Food Chemistry 174 (2015) 173–179

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Optimisation of the reaction conditions for the production of cross-linked starch with high resistant starch content

Kevser Kahraman [a], Hamit Koksel [b,*], Perry K.W. Ng [c]

[a] Department of Food Engineering, Faculty of Natural Science and Engineering, Abdullah Gul University, 38039, Melikgazi, Kayseri, Turkey
[b] Department of Food Engineering, Faculty of Engineering, Hacettepe University, 06800, Beytepe, Ankara, Turkey
[c] Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form 19 October 2014
Accepted 5 November 2014
Available online 11 November 2014

Keywords:
Cross-linked starch
RS4 type starch
Resistant starch
Response surface methodology

ABSTRACT

The optimum reaction conditions (temperature and pH) for the preparation of cross-linked (CL) corn and wheat starches with maximum resistant starch (RS) content were investigated by using response surface methodology (RSM). According to the preliminary results, five levels were selected for reaction temperature (38–70 °C) and pH (10–12) in the main study. RS contents of the CL corn and wheat starch samples increased with increasing temperature and pH, and pH had a greater influence on RS content than had temperature. The maximum RS content (with a maximum *p* value of 0.4%) was obtained in wheat starch cross-linked at 38 °C and pH 12. In the case of CL corn starch, the optimum condition was 70 °C and pH 12. CL corn and wheat starch samples were also produced separately under the optimum conditions and their RS contents were 80.4% and 83.9%, respectively. These results were also in agreement with the values predicted by RSM.

1. Introduction

Resistant starch (RS) has been defined as the starch fraction which escapes digestion in the small intestine of healthy individuals (Englyst, Kingman, & Cummings, 1992). Due to its similar physiological properties, it is considered as a constituent of dietary fibre. As it is fermented to short chain fatty acids in the colon, it may improve colonic health (Huth, Dongowski, Gebhardt, & Flamme, 2000; Wollowski, Rechkemmer, & Pool-Zobel, 2001). It lowers plasma cholesterol and lipids and improves glucose tolerance (Niba & Hoffman, 2003; Voragen, 1998). For a healthy diet, it is recommended to decrease fat intake and increase dietary fibre consumption (Wolf, Bauer, & Fahey, 1999). RS can be found naturally in foods in the range of 0–4%; however, a higher amount of RS in the diet is recommended, due to its preventative and therapeutical health effects (Lehmann, Rössler, Schmiedl, & Jacobasch, 2003). Therefore, there has been a great interest in increasing RS content of foods by various modification techniques. RS is classified into four types: physically inaccessible starch (RS1), native granular starch (RS2), retrograded starch (RS3), and chemically modified starch (RS4) (Eerlingen, Crombez, & Delcour, 1993; Shamai, Bianco-Peled, & Shimoni, 2003; Patil, 2004).

Cross-linking (CL) is one of the chemical modification techniques that have been used to improve functional properties, and freeze-thaw and cold storage stabilities of starch pastes (Chung, Woo, & Lim, 2004; BeMiller, 2011). It has also been reported that cross-linking of starches, using reagents such as STMP (sodium trimetaphosphate), or an STMP–STPP (sodium tripolyphosphate) mixture, caused resistance to digestion and therefore these resultant starches fit within the RS4 category (Woo, 1999; Woo, Maningat, & Seib, 2009). Cross-linking is generally performed by treatment of granular starch with multifunctional reagents capable of forming ether or ester linkages between hydroxyl groups on starch molecules (Rutenberg & Solarek, 1984; Wurzburg, 1986a,b; Singh, Kaur, & McCarthy, 2007). Cross-linking is intended to add intra- and inter-molecular bonds at random locations in the starch granule that stabilize and strengthen the granule (Acquarone & Rao, 2003; Singh et al., 2007). Xie and Liu (2004) indicated that CL starches cannot be completely digested since the substituents hinder enzymatic attack.

The chemical and functional properties of the CL starches depend on the starch source, reaction conditions (reaction time, temperature, pH, presence of catalyst) and type/concentration of reactant (Lim & Seib, 1993; Hirsch & Kokini, 2002; Wang & Wang, 2002; Singh et al., 2007). Woo and Seib (2002) reported that the RS contents of CL wheat, potato, corn and rice starches reached 75.7%, 72.8%, 57.8% and 5.4%, respectively, after a 3 h cross-linking reaction at 45 °C and pH 11.5, using STMP:STPP (12%, sb: starch

* Corresponding author. Tel.: +90 312 297 7107; fax: +90 312 299 2123.
E-mail addresses: kevser.kahraman@agu.edu.tr (K. Kahraman), koksel@hacettepe.edu.tr (H. Koksel), ngp@msu.edu (P.K.W. Ng).

http://dx.doi.org/10.1016/j.foodchem.2014.11.032
0308-8146/

basis) mixture (99:1). Yeo and Seib (2009) had cross-linked wheat starch under the same cross-linking conditions as Woo and Seib (2002) and achieved an RS content of 80%. Woo and Seib (2002) also investigated the effect of reaction time on the RS content of CL starches and reported that the RS contents of the CL wheat starches increased as the reaction time increased. Chung et al. (2004) investigated the effect of STMP:STPP (99:1) amount on the RS content of CL wheat starches. The RS contents of the CL starches cross-linked at 45 °C, pH 11.5, for 3 h, using 4%, 8% and 12% (sb) STMP:STPP (99:1), were increased to 24.5%, 60.0% and 81.6%, respectively (Chung et al., 2004).

