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ORIGINAL ARTICLE

Modelling the Effects of Debranching and Microwave Irradiation Treatments on the Properties of High Amylose Corn Starch by Using Response Surface Methodology

Selime Mutlu[1] · Kevser Kahraman[2] · Sebnem Severcan[2] · Serpil Öztürk[1]

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Abstract
Response surface methodology was applied to determine the effects of pullulanase debranching, microwave irradiation time (2–4 min) and power (20–100%) on resistant starch (RS) formation and in-vitro glycemic index (GI) values in high amylose corn starch, Hylon VII. Starch:water (1:10) suspensions were cooked and autoclaved, debranched with pullulanase (1000 PUN/g; 1500 U/kg starch) at 60 °C and then different microwave-storing cycles and drying (oven or freeze drying) processes were applied. In order to describe the relationship between the dependent and independent variables (microwave power and irradiation time), the response values were fitted by first order polynomial regression models. Significance analysis showed that microwave irradiation time had significant effect on RS content and GI value of the samples treated with one cycle of microwave-storing prior to freeze-drying. Microwave power had significant factor on the GI value of the samples that were oven-dried after one cycle of microwave-storing. Solubility and water binding capacity values of all heat treated samples were higher than those of native starch. On the other hand, RVA viscosity values were lower than native starch for oven-dried samples. Water binding capacity, solubility and final viscosity values of the freeze-dried samples were higher than those of oven-dried ones.

Keywords Resistant starch · In-vitro glycemic index · Pullulanase debranching · Microwave irradiation · High amylose corn starch, response surface methodology

Introduction

Starch contains only α-glycosidic linkages and potentially can be digested in the human digestive tract by amylolytic enzymes [1–4] . Native starch is in the form of semi-crystalline granules with a very complex structure composed of two types of alpha-glucan (approximately 98–99% of the dry weight), amylose and amylopectin [5, 6]. Amylose is an essentially linear polymer of (1,4)-linked D-glucopyranose unit while amylopectin is a highly branched polymer consisting of (1,4)-linked D-glucopyranose units and by ~5% of (1,6)-glycosidic branch-linked bonds [7–9].

Starch species exist with varying amylose and amylopectin contents which strongly affect their physicochemical properties and susceptibility to enzymes hydrolysis [10, 11]. Although naturally occurring starches typically have a range of 28% amylose and 72% amylopectin, there are also breeds of plants that produce starches with high-amylose contents of more than 50% of amylose through to practically zero containing only amylopectin [12–14]. Studies have shown that starch with a higher amylose content has less susceptibility to enzyme hydrolysis, higher gelatinization temperature (100–160 °C), higher melting temperature and increased double-helical crystallites formation [13, 15–17]. Linear amylose segments can be increased by treatments with debranching enzymes such as pullulanase, in the amylopectin branching points [18, 19]. Pullulanase can selectively hydrolyze (1,6)-α-D-glucosidic bonds, thus many short linear glucan chains can be obtained [11]. Debranching increases double-helical crystalline formations and changes the physicochemical properties of starch [17, 20–22].

✉ Serpil Öztürk
sozturk@sakarya.edu.tr

[1] Department of Food Engineering, Faculty of Engineering, Sakarya University, Esentepe Campus, 54187 Sakarya, Turkey

[2] Department of Material Science and Nanotechnology Engineering, Faculty of Engineering, Abdullah Gul University, Kocasinan, 38200 Kayseri, Turkey

Glycemic index (GI) is the value that illustrates the increase in blood glucose after the intake of carbohydrate containing foods, compared to the increase in blood glucose following the ingestion of white bread [23]. Among the staple foods, starch is the most glycemic carbohydrate and the glucose released from its digestion plays a major role in the energy metabolism [24, 25]. Classification of starch from the nutritional point of view according to enzymatic gastrointestinal tract digestion can be divided into three major fractions: (i) rapidly digestible starch (RDS), (ii) slowly digestible starch (SDS), and (iii) resistant starch (RS). The RS is the starch portion that cannot be digested in the small intestine, but produces short-chain fatty acids in the large intestine through fermentation [26–28]. The metabolism of RS occurs 5–7 h after consumption in contrast to normally cooked starch which is digested almost immediately [18, 20, 29]. Due to its low digestibility, RS improves glucose tolerance [30, 31], therefore lowering postprandial glucose response [32].

There are five types of RS, among these types, retrograded or non-granular crystalline post heat treatment product, RS_3, is the most popular within the research topics about resistant starches [33]. High thermal stability of RS_3 makes it stable in normal cooking conditions and consequently increase the usage of it as an ingredient in foods [5]. The combinations of heat treatments (cooking or autoclaving) with other modification methods (acid or enzyme modifications) are usually used to increase the RS_3 content of starches. In modifications conducted with enzyme treatment, debranching by pullulanase is an important method to produce linear chains which helps recrystallization and RS_3 formation [17, 34, 35]. Also, there is an increasing trend towards usage of microwave irradiation in food processing compared to conventional heat treatments. The microwave energy as a heat source is efficient enough to increase RS_3 content [36]. In the present study, high amylose corn starch, Hylon VII, was treated with combined modification methods according to the reaction parameters designed by Response Surface Methodology (RSM) and impact of treatments over RS_3 formation, in-vitro glycemic index value and physicochemical properties were investigated. Hylon VII was debranched with pullulanase followed by different microwave-storing cycles and drying (oven or freeze drying) treatments. The RSM was used to optimize the reaction conditions (microwave power and time) within given reaction parameters to obtain high RS containing starches with low GI value.

