium glycemic index food for diabetic patients.

Keywords: Corn starch phosphodiester, Digestion resistance, Sodium trimetaphosophate, In vitro digestibility, Glycemic index

INTRODUCTION

With rapid socio-economic growth, diabetes mellitus has become one of the chronic diseases with the greatest toll on human health while incurring a significant proportion of the government's health budget for disease management, treatment, and consequences [1]. Epidemiological research found that diabetes mellitus can be directly, or indirectly, diet-induced and is affected by insufficient dietary fibre intake [2]. However, dietary fibre consumption has disadvantages such as odor, rough structure, and undesirable taste, all of which make it unattractive to consumers, leading to disincentives in its marketing, promotion, and uptake [3]. 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 in Type 2 diabetic patients and in the management and control of their condition [4].

Chung *et al* has indicated that starch phosphodiester possesses a certain digestion resistance [5], and the distarch phosphate products prepared to use STMP as the raw material all met high edible safety standards. Currently, the studies on the preparation technology of starch phosphodiester 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.

Based on the single-factor experiment, the response surface method was used to optimize the process conditions of corn starch phosphodiester prepared by STMP with starch digestion resistance as a research objective. An *in vitro* digestion kinetic method proposed by Goni [8] was used to simulate the human gastrointestinal system in order to further investigate the in vitro digestion performances of corn starch phosphodiester. This research aimed to provide a reference for the industrial production of digestion resistance corn starch phosphodiester, and promote its application in the food and pharmaceutical industry.

EXPERIMENTAL

Materials

Corn starch was provided by Agriculture Development Limited Company, Hezai, Henan. α-amylase and glucoamylase were provided by Fuyuan Biological Science and Technology Limited Company, Zhengzhou, Henan. The other chemicals were of analytical grade.

Preparation of starch phosphodiester

The preparation of starch phosphodiester was carried out according to a previous report [9]. A quantity of corn starch was mixed with a certain amount of STMP, sodium chloride, sodium hydroxide, urea, and water in sequence. The resulting solution was kept at 40 °C and continuously stirred for 2 h, thereafter, its pH was adjusted to approximately 6.5. After water-washing and four centrifugation stages, it was dried at 40 °C, crushed, and sieved (through a 180 μm sieve).

Measurement of starch digestion resistance

Starch digestion resistance was measured according to a published method [10]. The starch sample (100 mg) was mixed with buffer solution. After its pH was adjusted to 1.5, pepsin solution was added. The resulting solution was maintained at 40 °C for 1 h, cooled to room temperature, and adjusted to a pH between 6.0 and 7.0. Thereafter the thermostable amylase was added, the resulting solution was then kept at 90 °C for 30 min, cooled to room temperature, and adjusted to the pH of 4.75. Glucoamylase solution was added to the solution, the obtained solution was kept at 60 °C for 1 h, cooled to room temperature and centrifuged. The remaining sediment was mixed with 4 M KOH solution to ensure complete dissolution, which was then neutralized with an HCl solution. After glucoamylase solution was added, the resulting solution was kept at 60 °C for 1 h, cooled to room temperature, and centrifuged. The supernatant was diluted to 100 mL with distilled water and reducing sugar content determined by 3, 5-dinitrosalicylic acid (DNS) method [11]. The obtained results were multiplied by 0.9, and this final yielding result was the resistant starch content. Starch digestion resistance (SDR) was calculated as the ratio of resistant starch to total corn starch phosphodiester, expressed as a percentage.

Single-factor experiment

By employing the single-factor experiment design with five pre-set levels, the effect of the four reacting factors: STMP concentration (according to corn starch), pH, esterification temperature, and urea addition (according to corn starch), on the anti-digestibility of corn starch phosphate diester was investigated. All experiments were carried out in triplicate and the mean taken.