Response surface methodology (RSM) is a statistical method, used to design experiments, build models, evaluate the effects of factors and search optimum conditions of factors for desirable responses (Myers & Montgomery, 1995). RSM generates a mathematical model that accurately describes the overall process with limited number of experiments (Senanayake & Shahidi, 2002). Although, various investigators (Woo & Seib, 2002; Chung et al., 2004; Yeo & Seib, 2009) have produced CL wheat starches at some reaction temperature, pH and time combinations, as well as utilising various STMP:STPP concentrations, investigating the effects of temperature and pH to obtain maximum RS content, use of RSM is expected to bring about additional significant information. In this study RSM was used to investigate the optimum reaction conditions (temperature and pH) for the preparation of cross-linked corn and wheat starches with high RS content with a permissible phosphorus level of 0.4%.

2. Materials and methods

2.1. Materials

Corn and wheat starches were obtained from Ingredion Inc. (formerly National Starch Chemical Co., Bridgewater, NJ, USA) and MGP Ingredients (Atchison, KS, USA), respectively. The chemicals used in the study were of analytical grade unless stated otherwise.

2.2. Methods

2.2.1. Production of cross-linked starch

Cross-linked starch samples were produced according to the method of Woo and Seib (2002) with some modifications of the reaction conditions. Corn and wheat starches (50 g, db) were dispersed in 70 ml of water containing 12% STMP:STPP (99:1) and 10% sodium sulphate, both based on starch weight. The dispersion was allowed to react at various temperature (T) and pH combinations (Table 1), using a magnetic stirrer equipped with a temperature controller (Heidolph Mei Tech Heater, Germany). After a 3 h reaction time, the pH of the dispersion was adjusted to 6.5 by adding 1.0 M NaOH in order to stop the reaction. The starch was collected by centrifugation, washed with distilled water (140 ml, 7 times), dried at 50 °C and ground to pass through a 212 μm sieve. Moisture contents of the samples were determined according to AACCI Approved Method 44-15A (AACCI, 2000).

Table 1
Independent variables and their levels used in the preliminary and main experiments.

Independent variables		Coded factor levels[a]				
		−1	−0.5	0	+0.5	+1
Preliminary experiments	pH (X_1)	8	9	10	11	12
	Temperature (X_2, °C)	25	35	45	55	65
Main experiments	pH (X_1)	10	10.5	11	11.5	12
	Temperature (X_2, °C)	38	46	54	62	70

[a] Independent variable levels were assigned coded factor designations for purposes of statistical analysis.

2.2.2. Experimental design for the cross-linked starch production

In this study, response surface methodology (RSM) was used to optimise the cross-linking conditions of corn and wheat 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. Resistant starch (RS) and phosphorus (P) contents of the cross-linked samples were selected as the dependent variables in order to estimate the RS production efficiency.

Preliminary experiments were conducted to select the approximate range for each independent variable before the optimisation process. In the preliminary experiments, five levels were selected for both reaction temperature and pH. Temperature and pH were varied from 25 °C to 65 °C and from 8 to 12, respectively (Table 1). Based on the preliminary experiments, narrower temperature and pH ranges were selected for the main experiments. For the main experimental design, five levels, for each factor, were selected and reaction temperature was varied from 38 °C to 70 °C and reaction pH was varied from 10 to 12 (Table 1).

2.2.3. Determination of RS content as total dietary fibre

In the method commonly used for the determination of RS content (AACCI 32–40; AACCI, 2000), the samples are digested using α-amylase and amyloglucosidase. The undigested part (RS) is dissolved with KOH and converted into glucose by using amyloglucosidase. Then RS content is estimated, based on the amount of glucose determined by GOPOD reagent. However, CL starches were not dissolved to any considerable extent, as has also been indicated in the literature (Wu & Seib, 1990; Hwang et al., 2009). Therefore, it was not possible to use AACCI Method 32–40 for the determination of RS content in CL starches. Hence, RS contents of the CL starches were determined according to "AOAC 991.43: Total dietary fibre determination method" (AOAC, 1998). Sequential enzymatic digestion was applied to the samples using heat-stable α-amylase, protease and amyloglucosidase to remove digestible starch and protein. Enzyme digestate was treated with alcohol before filtering, and total dietary fibre residue was washed with alcohol and acetone, dried, weighed and expressed as % (g RS/100 g dry sample). RS contents of the native corn and wheat starch samples were also determined, using the same procedure.

2.2.4. Determination of phosphorus content

Phosphorus (P) contents of the native and CL starch samples were determined according to the AOAC 986.24 Method (AOAC, 1998) with some modifications. A starch sample (1 g) was weighed into a porcelain crucible and ignited in a muffle furnace at 700 °C for 16 h. After cooling, 10 ml of HCl (25% v/v) and 1 ml of HNO_3 were added to each crucible. The mixture was brought to boil on a hot plate, cooled and transferred quantitatively into a 100 ml flask and diluted to volume with distilled water. One ml of this solution was mixed with 1 ml of vanadate-molybdate solution (Fluka, Switzerland) and 3 ml of distilled water. The absorbance of the solution was measured at 400 nm (Ultraspec III, Pharmacia LKB Biochrom Ltd., England) after the solution was allowed to stand for 10 min at room temperature. A calibration curve was prepared with standard phosphate solutions (KH_2PO_4) containing 0.1–0.6 mg of phosphorus per ml. Phosphorus contents were calculated as follows:

$$P = \frac{A}{16.76} \quad (1)$$

where A is the absorbance of the sample solution.