Materials and Methods

Materials

High amylose corn starch sample Hylon VII (about 70% of amylose) was supplied from Ingredion Incorporated (Westchester, IL, USA) and pullulanase (E2412, 1000 PUN/g) was purchased from Sigma-Aldrich (Taufkirchen, Germany). The chemicals used in the study were of analytical grade.

Experimental Design for Resistant Starch (RS) Formation

Response surface methodology (RSM) was used to investigate the effect of microwave irradiation on RS content and estimated glycemic index (GI) value of debranched Hylon VII by using Design Expert Software (Stat-Ease, Minneapolis, MN, USA). Nine experimental combinations were created with two points in the center (Tables 1 and 2). Microwave power and time were chosen as two independent variables. The RS contents and estimated GI values of the samples were selected as the dependent variables. The microwave power was varied from the lowest as 20% (160 W) to the highest as 100% (800 W) and the irradiation time was varied from 2 to 4 min.

Resistant Starch (RS) Formation and Determination

Native Hylon VII starch sample was suspended in distilled water at a ratio of 1/10 (630 g starch/ 6300 mL water). A starch/water ratio was maintained as constant by adding of vaporized amount of water after each heat treatment. The suspension was cooked for 45 min by stirring in a pan on an electric element to disperse the starch sample. The mixture was divided to 21 jars as equal amounts and autoclaved at 121 °C for 30 min. After autoclaving, the samples were cooled to 60 °C, which was the optimum temperature for pullulanase enzyme activity. In a previous study [17], Hylon VII starch was debranched with pullulanase for different periods and 48 h of debranching yielded significantly higher amounts of RS. Therefore in this study, starch samples were debranched with pullulanase (1500 U/kg starch) at 60 °C for 48 h on occasional stirring. Following enzyme modification, the samples were treated with microwave irradiation (Fakir MW80200, Turkey) at the conditions of designed experiment times (2–4 min) and powers (160–800 W). After microwave treatment, the samples were stored in oven at 95 °C for 24 h in closed jar for retrogradation. This microwave-storing treatment was applied either one (one-cycle) or three times (three-cycle). Then the samples were oven-dried at 50 °C or freeze-dried. Prior to freeze-drying, the samples were immediately placed on −18 °C for freezing. Finally, the dried samples were ground to pass a 212 μm sieve and stored at room temperature until further analyses.

The RS contents of the samples were measured by using the Megazyme Resistant Starch Kit (Megazyme Int. Ireland Ltd. Co., Wicklow, Ireland) according to the Approved Method 32–40 [37]. The results were reported as means of duplicate analyses.

Table 1 RS contents of treated Hylon VII samples for one and three cycles of microwave-storing and different drying

Sample	Reaction conditions		RS content (%)			
	MP (%)	Time (min)	One cycle		Three cycles	
			Oven-Dried	Freeze-Dried	Oven-Dried	Freeze-Dried
Cooked	–	–	32.0 e	30.9 d	32.0 b	30.9 c
Autoclaved	–	–	33.1 ce	29.1 e	33.1 b	29.1 c
5	20 (−1)	2.0 (−1)	46.7 ab	36.9 b	45.1 a	42.0 a
7	100 (+1)	2.0 (−1)	44.0 c	39.1 a	44.1 a	42.1 a
3	40 (−0.5)	2.5 (−0.5)	43.8 c	39.6 a	42.5 a	41.8 a
4	80 (+0.5)	2.5 (−0.5)	43.6 c	38.5 a	44.2 a	39.4 a
1	60 (0)	3.0 (0)	44.5 bc	35.5 bc	43.1 a	40.0 a
8	40 (−0.5)	3.5 (+0.5)	38.8 d	34.8 c	44.8 a	36.5 b
6	80 (+0.5)	3.5 (+0.5)	45.1 abc	36.4 b	43.9 a	40.9 a
2	20 (−1)	4.0 (+1)	47.2 a	34.2 c	44.0 a	39.6 a
9	100 (+1)	4.0 (+1)	44.0 c	36.4 b	44.5 a	40.6 a

Coded values are shown in pharenthesis

For each sample, means with different letters within each column are significantly different ($p < 0.05$)

RS, resistant starch; MP, microwave power

Estimation of In-Vitro Glycemic Index Value

The samples were digested according to the method of Englyst et al. (1992) with some modifications based on the method of Regand et al. (2011) [38, 39]. For this purpose, samples (100 mg) were weighed into 50 mL tubes with 10 glass beads (5 mm diameter) added to each tube. Two milliliters of 0.05 M hydrochloric acid (HCl) and 10 mg of pepsin (Sigma, P7000) were added and the tubes were incubated at 37 °C in a shaking water bath for 30 min. Then, 4 mL of sodium acetate buffer (0.5 M, pH 5.2) was added to each tube. One milliliter of freshly prepared enzyme solution containing 0.104 g pancreatin (Sigma-Aldrich, P7545) and 14.45 U amyloglucosidase (3300 U/mL, Megazyme Int., Ireland) was added and the tubes were incubated vertically at 37 °C in a shaking water bath. Aliquots (100 μL) were taken at 0, 10, 20,

Table 2 Estimated glycemic index values of treated Hylon VII samples for one and three cycle of microwave-storing and different drying