Response surface experiment

The response surface experiment for the four factors at their three levels was designed by Design-Expert (Version 7.1.3) software. Moreover, starch digestion resistance, as a response value, was analyzed. The model could be expressed as a quadratic polynomial, least-squares, or best-fit (see Eq 1).

$$Y = b_0 + \sum_{n=1}^{4} b_n x_n + \sum_{n=1}^{4} b_{nn} x_n^2 + \sum_{n \neq m-1}^{4} b_{tm} x_n x_m \ \cdots (1)$$

where Y was the response value; b_0, b_n, b_{nn}, and b_{nm} refer to the coefficients, x_n, x_m ($n \neq m$) were the coded values of the independent variable. The goodness-of-fit of the polynomial was expressed by its coefficient of determination R^2. Its statistical significance was tested by F-test, and Design-Expert (Version 7.1.3) software was used for the analysis of variance [12].

Fourier transform infrared spectroscopy (FT-IR)

In order to further determine the structure of the corn starches, the 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 400 cm^{-1} (mid-infrared region) at a resolution of 0.44 cm^{-1}.The sample was mixed with KBr (1:100, w/w) prior to data acquisition and the background value of pure KBr was acquired prior to scanning the sample.

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 gelatinized 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 [13]. The mass fraction of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistance starch (RS) in the test samples was computed as in Eqs 2 – 4, respectively.

$$RDS(\%) = \{[(G_{20}-FG) \times 0.9]/TS\} \times 100 \dots\dots (2)$$
$$SDS(\%) = \{[(G_{120}-G_{20}) \times 0.9]/TS\} \times 100 \dots (3)$$
$$RS(\%) = \{[TS-(RDS+SDS)]/TS\} \times 100 \dots\dots (4)$$

where G_{20} denotes the glucose content after conducting amylase hydrolysis for 20 min; G_{120} denotes the glucose content after conducting amylase hydrolysis for 120 min; while FG refers to the free glucose content in starch before conducting enzymatic hydrolysis; TS represented the total starch content in 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 hydrolysis, a reaction solution of 1 mL was removed and the removal time was recorded until the reaction was stopped. DNS method was applied to determine reducing sugar content and calculate starch hydrolysis rate (SHR%) as in Eq 5 [14].

$$SHR (\%) = [(G_t \times 25 \times 0.9)/200] \times 100 \dots\dots (5)$$

Where G_t denoted glucose content after conducting amylase hydrolysis for t min.

Statistical analysis

Statistical analysis was carried out using DPS 7.05 software (Zhejiang University, Hangzhou, China). All measurements were repeated three times and mean ± standard deviation obtained. Statistical comparisons were carried out using Dixon test. $P < 0.05$ was considered statistically significant.

RESULTS

Analysis of single factor experiment

The factors and levels, except the research objective itself, were set at an STMP concentration of 3 %, a pH of 9, an esterification temperature of 40 °C, and urea addition of 2 %, through which, single factor test results were acquired (Table 1).

The starch digestion resistance of corn starch phosphodiester increased with the increase of STMP concentration. However, when STMP

Table 1: Single-factor experiment protocols and results

STMP (%)	Digestion resistance (%)	pH	Digestion resistance (%)	Temperature (℃)	Digestion resistance (%)	Urea (%)	Digestion resistance (%)
1	44.07	7	40.86	20	51.77	1	50.81
2	52.46	8	50.23	30	53.65	2	55.86
3	55.12	9	55.40	40	55.78	3	56.76
4	55.39	10	59.37	50	56.54	4	56.93
5	53.87	11	56.42	60	55.89	5	55.47

concentration exceeded 4 %, its starch digestion resistance declined. When the pH was 10, the starch digestion resistance reached its highest value. With the increase of reaction temperature and urea addition, the starch anti-digestibility rose, but when the reaction temperature reached 40 °C a urea addition of 2 %, its rate slowed.

Regression model for the preparation of corn starch phosphodiester

Based on single factor experimental data, and with due regard to the starch digestion resistance and economic cost, the middle experimental level of the independent variables for the response surface experiment were set at an STMP concentration of 3 %, a pH of 10, a reaction temperature of 40 °C, and 2 % urea addition. The four factors were represented by: X_1, X_2, X_3, and X_4 respectively: the low, middle, and high experimental levels of each independent variable were separately coded as: -1, 0, and 1 (Table 2).