2.2.5. Statistical analysis

All experiments were performed in duplicate and mean values were recorded. RS and P contents of the samples were analysed, 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 paired t-tests were carried out to compare the RS and P contents of cross-linked corn and wheat starches.

In order to describe the relationship between the dependent variable (RS or P contents) and the independent variables (T and pH), the response values (RS or P contents) were fitted by a second-order polynomial (quadratic) regression model, using a multiple regression analysis (Standard Least Square Fitting) using Design Expert (Stat-Ease, Minneapolis, MN, USA) (Eq. (2));

$$Y = b_0 + \sum_{i=1} b_i X_i + \sum_{i=1} b_{ii} X_{ii}^2 + \sum_{i}^{i<j} \sum_{j} b_{ij} X_i X_j \quad (2)$$

where Y is the response (RS or P content), b_0 is a constant value, X_i and X_j are the independent variables (T and pH, respectively) in coded values (see designations in Table 1). b_i, b_{ii} and b_{ij} are the regression coefficients of the model and show the linear (main) effect, the quadratic effects of X_i, and the interaction effect between X_i and X_j, respectively, for the responses. To describe both individual and interactive effects of the independent variables of cross-linked starch production on RS and P contents, three-dimensional response surface plots were also developed by Design Expert (Stat-Ease, Minneapolis, MN, USA).

3. Results and discussion

3.1. Preliminary study: selection of experimental design

In order to select the appropriate range for the optimum reaction conditions, preliminary experiments were conducted according to the experimental design shown in Table 2. The experimental points generated by Design Expert Software, their coded levels, and the observed responses (RS and P contents), for the CL corn and wheat starch samples produced in the preliminary experiments, are shown in Table 2.

As can be observed from Table 2, the RS contents of CL wheat starches were generally higher than those of the CL corn starches. This was also revealed by the paired t-test analysis ($p < 0.05$). RS contents were relatively low for the CL starch samples prepared from both types of starches at the pH range of 8–10. At the reaction temperature of 45 °C, RS contents of CL corn starches were low and not significantly different for the reaction pHs of 9 and 10, while the RS contents significantly increased as the reaction pH was increased to 11 ($p < 0.05$), indicating higher levels of cross-linking at pH 11. A similar trend was also observed in CL wheat starches produced at 45 °C. Substantial increases were observed in the RS contents of the CL starches produced at pH 12 as the reaction temperature increased from 25 °C to 65 °C ($p < 0.05$).

The P contents of the CL wheat starches were significantly higher than those of CL corn starches, as revealed by the paired t-test ($p < 0.05$). Similar to the RS results, P contents of the CL starch samples, prepared from both types of starches, were lower in samples produced at the low reaction pH range (pH 8–10). At the reaction temperature of 45 °C, P contents of CL corn and wheat starches were low and not significantly different for the reaction pHs of 9 and 10. The P contents significantly increased as the reaction pH was increased to 11 ($p < 0.05$), indicating higher levels of cross-linking at pH 11. Similar to the RS results, considerable increases were observed in the P contents of the CL starches produced at pH 12 as the reaction temperature increased from 25 °C to 65 °C ($p < 0.05$).

Table 2
RS (%) and P (%) contents of the CL corn starch and wheat starch samples prepared in the preliminary experiments.[a,b,c]

Run	Reaction conditions		CL corn starches		CL wheat starches	
	T (°C)	pH	RS (%)	P (%)	RS (%)	P (%)
	Control (native starch)		2.5 d	0.01 g	3.0 d	0.05 c
1	25 (−1.0)	8 (−1.0)	2.9 d	0.05 f	2.0 d	0.06 c
7	25 (−1.0)	12 (+1.0)	17.3 b	0.28 b	62.8 b	0.39 b
6	35 (−0.5)	10 (0)	3.0 d	0.08 e	3.1 d	0.09 c
4	45 (0)	9 (−0.5)	2.5 d	0.08 e	2.7 d	0.06 c
2	45 (0)	10 (0)	2.8 d	0.10 e	2.1 d	0.11 c
5	45 (0)	11 (+0.5)	11.3 c	0.20 c	26.9 c	0.32 b
3	55 (+0.5)	10 (0)	3.9 d	0.10 e	3.2 d	0.13 c
9	65 (+1.0)	8 (−1.0)	2.3 d	0.13 d	2.1 d	0.12 c
8	65 (+1.0)	12 (+1.0)	70.8 a	0.36 a	85.2 a	0.48 a

[a] Coded values are shown in parentheses.
[b] For each sample, means with different letters within each column are significantly different ($p < 0.05$).
[c] CL, cross-linked; RS, resistant starch; P, phosphorus.

3.2. Main study: effect of reaction conditions on RS and P contents of the CL starches

Preliminary cross-linking experiments had shown that a high content of RS was obtained at higher temperature and pH ranges. Therefore, for the main study, the reaction temperature and pH ranges were selected as 38–70 °C and 10–12, respectively. The experimental points for the main study generated by Design Expert Software, their coded levels and the observed responses (RS and P contents) for the CL corn and wheat starch samples, are shown in Table 3.