Sample	Reaction conditions		Estimated GI[a]			
	MP (%)	Time (min)	One cycle		Three cycles	
			Oven-dried	Freeze-dried	Oven-dried	Freeze-dried
Native	–	–	48.2	48.2	48.2	48.2
Cooked	–	–	67.8	71.5	67.8	71.5
Autoclaved	–	–	63.7	68.5	63.7	68.5
5	20 (−1)	2.0 (−1)	59.7	67.5	65.6	67.9
7	100 (+1)	2.0 (−1)	63.2	68.5	67.3	74.3
3	40 (−0.5)	2.5 (−0.5)	61.2	67.5	68.8	67.4
4	80 (+0.5)	2.5 (−0.5)	62.1	67.5	65.6	68.2
1	60 (0)	3.0 (0)	61.0	70.1	64.8	66.6
8	40 (−0.5)	3.5 (+0.5)	60.1	68.9	64.3	68.4
6	80 (+0.5)	3.5 (+0.5)	60.4	69.6	67.3	62.7
2	20 (−1)	4.0 (+1)	59.0	68.5	63.8	67.9
9	100 (+1)	4.0 (+1)	64.8	70.6	66.3	60.1

Coded values are shown in pharenthesis

GI, glycemic index; MP, microwave power

[a] White bread is the reference material with an estimated GI of 100

30, 60, 90, 120, and 180 min intervals and mixed with 1 mL of absolute ethanol. These solutions were centrifuged at 800 x *g* for 10 min, and glucose content of the supernatant was measured with glucose oxidase-peroxidase (GOPOD) reagent (Megazyme Int., Ireland) by using spectrophotometer (Shimadzu 1601, Japan) at 510 nm wavelength. Total starch hydrolysis (%) in the samples was calculated as follows (Eq. 1)

$$\textit{Total Starch Hydrolysis}\ (\%) = \left[\frac{\left(\textit{Released Glucose Weight} \times \frac{160}{182}\right)}{\textit{Total Starch Weight}}\right] \times 100 \quad (1)$$

Several researchers showed a high correlation between the rate of starch digestion and the glycemic response by various in-vitro digestion methods that imitate the in-vivo methods [1, 39–43]. The in-vitro glycemic index has been called as "Estimated Glycemic Index".

Goni et al. (1997) stated that the kinetics of in-vitro digestion is followed by a nonlinear model with a first order equation of $C = C_\infty(1 - e^{-kt})$, where C_∞ is the percentage of starch hydrolyzed at time t (min), C_∞ is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant [42]. Total starch hydrolysis (%) values of the samples were plotted against time (min) and the area under the curve was calculated using Microsoft Excel. Hydrolysis index (HI) represents the rate of starch digestion in the samples in relation to the digestibility of starch in a reference material, white bread (75.4% starch content). The HI was calculated as follows (Eq. 2);

$$HI = \frac{\textit{Area under the curve of the sample}}{\textit{Area under the curve of reference sample (white bread)}} \quad (2)$$

The estimated GI was calculated by using the equation of Goni et al. (1997) [42] (Eq. 3);

$$GI = 39.71 + 0.549 \times HI \quad (3)$$

Birefringence of Starch Samples

The birefringence of the starch samples were observed by polarized light microscope Olympus (BX53-P, Tokyo, Japan). Powder starch sample and distilled water were placed on the microscope slide with a glass coverslip. The field was then viewed under polarized light microscope.

Solubility and Water Binding Capacity of Starch Samples

Solubility and water binding capacity (WBC) values of the starch samples were determined by Mutlu et al. (2017) [36]. For each sample, 5 mL of distilled water was added to 0.5 g of starch sample and the mixtures were held for 40 min by vortexing for 15 s every 5 min. Then, the samples were centrifuged (Heraus Labofuge, Germany) at 2100 *g* for 10 min, and supernatant and precipitate were dried at 100 °C separately. Solubility (%) and WBC (%) values were calculated as in Eqs. (4) and (5), and the results were reported as means of duplicate analyses.

$$\textit{Solubility}\ (\%) = \frac{\textit{weight of dry supernatant}}{\textit{sample weight}} \times 100 \quad (4)$$

$$WBC\ (\%) = \frac{\textit{weight of wet precipitate} - \textit{weight of dry precipitate}}{\textit{sample weight}} \times 100 \quad (5)$$

Pasting Properties of Starch Samples

Pasting properties of the starch samples were tested by using a Rapid ViscoAnalyzer (RVA 4, Newport Scientific, Australia). In this assay, 4 g (14% moisture basis) starch and 25 g distilled water (corrected for sample moisture) were placed in an aluminum canister. The RVA pasting curve was obtained by using a 28 min test profile with 160 rpm mixing speed according to the method developed by Mutlu et al. (2017) [36]. The results were evaluated with the data analysis software (Thermocline for Windows, Newport Scientific, Australia) and reported as means of duplicate analyses for cold viscosity, peak/maximum viscosity, breakdown and final viscosity values, and pasting temperatures.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze data. When significant ($p < 0.05$) differences were found, Duncan's test was used to determine the differences among means. Paired *t-test* was performed to compare the properties of oven-dried and freeze-dried samples, and also the samples treated for one and three cycles of microwave applications.

The response values were fitted by regression models using Design Expert (Stat-Ease, Minneapolis, MN, USA) to describe the relationship between dependent variables (RS and GI) and independent variables (microwave power and irradiation time). Three-dimensional response surface plots were also developed.