Table 2: Variables and their levels of central composite design

Variable	Symbol	Coded-variable level		
		-1	0	1
STMP (%)	X_1	2	3	4
pH	X_2	9	10	11
Temperature (°C)	X_3	30	40	50
Urea (%)	X_4	1	2	3

The four-factor, three-level experiment was carried out according to typical protocols for a central combination of response surface experiment. Each treatment was replicated three times, and an average value taken. The experimental data obtained were used to perform a multiple regression fit using Design-Expert software, thereby acquiring the quadratic regression equation with starch digestion resistance as its objective function.

$Y= -367.4678 + 35.3399 \times x_1 + 67.6946 \times x_2 + 2.5763 \times x_3 + 9.1354 \times x_4 - 0.5600 \times x_1 \times x_2 - 0.1645 \times x_1 \times x_3 - 0.8225 \times x_1 \times x_4 - 0.0718 \times x_2 \times x_3 + 0.3150 \times x_2 \times x_4 - 0.0700 \times x_3 \times x_4 - 3.2954 \times x_1^2 - 3.5054 \times x_2^2 - 0.0148 \times x_3^2 - 1.4054 \times x_4^2$ ....(6)

It was revealed by the regression equation that, the pH and STMP concentration showed the most obvious influence on the starch digestion resistance of the phosphodiester of corn starch. To verify the effectiveness of the equation, an analysis of variance was carried out on the mathematical model of the preparation process for corn starch phosphodiester. The results were listed in Table 3.

The lack of fit term (F = 0.66) in the variance analysis suggested that the lack of fit of the test to the regression equation was not significant, indicating that those unknown factors presented little disturbance to the experimental results. The F value of 13.73 for the model explained that the regression equation was statistically significant ($p < 0.01$). Relative coefficients of determination, R^2 = 0.9276, expressed the fact that 92.76 % of the fluctuation of the response value (starch digestion resistance) stemmed from the selected variables. Therefore, the regression equation represented a good fit to experimental conditions with acceptably low test errors. Furthermore, it could more properly illustrate the real relationship between each factor and the response value and may thus be used to analyze experimental data as a simulated substitute for full-scale, real-life cases.

Response surface plots

Through the regression equation, the response surface and contour plots of each factor on the starch digestion resistance were obtained by using Design-Expert software. In Figure 1, the effect of STMP concentration and pH on the indigestibility of starch was demonstrated.
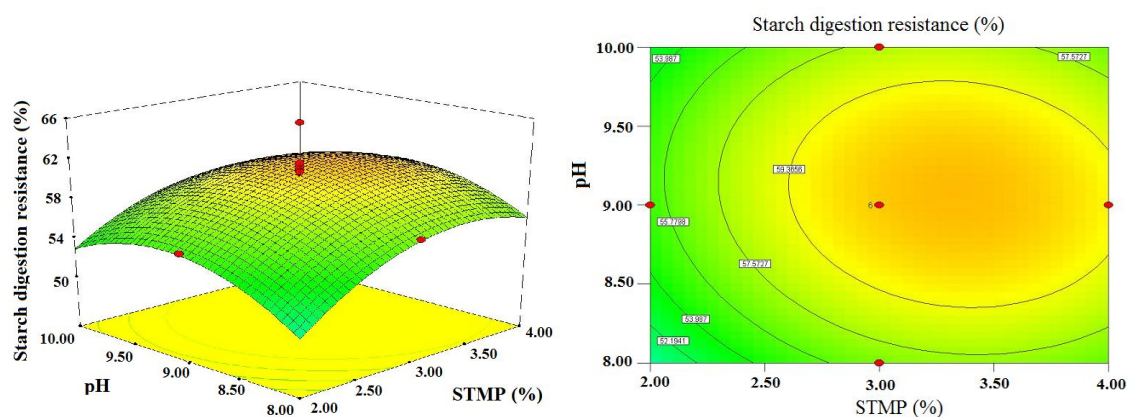
Table 3: ANOVA for the fitted model