The RS and P contents of CL wheat starches were significantly higher than those of the CL corn starches, as revealed by the paired t-test ($p < 0.05$). RS contents of the CL starches produced at the low reaction pH (pH 10 and 10.5) from both types of starches (corn and wheat) were comparable to the RS content of the respective native starches. There were no significant differences in the RS contents of the native corn starch and the CL corn starches produced at pH 10 and 10.5, regardless of the reaction temperature. It was also indicated in the literature that higher alkaline pH ranges are required

Table 3
RS (%) and P (%) contents of the CL corn starch and wheat starch samples prepared in the main study.[a,b,c]

Run	Reaction conditions		CL corn starches		CL wheat starches	
	T (°C)	pH	RS (%)	P (%)	RS (%)	P (%)
	Control (Native starch)		2.5 h	0.01 i	3.0 i	0.05 e
5	38 (−1)	10 (−1)	2.3 h	0.08 h	2.7 i	0.10 g
9	38 (−1)	11 (0)	5.2 g	0.20 d	15.9 g	0.30 d
3	38 (−1)	12 (+1)	49.4 c	0.30 b	85.2 b	0.44 b
8	46 (−0.5)	10.5 (−0.5)	2.3 h	0.14 f	2.1 i	0.16 f
13	46 (−0.5)	11.5 (+0.5)	18.9 e	0.28 bc	56.0 d	0.43 b
7	54 (0)	10 (−1)	3.3 h	0.07 h	2.7 i	0.09 g
11	54 (0)	11 (0)	12.9 f	0.21 d	39.5 f	0.32 d
1	54 (0)	12 (+1)	59.6 b	0.36 a	92.2 a	0.48 a
12	62 (+0.5)	10.5 (−0.5)	3.9 h	0.17 e	8.8 h	0.20 e
10	62 (+0.5)	11.5 (+0.5)	39.6 d	0.34 a	71.7 c	0.45 b
6	70 (+1)	10 (−1)	2.1 h	0.11 g	2.4 i	0.14 f
2	70 (+1)	11 (0)	16.6 e	0.26 c	49.3 e	0.37 c
4	70 (+1)	12 (+1)	78.4 a	0.37 a	95.8 a	0.50 a

[a] Coded values are shown in parentheses.
[b] For each sample, means with different letters within each column are significantly different ($p < 0.05$).
[c] CL, cross-linked; RS, resistant starch; P, phosphorus.

for the production of CL starches (Lim, 1990; Woo & Seib, 2002). In this study, the increase in the reaction pH generally caused increases in RS contents of the samples produced at the same temperature and the increases were more noticeable for the CL wheat starch samples. The RS contents of the CL corn starch samples produced at 54 °C were 12.9% and 59.6% when the reaction pHs were 11 and 12, respectively. At the same reaction temperature (54 °C), the RS contents of the CL wheat starch samples were considerably higher than the counterpart corn starch samples (39.5% and 92.2% for pHs 11 and 12, respectively). Increasing reaction temperature generally caused increases in the RS contents of the CL starch samples produced at the same pH (Table 3). The RS contents of the CL wheat starch samples produced at pH 11, were 15.9%, 39.5% and 49.3% when the reaction temperatures were 38 °C, 54 °C and 70 °C, respectively.

The paired *t*-test revealed that P contents of the CL wheat starches were significantly higher than those of CL corn starches ($p < 0.05$). Similar to RS results, P contents of the CL starch samples prepared from both types of starches were lower at the low reaction pHs (pH 10 and 10.5). The increase in the reaction pH resulted in significant increases in the P contents of the CL wheat and corn starches produced at all temperatures studied ($p < 0.05$). At the reaction temperature of 38 °C, the differences among the P contents of the CL wheat starches, produced at pH 10, 11 and 12, were significant ($p < 0.05$). Overall RS and P results indicated that wheat starch seemed to have a better cross-linking ability than had corn starch. This is also in agreement with the results of Woo and Seib (2002) and Shin et al. (2004).

3.3. Optimisation of reaction conditions by RSM

In order to describe the relationship between the dependent variables (RS or P content) and the independent variables (*T* and pH), the response values (RS or P content) were fitted by second-order polynomial (quadratic) regression models. ANOVA analyses for the models (of RS and P contents of CL corn starch and wheat starch samples) are presented in Table 4.

In the case of RS content of CL corn starch samples, the *F* value (135.21), the *p* value (<0.0001) and R^2 (0.9898) of the model indicated that the model was reliable. The regression coefficient (R^2) of the model, provided by the ANOVA, indicated that only 1.02% of the total variation could not be explained by the model. Significance analysis of coefficients of each factor showed that the pH (coded as X_2) had the largest influence on the RS content of the CL corn starches ($p < 0.0001$), linear (X_2) and second order (X_2^2) coefficients of pH were both significant. Reaction temperature (coded as X_1) also had a significant effect on the RS content ($p = 0.0007$). The coefficient of interaction term between the linear effects of temperature and pH (X_1X_2) was also significant ($p = 0.0019$). In order to minimise error, all significant and nonsignificant coefficients given in Table 4 were considered in the model. The final estimative response model equation for the RS content of CL corn starch, in terms of the coded factors, was generated by Design Expert Software and given as follows (Eq. (3));

$$Y_1 = 10.96 + 7.34X_1 + 29.41X_2 + 7.99X_1X_2 + 0.58X_1^2 + 21.13X_2^2 \quad (3)$$

in which Y_1 is the RS content (%) of CL corn starch, and X_1 and X_2 are the coded values of independent factors (*T* and pH, respectively).