Results and Discussion

Resistant Starch (RS) Content

The RS contents of the samples are shown in Table 1. The relatively high RS content (42.7%) found in native Hylon VII starch sample compared to cooked and autoclaved samples was due to its granular form and was expected to be in the form of RS_2, which could be degraded with sufficient heat treatment to form RS_3. Therefore, the decreases in the RS contents of the samples cooked and autoclaved at 121 °C, following oven-drying (32.0% and 33.1%) and freeze-drying (30.9% and 29.1%) were anticipated. The heating processes (cooking and autoclaving) lowered the natural RS_2 found in native Hylon VII, but formed RS_3 during drying period by retrogradation. The samples that were freeze-dried after cooking and autoclaving had lower RS contents than their respective oven-dried samples.

Debranching with pullulanase followed by microwave-storing treatments increased the RS content of native Hylon VII starch compared to cooked and autoclaved samples significantly indiscriminating the one and three microwave-storing cycle numbers or drying methods. Our previous study [36] conducted with same experimental conditions (microwave power and time) but without pullulanase debranching showed lower RS contents compared to the debranched samples. The increases in RS content could be associated to increased amylose chain numbers due to pullulanase debranching which promoted the possibility to aggregate and organize double helixes by hydrogen bonding during microwave treatment. The increasing effect of pullulanase on RS content were also found in the related studies [11, 34, 44, 45] reported that the different starch samples treated by pullulanase became more resistant to pancreatic amylase hydrolases.

Paired *t-test* revealed that the RS contents of three cycles of microwave-storing treated samples were higher than those of one cycle of microwave-storing treated samples only for freeze drying ($p < 0.05$), while there was no significant difference for oven dried samples. Debranched samples developed more stabile double helix aggregates during longer microwave-storing periods in three cycles treatment, so that the temperature and mechanism related to drying method differences towards the RS contents were minor. On the other hand, it was thought that following the first microwave-storing cycle the most of the double helical bound aggragation took place during drying period at 50 °C. Because of these reactions, the remaining low number of single chain to aggragate might be the main reason of approximate values in the RS contents with different microwave cycle treatments. The highest RS value (47.2%) was obtained by oven drying after one cycle of microwave treatment at 20% power for 4 min.

The RS contents of the oven-dried samples for one microwave-storing cycle were significantly higher ($p < 0.05$) than those of the freeze-dried samples due to suitable conditions for retrogradation. It was estimated that, re-association of starch molecules and formation of tightly packed structures stabilized by hydrogen bonding during drying period in oven at 50 °C were more likely to develop and thus limited the accessibility of digestive enzymes. On the other hand, during freeze-drying, starch chains had limited mobility to interact with each other. Hence, the molecular structure was not as resistant and packed in as the structure formed during oven-drying. Similar results were also found in the previous studies [21, 46].

Estimated In-Vitro Glycemic Index Values

In-vitro starch digestibility values of white bread and some of the selected starch samples are shown in Fig. 1. The starch hydrolysis of the samples increased as digestion time increased. The starch digestion rate of native Hylon VII was the lowest, as it contained RS_2, which was considered as native starch granule protected from digestion by the conformation or structure of the granule. Microwave treated samples had lower starch digestibility than white bread. The starch digestibility can be influenced by starch source, amylose/amylopectin ratio, granule size, crystallinity, degree of gelatinization, modification, etc. [6, 47, 48]. High amylose and RS contents have been associated with reduced digestibility, providing prolonged and slow glucose release, therefore a low GI compared to regular starch. In this study, the microwave treated samples had lower digestibility than the control sample (white bread), in accordance with their RS contents.

The estimated GI values of the samples that were autoclaved for one cycle, microwave treated and oven-dried ranged from 59.0 to 64.8, whereas the estimated GI values of the freeze-dried samples were quite higher (67.5–70.6). In accordance with the inverse relationship between RS content and GI value, RS contents of the freeze-dried samples were lower than those of the oven-dried ones (Table 1).

Investigation of Heat Moisture Treatments by Response Surface Methodology (RSM)

In order to describe the relation between the independent variables (microwave power and time) and dependent variables (RS content and GI), the response values were fitted by first order polynomial regression models. ANOVA analyses for the models of the samples are presented in Table 3.

In case of the RS contents of the samples, the response values (RS content) were fitted by first order (linear) regression model for debranched, one cycle of microwave-storing treated and freeze-dried samples. However, the RS data obtained for the samples produced by one cycle of microwave-storing and oven-drying and by three cycles of microwave-storing and both drying methods were not fitted by a regression model ($p > 0.05$). The F and p (<0.05) values of the model indicated that the