Source	Sum of squares	d_f	Mean square	F value	Prob＞F
Model	768.02	14	54.86	13.73	＜0.0001
Residual	59.94	15	4.00		
Lack of Fit	34.20	10	3.42	0.66	0.7282
Pure error	25.74	5	5.15		
Cor total	827.96	29			
			R^2 = 0.9276		

Fig 1: Effect of STMP concentration and pH on starch indigestibility of corn starch phosphodiester

As shown in Figure 1, when the esterification temperatures were at their central level of 40 °C and for a urea addition of 2 %, the interaction of the STMP concentration and pH was apparent. Moreover, within the range of 2 % ≤ STMP ≤ 3.5 %, the digestion resistance of the starch sample grew with increasing STMP concentration. However, when the STMP concentration was too high, it subsequently decreased.

Verification of the process

Within the range of each selected factor, the optimum preparation process of the phosphodiester of anti-digestive corn starch was obtained according to the regression model and analysis by Design-Expert software. The optimum conditions were determined as following: STMP concentration 3.3 %, pH 9.7,

esterification temperature 42 °C, urea concentration 2 %.

Under the optimum conditions, the average starch digestion resistance over these three experiments was 66.02 % ± 1.63 %, which was in agreement with the predicted value of 66.71 %. The model, therefore represented a good fit to reality and indicated its validity.

Infrared scanning analysis

The IR spectra of corn starch, corn starch phosphodiester (phosphorus content 0.021 %) and corn retrogradation resistant starch (RS = 24 %) were shown in Figure 2.

The corn starch phosphodiester had similar infrared absorption characteristics to those of raw starch and retrogradation starch, mainly presented a small adsorption peak at 1028 cm^{-1}.
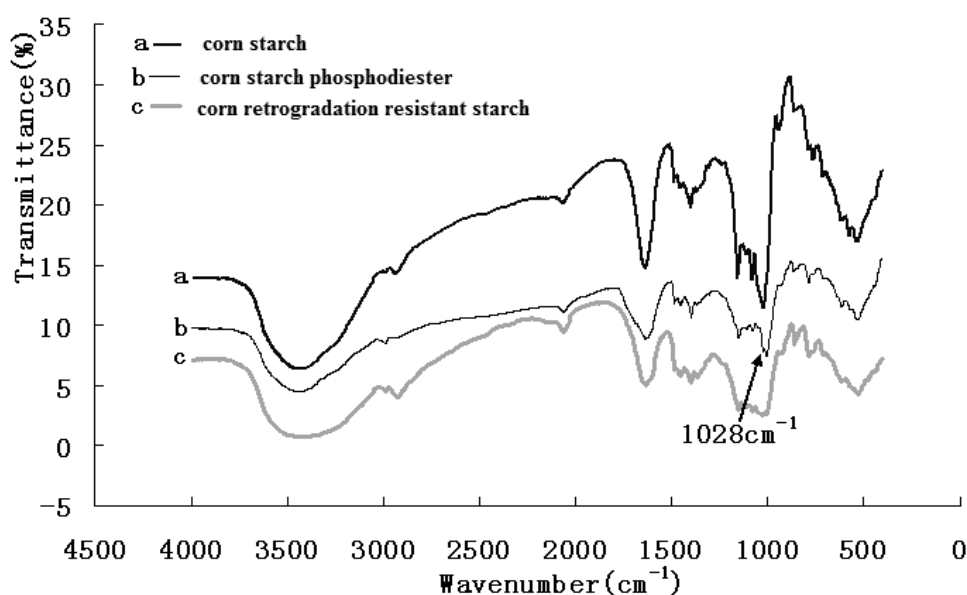

Fig 2: FTIR spectra of corn starch and its phosphodiester

By an esterification cross-linking reaction of corn starch and STMP, the increased strength of the adsorption peak at 1028 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 corn starch and STMP introduced phosphate groups into the corn starch with small adsorption peaks present. Moreover, the infrared spectra showed that the characteristic absorption peaks of other groups underwent no apparent change, which indicated that the esterification cross-linking reaction merely added some new groups to the starch chain and did not damage its underlying chemical structure.