The higher influence of pH than of temperature on the RS content of CL samples can also be seen from Table 3. The CL corn starch sample produced at 46 °C and pH 11.5 had an RS content of 18.9%, while the CL corn starch sample, produced at 38 °C and pH 12, had an RS content of 49.4% (Table 3). Although, the reaction temperature was 8 °C lower (38 °C instead of 46 °C), the RS content was almost 30% higher, due to increase in pH by 0.5 of a unit (pH 12 instead of 11.5). Hence, pH seems to have a greater influence than temperature on RS formation in accordance with the ANOVA analysis for the response surface quadratic model of RS contents of CL corn starch samples (Table 4).

ANOVA analysis for the response surface quadratic model of P contents of CL corn starch samples is presented in Table 4. The *F* value (75.25), the *p* value (<0.0001) and R^2 (0.9817) of the model indicated that the model was sufficiently reliable. The regression coefficient (R^2) of the model indicated that 98.17% of the total variation could be explained by the model. Similar to the RS results, significance analysis on coefficients of each factor showed that the pH (coded as X_2) had the largest influence on the P content of the CL corn starch ($p < 0.0001$) and that the linear coefficient of pH was significant. Reaction temperature (coded as X_1) had a smaller but significant effect on the P content ($p = 0.0041$). In order to minimise the error, all significant and nonsignificant coefficients given in Table 4 were considered in the model. The final estimative response model equation for the P content of CL corn starch, in terms of the coded factors, was generated by Design Expert Software and given as follows (Eq. (4));

$$Y_2 = 0.23 + 0.029X_1 + 0.13X_2 + 0.011X_1X_2 + 1.201E - 003X_1^2 - 0.014X_2^2 \quad (4)$$

in which Y_2 is the P content (%) of CL corn starch, and X_1 and X_2 are the coded values of independent factors (*T* and pH, respectively).

Table 4
Significance of the regression models (*F* values) and the effects of variables on RS and P contents of CL corn starch and wheat starch samples.[a]

Source of variance	Degrees of freedom	CL corn starches				CL wheat starches			
		RS		P		RS		P	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Linear									
X_1 (*T*)	1	32.56	0.0007*	17.48	0.0041	5.86	0.0460*	6.51	0.0380*
X_2 (pH)	1	523.18	<0.000*	355.89	<0.0001	203.93	<0.0001*	296.61	<0.0001*
Interaction									
X_1X_2	1	23.47	0.0019*	1.55	0.2538	0.55	0.4809	0.060	0.8130
Quadratic									
X_1^2	1	0.069	0.7997	0.010	0.9230	0.046	0.8371	0.52	0.4941
X_2^2	1	92.13	<0.0001*	1.32	0.2877	6.07	0.0432*	3.56	0.1012
Residual	7								
Model	5	135.21	<0.0001*	75.25	<0.0001*	43.30	<0.0001*	61.37	<0.0001*

[a] CL, cross-linked; RS, resistant starch; P, phosphorus.
* Significant factors ($p < 0.05$).

In the case of RS content of CL wheat starch samples, the *F* value (43.30), the *p* value (<0.0001) and R^2 (0.9687) of the model indicated that the model was sufficiently reliable (Table 4). The regression coefficient (R^2) of the model indicated that 3.13% of the total variation could not be explained by the model. The results of CL wheat starches were quite similar to those of CL corn starches. Significance analysis of coefficients of each factor showed that the pH (coded as X_2) had the largest influence on the RS content of the CL wheat starches ($p < 0.0001$); linear (X_2) and second order (X_2^2) coefficients of pH were all significant. Reaction temperature (coded as X_1) had a smaller but significant effect on the RS content ($p = 0.0460$). In order to minimise error, all significant and nonsignificant coefficients given in Table 4 were considered in the model, and the final estimative response model equation in terms of the coded factors generated by Design Expert Software was given as follows for the RS content of CL wheat starch (Eq. (5));

$$Y_3 = 33.61 + 7.84X_1 + 46.26X_2 + 3.09X_1X_2 - 1.18X_1^2 + 13.67X_2^2 \quad (5)$$

in which Y_3 is the RS content (%) of CL wheat starch, and X_1 and X_2 are the coded values of independent factors (*T* and pH, respectively).

Similar to the results of CL corn samples, the higher influence of pH on the RS content of CL wheat starches can also be seen from Table 3. The CL wheat starch sample produced at 46 °C and pH 11.5 had an RS content of 56.0%, while the CL wheat starch sample, produced at 38 °C and pH 12, had a substantially higher RS content of 85.2% (Table 3). Although, the reaction temperature was 8 °C lower (38 °C instead of 46 °C), the RS content was almost 30% higher, due to the increase in pH by 0.5 of a unit (pH 12 instead of 11.5). Thus, pH seems to have a greater influence than temperature on RS formation, in accordance with the ANOVA analyses, for the response surface quadratic model of RS contents of CL wheat starch samples.

In the case of P contents of CL wheat starch samples, the *F* value (61.37), the *p* value (<0.0001) and R^2 (0.9777) of the model indicated that the model was sufficiently reliable (Table 4). The regression coefficient (R^2) of the model indicated that 97.77% of the total variation could be explained by the model. Significance analysis of coefficients of each factor showed that the pH (coded as X_2) had the largest influence on the P content of the CL wheat starches ($p < 0.0001$); the linear coefficient of pH was significant. Reaction temperature (coded as X_1) had a smaller but significant effect on the P content ($p = 0.0380$). In order to minimise error, all significant and nonsignificant coefficients given in Table 4 were considered in the model. The final estimative response model equation for the P content of CL wheat starch, in terms of the coded factors generated by Design Expert Software, was given as follows (Eq. (6));

$$Y_4 = 0.32 + 0.029X_1 + 0.19X_2 + 3.529E - 003X_1X_2 + 0.014X_1^2 - 0.036X_2^2 \quad (6)$$

in which Y_4 is the P content (%) of CL wheat starch; X_1 and X_2 are the coded values of independent factors (*T* and pH, respectively).