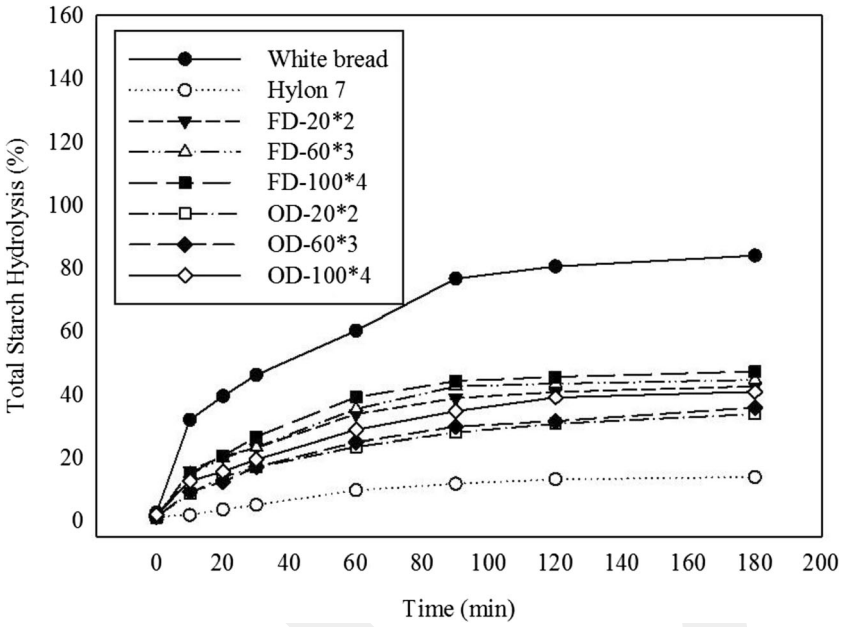

Fig. 1 Total starch hydrolysis rate of some of the samples. OD: oven-dried; FD: freeze-dried; 20 × 2: treatment for 2 min at 20% microwave power; 60 × 3: treatment for 3 min at 60% microwave power; 100 × 4: treatment for 4 min at 100% microwave power

selected model for the samples that were debranched, one cycle of microwave-storing treated and freeze-dried was reliable. The microwave irradiation time (t as *B*) had significant effect on the RS contents of the samples that were freeze-dried after one cycle of microwave-storing ($p = 0.0171$). The regression coefficient (R^2) of the model, indicated 30.6% of the total variation could not be explained by the models. The final estimative model equation for the RS contents of samples treated with one cycle of microwave-storing prior to freeze-drying, in terms of coded factors, was generated by Design Expert Software and given at Eq. (6), in which Y_1 is the RS content (%) of the samples; *A* and *B* are the coded values of independent variables (microwave power and irradiation time, respectively). As, the RS contents of the debranched samples produced by one cycle of microwave-storing and oven-drying and by three cycles of microwave-storing and both drying methods were not fitted by a regression model, final estimative response model equations were not created.

$$Y_1 = 36.82 + 0.93\ A\ 1.77\ B \quad (6)$$

In case of the estimated glycemic index values of the samples, the *F* and *p* (<0.05) values of the models indicated that the selected models for the samples that were debranched, one cycle of microwave-storing treated, freeze-dried as well as oven-dried were reliable. The linear effect of microwave irradiation time (t as *B*) had significant effect on the GI values of the samples that were freeze-dried after one cycle of microwave-storing ($p = 0.0313$), whereas microwave power (MP as *A*) did not. The regression coefficient (R^2) of the model, indicated 34.3% of the total variation could not be explained by the models. In contrast to the samples that were treated with one cycle of microwave-storing prior to freeze-drying, the linear effect of microwave power (MP as *A*) had the only factor that had significant effect on the GI values of the samples that were oven-dried after one cycle of

Table 3 Significance of the regression models (F values) and the effects of variables on RS contents and estimated GI value of the treated samples for one cycle of microwave-storing

		RS		Estimated GI			
		Freeze-dried		Freeze-dried		Oven dried	
Source of variance	Degrees of freedom	*F*	*p*	*F*	*p*	*F*	*p*
A (MP)	1	2.95	0.1369	3.68	0.1035	16.21	0.0069*
B (t)	1	10.67	0.0171*	7.82	0.0313*	0.039	0.8493
Residual	6						
Model	2	6.81	0.0286*	5.75	0.0403*	8.13	0.0196*
R^2		0.6942		0.6571		0.7304	

RS, resistant starch; GI, glycemic index; MP, microwave power; t, irradiation time

*Significant factors ($p < 0.05$)

microwave-storing ($p = 0.0069$). The regression coefficient (R^2) of the model, indicated 27.0% of the total variation could not be explained by the models. The final estimative response model equations for the estimated GI values of samples treated with one cycle of microwave-storing prior to freeze-drying and oven-drying, in terms of coded factors, was generated by Design Expert Software and given at Eqs. (7 and 8), in which Y_2 and Y_3 are the estimated GI values of the samples (freeze-drying and oven-drying, respectively); A and B are the coded values of independent variables (microwave power and irradiation time, respectively). The GI values determined for the samples produced by three cycles of microwave-storing prior to freeze-drying and oven-drying were not fitted by a regression model ($p > 0.05$), therefore final estimative response model equations for these data were not created.

$$Y_2 = 61.27 + 1.96\ A\ 0.097\ B \quad (7)$$

$$Y_3 = 68.75 + 0.68\ A + z1.0\ B \quad (8)$$

The Eqs. 6, 7, and 8 were plotted as 3D response surface plots and are shown in Fig. 2a, b, and c, respectively. Figure 2a represents the effect of microwave treatment on the RS content of the samples debranched, one cycle of microwave-storing treated and freeze-dried. It was expected in response surface method that by changing of independent variables, the dependent variables reached to a minimum or maximum (optimum) value. It can be seen in Fig. 2a that the RS contents changed by changing of the irradiation time and power variables but could not reach to an optimum point since the optimum point was probably out of the experimental designed values. Similar results were also observed in the previous studies [36, 49]. The RS determination methodology [37] cannot differ RS_2 from RS_3 and estimates overall RS content, thus the RS_3 could not be determined certainly. Therefore, the RS content did not show apparent increase or decrease so it was not possible to reach a maximum RS value. Degradation of RS_2, and therefore the formation of RS_3, increased with microwave treatment in low intensity conditions. However, high amount of RS_2 in granule made the determined RS values to be high. On the other hand, high degradation of RS_2 at higher power and longer time treatments reduced the calculated values of overall RS contents even the formation of RS_3 increased at same conditions. This was supported by the birefringence analysis results. The images of the selected starch samples under polarized light microscope are given in Fig. 3. It was observed that there were granular starches or Maltese crosses in all heat treated samples. High amylose starches had higher gelatinization temperature (around 150 °C) than the regular starches [21]. Therefore, the granular structure of the native Hylon VII could only be degraded with sufficient heat treatment. Cooking, autoclaving and also microwave treatments were not sufficient to degrade all native granules.