Starch digestion performance

The *in vitro* digestion performances of corn starch, corn starch phosphodiesters and corn retrogradation resistant starch were listed in Table 4.

As shown in Table 4, the RDS content of corn starch was the highest (86.6 %), while the RS content was approximately 2 %. With the increase of phosphorus content, the RS content of corn starch after modification by

phosphodiester increased from 1.9 to 66.0 %, while RDS content decreased from 86.6 to10.9 %, which suggested that the nutritional quality of corn starch phosphodiester had been significantly improved.

In vitro digestion rate

By referring to fresh white bread, the *in vitro* digestion rates of corn starch, corn starch phosphodiesters and corn retrogradation resistant starch were determined (Figure 3). The *in vitro* digestion rate was defined as the percentage of starch hydrolysis at various times. In Figure 7, the hydrolysis rates of corn starch rapidly increased during the first 30 min and stabilized. The corn starch was likely to be hydrolyzed by amylase to therefore produce glucose. The corn starch phosphodiesters exhibited a slower absorption than corn starch and fresh white bread. During the first 120 min, the hydrolysis rate of corn starch phosphodiester (phosphorus content 0.026 %) increased slowly, and then decreased at a slower rate. After introducing phosphate groups into corn starch, the digestion ability of starch was greatly reduced due to the combination of amylase and starch being inhibited.

Table 4: *In vitro* digestibility of test samples

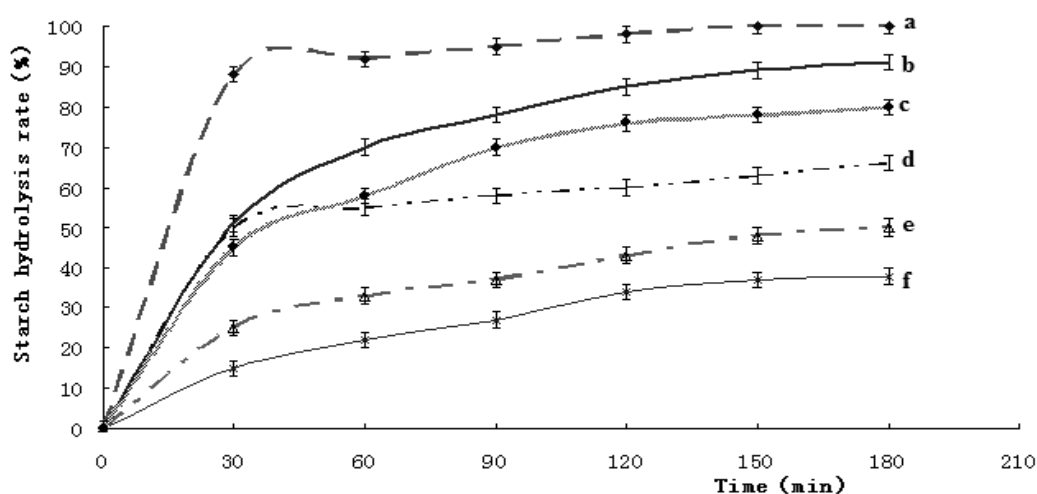| Sample	RDS (%)	SDS (%)	RS (%)
Corn starch	86.6±1.9	11.5±1.1	1.9±1.0
Corn starch phosphodiester (phosphorus content, 0.013 %)	41.7±1.6	18.1±1.1	40.2±1.4
Corn starch phosphodiester (phosphorus content, 0.021 %)	22.2±1.2	20.7±1.3	57.1±1.4
Corn starch phosphodiester (phosphorus content, 0.026 %)	10.9±0.9	23.1±1.3	66.0±1.6
Corn retrogradation resistant starch (RS 24 %)	41.8±1.6	34.2±1.3	24.0±1.4

Fig 3: *In vitro* starch hydrolysis rate of the samples. ***Key:*** a = corn starch; b-fresh white bread; c = corn retrogradation resistant starch (RS 24 %); d = corn starch phosphodiester(phosphorus content 0.013 %); e = corn starch phosphodiester(phosphorus content 0.021 %); f = corn starch phosphodiester(phosphorus content 0.026 %)

DISCUSSION

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. As one of the new, functional, low-calorie foodstuffs, it has become one focus of food science research [15,16].