Eqs. (3)–(6) are plotted in Figs. 1(a), 2(a), 1(b), and 2(b), respectively as 3D response surface plots. Fig. 1(a) and (b) represent the effects of reaction pH and temperature on RS contents of CL corn and wheat starches, respectively. In RSM, the dependent variable (response) approaches a minimum or a maximum value as the independent variables are changed. Further changes in the independent variables are expected to cause deflection in response from this minimum or maximum value; hence this minimum or maximum value can be considered as the optimum. On the other hand, in certain cases, response just reaches a minimum/maximum value and does not increase/decrease as the independent variables are changed. As can be observed from Fig. 1(a) and (b), increasing the reaction pH and temperature caused an increase in the RS

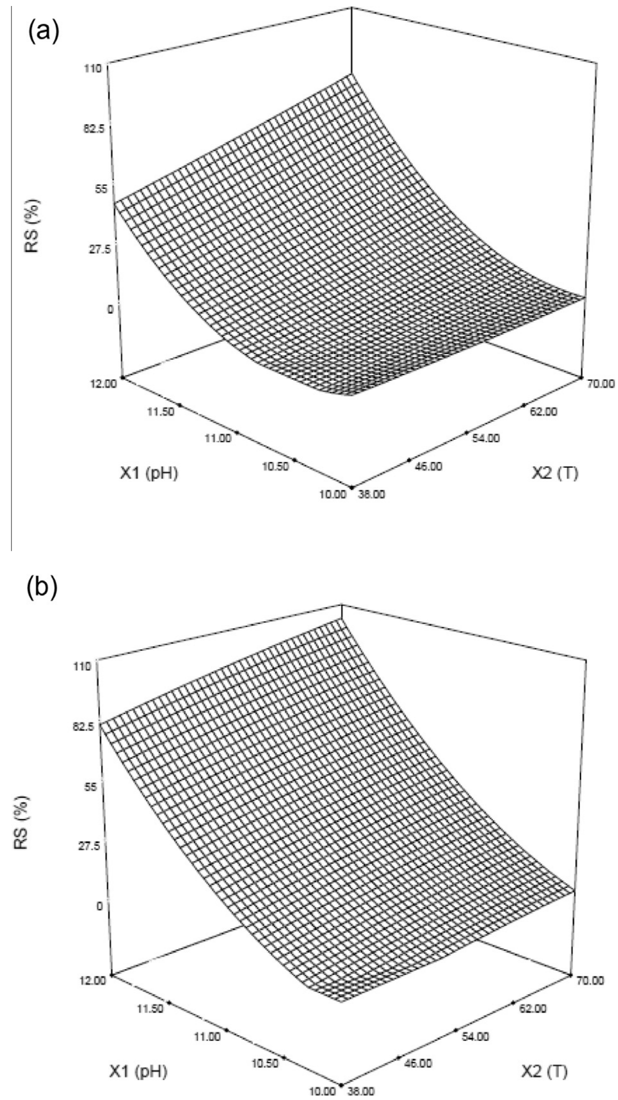

Fig. 1. 3D response surface plots of the predicted resistant starch (RS) contents of cross-linked (a) corn starch samples and (b) wheat starch samples as a function of reaction pH (X_1) and temperature (*T*; X_2).

contents of the CL starches. However at the reaction conditions used in this study, the response (RS content) reached a maximum value for the CL starches produced from both types of starches (corn and wheat) instead of reaching the optimum value. It was observed, in the preliminary experiments, that increasing the reaction temperature and pH caused increases in the RS content of cross-linked starches. Therefore, at the beginning of the main experiments, it was decided to increase the reaction temperature and pH above 70 °C and 12, respectively. However, in the preliminary studies the reaction pHs above 12 caused degradation of starch granules and increased starch solubility, resulting in gel formation. Therefore it was not possible to conduct the cross-linking reactions above pH 12 and the maximum levels for temperature and pH were chosen as 70 °C and 12, respectively.

Fig 2(a) and (b) represents the effects of reaction temperature and pH on P content of CL corn and wheat starches, respectively. Similar to the RS results, increases in the reaction temperature and pH caused increases in the P content of CL corn and wheat starches (Fig. 2), and P content reached a maximum value instead of optimum value under the reaction conditions used in this study.

According to the Code of Federal Regulations in the United States, residual phosphate in modified starches cross-linked with