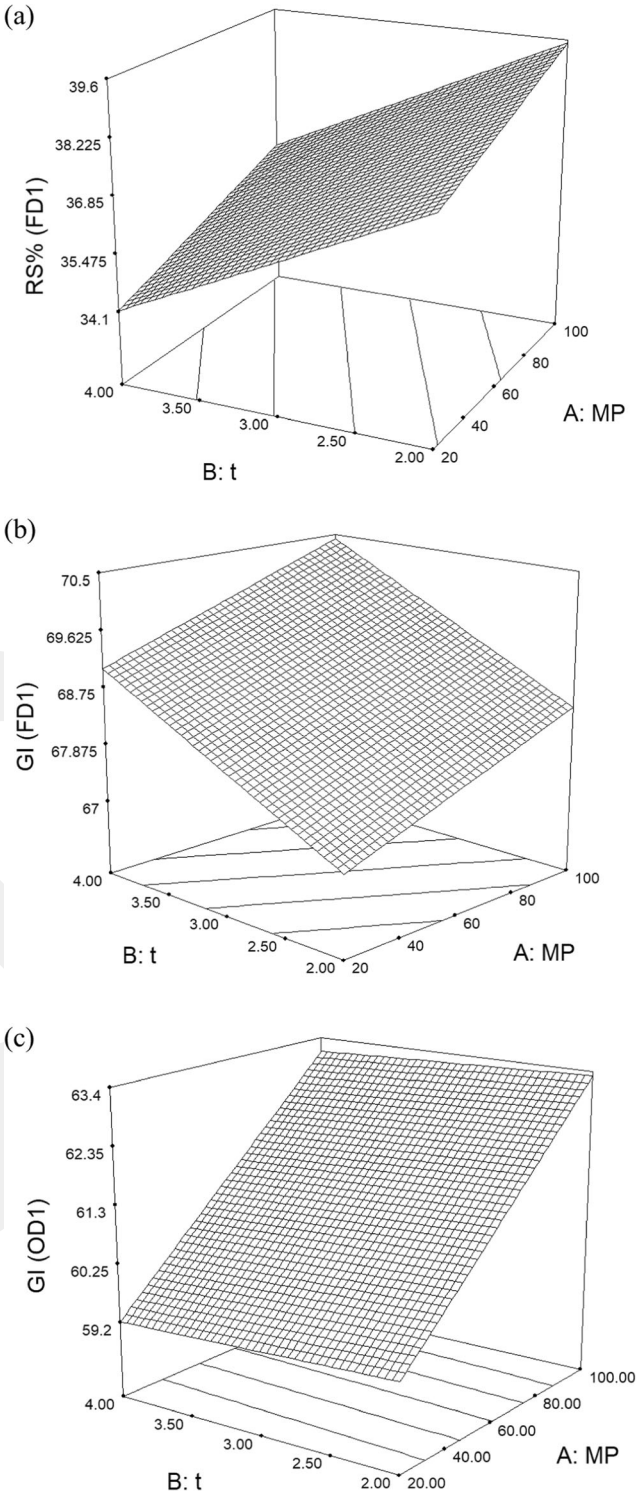

Fig. 2 3D response surface plots of RS content (**a**) and estimated GI values (**b**, **c**) as a function of time (t, min) and microwave power (MP, %). FD1: 1 cycle of microwave prior to freeze drying, OD1: 1 cycle of microwave prior to oven drying

Solubility and Water Binding Capacity Values

The effects of debranching, microwave-storing treatments and different drying conditions on solubility and water binding

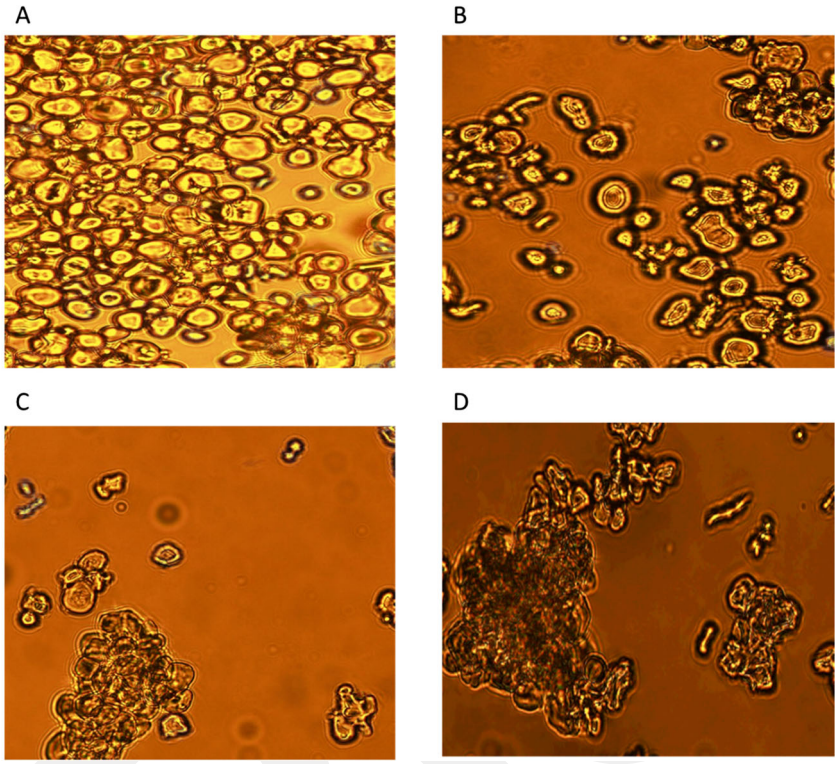

Fig. 3 Polarized light microscope images of the starch samples; (**a**): native Hylon VII; (**b**): cooked; (**c**): autoclaved; (**d**): debranched and 3 cycles of microwave treated at 100% power for 4 min