Starch phosphate is derived from the phosphorylation of starch. It can be sub-divided into two types: monoester and diester. Generally, it can be produced from orthophosphate, tripolyphosphate, metaphosphate, etc. Reviewing the preparation and application history of starch ester, it is observed that those with commercial value are the products with sol stability and low price [17,18]. The distarch phosphate products prepared by using STMP as the raw material all met high edible safety standards. The Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) regard the product as edible without setting an acceptable daily intake (ADI) value.

The mean starch digestion resistance of corn starch phosphodiester under the optimized conditions employed was 66.02 ± 1.63 %. The infrared spectrum analysis showed that corn starch had an absorption peak at 1028 cm^{-1} after esterification by STMP, which indicate that phosphodiester was formed. The digestion ability of the starch was reduced by the introduction of phosphate groups.

CONCLUSION

Based on the Box-Behnken central composition design and a standard response surface method, the preparation conditions for the production of phosphodiester of digestion-resistant corn starch were optimized, and the nutritional quality of corn starch after esterification by STMP was improved significantly. Thus, corn starch phosphodiester has good application prospects in the functional foods and pharmaceutical industry.

ACKNOWLEDGEMENT

REFERENCES

1. *Nuevo R, Chatterji S, Fraguas D, Verdes E, Naidoo N, Arango C, Ayuso-Mateos JL. Increased risk of diabetes mellitus among persons with psychotic symptoms: results from the WHO world health survey. J Clin Psychiatry 2011; 72(12): 1592-1599.*
2. *Slavin JL. Dietary fiber and body weigh. Nutrition 2005; 21: 411-418.*
3. *Brouns F, Kettlitz B, Arrigoni E. Resistant starch and "the butyrate revolution". Trends Food Sci Tech 2002; 13(8): 251-261.*
4. *Robertson MD, Bickerton AS, Dermis AL, Vidal H, Frayn KN. 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.*
5. *Jenkins DJA, Wolever TMS, Taylor RH. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34: 362-366.*
6. *Lars P, Falk L, Klaus F. Synthesis and properties of novel hydrogels from cross-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. Nutr Res 1997; (17): 427-437.*
9. *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.*
10. *Goni I, Garcia D. Anlysis of resistant starch: a method for foods and food product. Food Chem 1996; 56(4): 445-449.*
11. *Teixeira RS, Silva AS, Ferreira-Leitão VS, Bon EP. Amino acids interference on the quantification of reducing sugars by the 3,5-dinitrosalicylic acid assay mislead carbohydrase activity measurements. Carbohydr Res 2012; 363:33-37.*
12. *Razali MAA, Sanusi N, Ismail H, Othman N, Ariffin A. Application of response surface methodology (RSM) for optimization of cassava starch grafted polyDADMAC synthesis for cationic properties. Starch/Stake 2012; 64(12): 935-943.*
13. *Ornanong S, Kittipongpatana NK . Physicochemical, in vitro digestibility and functional properties of carboxymethyl rice starch cross-linked with epichlorohydrin. Food Chem 2013; 141(2): 1438-1444.*
14. *Zabidi MA, 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.*
15. *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.

16. *Zhang J, Wang ZW. Optimization of reaction conditions for resistant Canna edulis Ker starch phosphorylation and its structural characterization. Ind Crop Prod 2009; 30(1): 105-113.*

17. *Lars P, Falk L, Klaus F. Starch phosphate hydrogels: synthesis by mono-phosphorylation and cross-linking of Starch. Starch/Stake 2009, 69(11): 621-627.*

18. *Rong H, Canpeng 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.*