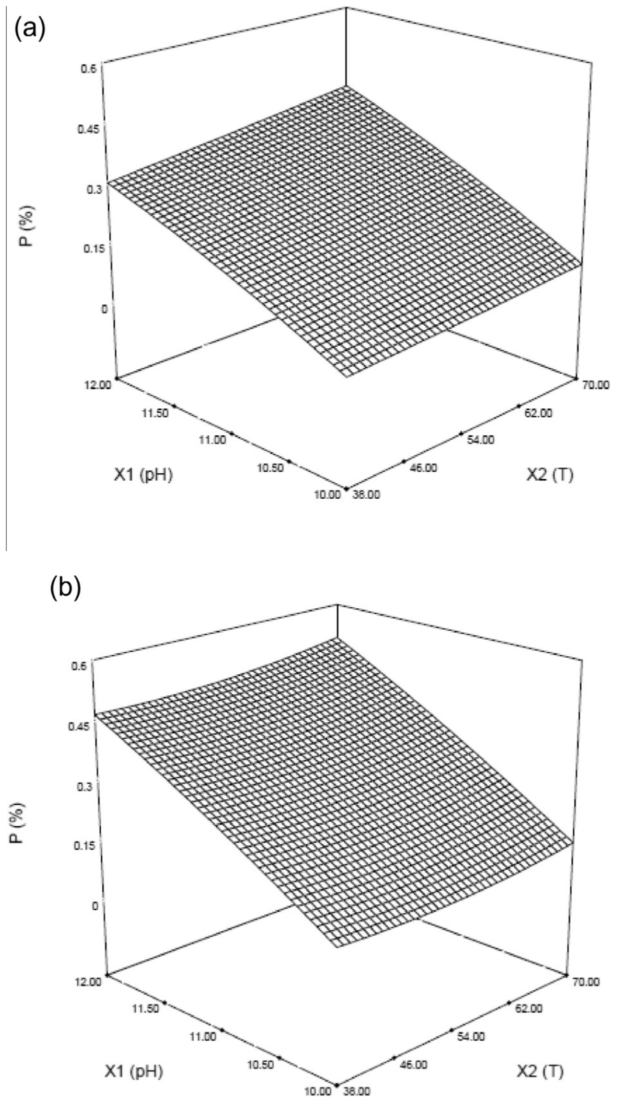

Fig. 2. 3D response surface plots of the predicted phosphorous contents of cross-linked (a) corn starch samples and (b) wheat starch samples as a function of reaction pH (X_1) and temperature (T; X_2).

STMP:STPP mixture should not exceed 0.4%, calculated as phosphorus (CFR, 2001). Therefore, besides maximising the RS content, this limitation in P content was also taken into account during the optimisation of the cross-linking conditions. By solving the regression equations (Eqs. (3)–(6)), using Design Expert Software, the optimal values of the independent variables to obtain the highest RS content (with a maximum P content of 0.4%) were estimated as follows: X_1 (T) = 70 °C and X_2 (pH) = 12 for the corn starch and X_1 (T) = 38 °C and X_2 (pH) = 12 for the wheat starch. However, much higher RS contents were obtained in wheat starches cross-linked at 54 or 70 °C and pH 12, but their P contents were above 0.4% (Table 3). The theoretical RS contents of CL corn and wheat starch samples predicted under the above optimal conditions were 79.3% and 82.4%, respectively. In order to verify this prediction, five confirmation experiments were conducted to compare the experimental results with the prediction under the optimal conditions. The RS contents of the CL wheat and corn starches were 83.6 ± 1.87% and 80.4 ± 1.77%, respectively, which are consistent with the predicted RS contents just mentioned.

Yeo and Seib (2009) and Chung, Woo, and Lim (2004) produced cross-linked starches from wheat starch using STMP:STPP (99:1) at 45 °C and pH 11.5 and RS contents of both studies rose to around 80%. The RS contents they achieved are comparable to those obtained in the present study at reaction conditions of 38 °C and pH 12, without exceeding the permissible phosphorus level of 0.4%. In other words, in the present study, comparable RS contents were obtained at a temperature 7 °C lower (38 °C instead of 45 °C) than that reported by Yeo and Seib (2009) and Chung, Woo, and Lim (2004) by slightly increasing the pH (from 11.5 to 12.0).

4. Conclusion

In this work, corn and wheat starches were cross-linked with STMP:STPP mixture for the production of RS. To obtain maximum RS content, response surface methodology (RSM) was employed to optimise the reaction temperature and pH. Second-order polynomial (quadratic) regression models were fitted to describe the relationship between the dependent variables/responses (RS/P content) and the independent variables (T and pH). The results revealed that RSM can be used to predict the relationship between responses and independent variables.

The optimal cross-linking reaction conditions to obtain maximum RS content (with a maximum P value of 0.4%) were 38 °C and pH 12 for CL corn starch and 70 °C and pH 12 for CL wheat starch. The verification experiments demonstrated that the RS contents of CL corn and wheat starch samples, predicted by using the regression models, were comparable to the RS contents of the actual samples prepared under the optimum predicted conditions. The RSM results indicated that reaction pH had a greater influence than had temperature on RS formation in both CL corn starch and wheat starch samples. Hence, it was possible to achieve a high RS content, comparable with the literature values, at a lower temperature by slightly increasing the pH. Overall, RS and P results indicated that wheat starch seemed to have better cross-linking ability than had corn starch under the same reaction conditions.

Acknowledgements

We would like to thank Michigan AgBio Research and Turkish Higher Education Council (YOK) for their financial support for Kevser Kahraman to do this research.

References

AACCI (2000). *American Association of Cereal Chemist International, Approved methods of the AACC International* (8th ed.). St. Paul, MN: The Association.

Acquarone, V. M., & Rao, M. A. (2003). Influence of sucrose on the rheology and granule size of cross-linked waxy maize starch dispersions heated at two temperatures. *Carbohydrate Polymers, 51*, 451–458.

AOAC (1998). *Official Methods of Analysis of The Association of Analytical Chemists*. Arlingo, VA: Association of Official Chemists.

BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch-hydrocolloid combinations (Review). *Carbohydrate Polymers, 86*(2), 386–423.

CFR (2001). Food starch modified Title: 21. Chapter 1, Part 172, Sect 172.892. In *Food additives permitted in food for human consumption*. Washington, DC: Government Printing Office.