capacity (WBC) values of Hylon VII sample for one and three cycles of microwave-storing are shown in Table 4. The solubility values of the cooked, autoclaved and microwave-storing applied starches were higher than that of the native Hylon VII sample ($p < 0.05$). Starch granules were not only degraded by heat treatments but also hydrolyzed with pullulanase enzyme treatment which increased free amylose chains that could dissolve easily. Even though the greater numbers of debranched free chains promoted the aggregation rate and double helix formation, the solubility values of treated samples were

Table 4 Functional properties of native and treated Hylon VII samples for one and three cycles of microwave-storing and different drying

Sample	Reaction conditions		Solubility (%)				Water binding (%)			
	MP	Time	One cycle		Three cycles		One cycle		Three cycles	
	(%)	(min)	OD	FD	OD	FD	OD	FD	OD	FD
Native	–	–	0.83 d	0.83 e	0.83 a	0.83 b	139.0 d	139.0 e	139.0 d	139.0 e
Cooked	–	–	1.46 bcd	1.60 d	1.46 a	1.60 b	264.3 ab	395.0 bcd	264.3 a	395.0 b
Autoclaved	–	–	1.80 abcd	0.87 e	1.80 a	0.87 b	259.8 abc	426.0 ab	259.8 ab	426.0 a
5	20 (−1)	2.0 (−1)	1.02 cd	4.17 a	1.51 a	4.25 a	279.4 a	438.7 a	250.2 abc	394.7 b
7	100 (+1)	2.0 (−1)	2.77 a	4.15 a	2.85 a	3.75 a	251.1 abc	428.3 ab	237.1 bc	370.7 bc
3	40 (−0.5)	2.5 (−0.5)	2.32 ab	3.94 ab	1.99 a	4.21 a	236.7 bc	397.4 bcd	257.9 ab	371.5 bc
4	80 (+0.5)	2.5 (−0.5)	2.26 ab	3.68 abc	1.30 a	3.68 a	248.3 bc	397.2 bcd	231.3 c	378.0 b
1	60 (0)	3.0 (0)	1.96 abcd	3.91 ab	1.78 a	3.76 a	238.8 bc	403.6 bc	248.3 abc	380.0 b
8	40 (−0.5)	3.5 (+0.5)	2.49 ab	4.01 a	1.99 a	4.10 a	230.8 c	422.4 ab	246.4 abc	386.6 b
6	80 (+0.5)	3.5 (+0.5)	1.57 abcd	3.10 c	2.60 a	3.80 a	241.6 bc	377.6 cd	244.6 abc	317.5 d
2	20 (−1)	4.0 (+1)	1.98 abcd	3.19 bc	2.36 a	3.98 a	235.4 bc	416.4 ab	232.2 c	392.0 b
9	100 (+1)	4.0 (+1)	2.06 abc	3.35 abc	1.86 a	4.12 a	246.6 bc	364.9 d	247.2 abc	343.6 cd

Coded values are shown in pharenthesis

For each sample, means with different letters within each column are significantly different ($p < 0.05$)

MP, microwave power; OD, oven-dried; FD, freeze-dried

Table 5 Pasting properties of native and treated Hylon VII samples for one cycle of microwave-storing and different drying

Sample	Reaction conditions		Oven-dried					Freeze-dried				
	MP (%)	Time (%)	CV (cP)	PV (cP)	B (cP)	FV (cP)	PT (°C)	CV (cP)	PV (cP)	B (cP)	FV (cP)	PT (°C)
Native	–	–	6 c	22 g	−22 h	62 c	95 a	6 f	22 g	−22 f	62 e	95 a
Cooked	–	–	17 ab	194 b	120 b	105 b	86 b	34 bc	100 def	24 de	198 b	87 b
Autoclaved	–	–	16 ab	239 a	130 a	130 a	92 a	45 a	522 a	107 a	1009 a	65 d
5	20 (−1)	2.0 (−1)	13 b	73 d	32 d	2 d	88 b	38 ab	130 b	62 b	113 cd	82 bc
7	100 (+1)	2.0 (−1)	13 b	67 de	31 d	45 e	87 b	36 bc	118 bcd	57 bc	109 cd	85 b
3	40 (−0.5)	2.5 (−0.5)	12 bc	56 f	21 fg	39 f	88 b	20 e	74 f	23 de	82 cde	94 a
4	80 (+0.5)	2.5 (−0.5)	12 bc	60 ef	29 de	39 f	79 c	25 de	101 de	41 cd	103 cde	87 b
1	60 (0)	3.0 (0)	14 ab	63 ef	26 def	42 ef	95 a	32 bcd	103 cde	37 de	117 c	85 b
8	40 (−0.5)	3.5 (+0.5)	15 ab	54 f	19 g	46 e	95 a	33 bc	111 bcd	24 de	193 b	82 bc
6	80 (+0.5)	3.5 (+0.5)	14 ab	55 f	21 fg	41 ef	94 a	29 cd	79 ef	19 e	86 cde	78 c
2	20 (−1)	4.0 (+1)	12 b	56 f	23 efg	45 e	88 b	39 ab	128 bc	69 b	113 cd	78 c
9	100 (+1)	4.0 (+1)	20 a	94 c	47 c	59 c	88 b	20 e	77 ef	24 de	73 de	86 b