Chung, H. J., Woo, K. W., & Lim, S. T. (2004). Glass transition and enthalpy relaxation of cross-linked corn starches. *Carbohydrate Polymers, 55*, 9–15.

Eerlingen, R. C., Crombez, M., & Delcour, J. A. (1993). Enzyme-resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry, 70*, 339–344.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, 33–50.

Hirsch, J. B., & Kokini, J. L. (2002). Understanding the mechanism of cross-linking agents ($POCl_3$, STMP, and EPI) through swelling behavior and pasting properties of cross-linked waxy maize starch. *Cereal Chemistry, 79*, 102–107.

Huth, M., Dongowski, G., Gebhardt, E., & Flamme, W. (2000). Functional properties of dietary fibre enriched extrudates from barley. *Journal of Cereal Science, 32*, 115–128.

Hwang, D. K., Kim, S. W., Kim, J. H., Ryu, J. H., Yoo, S. H., Park, C. S., et al. (2009). In vitro digestibility of hydroxypropylated and cross-linked waxy and non-waxy rice starches. *Starch/Stärke, 61*, 20–27.

Lehmann, U., Rössler, C., Schmiedl, D., & Jacobasch, G. (2003). Production and physicochemical characterization of resistant starch type III derived from pea starch. *Nahrung/Food, 47*(1), 60–63.
Lim, H., & Seib, P. A. (1993). Location of phosphate esters in a wheat starch phosphate by P-nuclear magnetic resonance spectroscopy. *Cereal Chemistry, 70*, 145–152.
Lim, S. T. (1990). *Preparation and properties of a thick-boiling, phosphorylated wheat starch for food use, and location of phosphate esters on starch by ^{31}P-NMR spectroscopy* (Ph.D. thesis). USA: Kansas State University.
Myers, R. H., & Montgomery, D. C. (1995). *Response surface methodology: Process and product optimization using designed experiments*. New York: John Wiley and Sons.
Niba, L. L., & Hoffman, J. (2003). Resistant starch and b-glucan levels in grain sorghum (*Sorghum bicolor* M.) are influenced by soaking and autoclaving. *Food Chemistry, 81*, 113–118.
Patil, S. K. (2004). Resistant starches as low-carb ingredients–current applications and issues. *Cereal Foods World, 49*, 292–294.
Rutenberg, M. W., & Solarek, D. (1984). Starch derivatives: Production and uses. In R. L. Whistler, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch: Chemistry and technology* (pp. 312–388). London: Academic Press.
Senanayake, S. P. J., & Shahidi, F. (2002). Lipase-catalyzed incorporation of docosahexaenoic acid (DHA) into borage oil: Optimization using response surface methodology. *Food Chemistry, 77*, 115–123.
Shamai, K., Bianco-Peled, H., & Shimoni, E. (2003). Polymorphism of resistant starch type III. *Carbohydrate Polymers, 54*, 363–369.
Shin, S. I., Choi, H. J., Chung, K. M., Hamaker, B. R., Park, K. H., & Moon, T. W. (2004). Slowly digestible starch from debranched waxy sorghum starch: Preparation and properties. *Cereal Chemistry, 81*, 404–408.
Singh, J., Kaur, L., & McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – A review. *Food Hydrocolloids, 21*, 1–22.
Voragen, A. G. J. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science and Technology, 9*, 328–335.
Wang, Y. J., & Wang, L. (2002). Characterization of acetylated waxy maize starches prepared under catalysis by different alkali and alkaline-earth hydroxides. *Starch/Stärke, 54*, 25–30.
Wolf, W. B., Bauer, L. L., & Fahey, G. C. Jr., (1999). Effects of chemical modification in vitro rate and extent of food starch digestion: An attempt to discover a slowly digested starch. *Journal of Food and Agricultural Chemistry, 47*, 4178–4183.
Wollowski, I., Rechkemmer, G., & Pool-Zobel, B. L. (2001). Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition, 73*, 451–455.
Woo, K. S., & Seib, P. A. (2002). Cross-linked resistant starch. Preparation and properties. *Cereal Chemistry, 79*, 819–825.
Woo, K. S., Maningat, C. C., & Seib, P. A. (2009). Increasing dietary fiber in foods: The case for phosphorylated cross-linked resistant starch, a highly concentrated form of dietary fiber. *Cereal Foods World*, 217–223.
Woo, K. S. (1999). *Cross-linked, RS4 type resistant starch: Preparation and properties* (Ph.D. thesis). Kansas State: University Manhattan, KS.
Wu, Y., & Seib, P. A. (1990). Acetylated and hydroxypropylated distarch phosphates from waxy barley: Paste properties and freeze-thaw stability. *Cereal Chemistry, 67*(2), 202–208.
Wurzburg, O. B. (1986a). Nutritional aspects and safety of modified food starches. *Nutrition Reviews, 44*, 74–79.
Wurzburg, O. B. (1986b). Cross-linked starches. In O. B. Wurzburg (Ed.), *Modified starches: Properties and uses* (pp. 41–53). Boca Raton, FL: CRC Press.
Xie, X., & Liu, Q. (2004). Development and physicochemical characterization of new resistant citrate starch from different corn starches. *Starch/Stärke, 56*, 364–370.
Yeo, L. L., & Seib, P. A. (2009). White pan bread and sugar-snap cookies containing wheat starch phosphate, a cross-linked resistant starch. *Cereal Chemistry, 86*, 210–220.