Coded values are shown in pharentheses

For each sample, means with different letters within each column are significantly different ($p < 0.05$)

MP,microwave power; CV, cold viscosity; PV, peak viscosity; B, breakdown; FV, final viscosity; PT, pasting temperature

generally still higher than those of the native, cooked and autoclaved samples. Similar results were obtained in the related studies [11, 50]. These results indicated that, the general belief of inversely correlation between solubility and RS formation might need reconsideration. Solubility values of debranched, microwave treated and freeze-dried samples were higher than those of the oven-dried samples ($p < 0.05$).

The WBC values of the microwave treated debranched samples changed significantly according to different drying methods ($p < 0.05$). The WBC values of microwave treated samples generally decreased as compared to cooked and autoclaved samples and increased as compared to native Hylon VII starch for both drying methods ($p < 0.05$). Anticipated increases of

Table 6 Pasting properties of native and treated Hylon VII samples for three cycles of microwave-storing and and different drying

Sample	Reaction conditions		Oven-dried					Freeze-dried				
	MP (%)	Time (min)	CV (cP)	PV (cP)	B (cP)	FV (cP)	PT (°C)	CV (cP)	PV (cP)	B (cP)	FV (cP)	PT (°C)
Native	–	–	6 d	22 g	−22 f	62c	95 a	6 g	22f	−22 h	62 def	95 a
Cooked	–	–	17 b	194 b	120 b	105 b	86 bcd	34 b	100 b	24 b	198 b	87 bc
Autoclaved	–	–	16 bc	239 a	130 a	130 a	92 a	45 a	522 a	107 a	1009 a	65 e
5	20 (−1)	2.0 (−1)	13 bc	55 cd	18 cd	59 c	86 bcd	24 cde	52 cd	10 cd	68 de	91 ab
7	100 (+1)	2.0 (−1)	11 cd	39 f	13 e	42 fg	81 f	16 ef	37 e	3 f	57 def	95 a
3	40 (−0.5)	2.5 (−0.5)	12 bc	48 de	15 de	59 c	75 g	16 ef	37 e	7 de	52 ef	94 a
4	80 (+0.5)	2.5 (−0.5)	10 cd	48 e	22 c	39 g	83 ef	15 f	42 de	9 cde	55 ef	91 ab
1	60 (0)	3.0 (0)	14 bc	48 de	20 cd	46 ef	86 bc	25 cd	49 cde	6 ef	77 de	85 bcd
8	40 (−0.5)	3.5 (+0.5)	17 b	59 c	19 cd	61 c	84 cde	23 cde	58 c	−3 g	119 c	79 d
6	80 (+0.5)	3.5 (+0.5)	15 bc	55 cd	19 cd	51 d	83 def	17 def	37 e	10 cd	42 f	85 bcd
2	20 (−1)	4.0 (+1)	23 a	57 c	22 c	48 de	88 b	29 bc	52 cd	7 de	81 d	80 cd
9	100 (+1)	4.0 (+1)	15 bc	48 de	19 cd	45 ef	88 b	17 def	49 cde	11 c	65 def	91 ab

Coded values are shown in pharentheses

For each sample, means with different letters within each column are significantly different ($p < 0.05$)

MP, microwave power; CV, cold viscosity; PV, peak viscosity; B, breakdown; FV, final viscosity; PT, pasting temperature

WBC in connection with hydrolyze treatment and higher free chain numbers were observed.

Increasing the number of microwave-storing treatment to three cycles reduced the WBC of freeze-dried samples while showed no significant changes in solubility values. Debranched amylose chains had higher possibility to aggregate and form double helixes than the branched structures containing chains [11, 51]. Decreasing of water binding could be caused by increasing of double helix formation among amylose chains during longer storing period in three cycles of treatment. There were significant ($p < 0.05$) differences between drying methods in terms of solubility and WBC values determined by paired *t-test*. This could be caused by different mechanisms of oven and freeze drying [11, 52, 53].

Pasting Properties of Starch Samples

The effects of microwave-storing treatments and different drying conditions on pasting properties of debranched Hylon VII starch sample for one and three cycles of microwave-storing applications are presented in Tables 5 and 6, respectively. All viscosity values of cooked, autoclaved, debranched and microwave-storing applied starch samples were higher than those of the native Hylon VII ($p < 0.05$). Yet, the microwave treated and oven-dried debranched samples had lower viscosities compared to cooked and autoclaved samples. This was in agreement with some of our previous studies [21, 36, 52], and was due to the excessive degradation of starch granules and increased solubility by debranching and heating. Liu et al. (2015) [11] also reported that the debranching of wheat and maize starches by pullulanese caused reduction in viscosities of the samples. Glycosidic bonds were reported to degrade because of the vibrations during microwave irradiation [36, 54], which could be another reason of reduction in viscosity. Similar results were also declared in numerous studies [55–57].

According to paired *t-test* results, significant ($p < 0.05$) differences were observed in viscosity values of the samples dried by different methods. Freeze-dried samples demonstrated cold viscosity values which were significantly ($p < 0.05$) higher compared to oven-dried samples. The difference was prognosticated to be caused by loose rearrangement of starch chains by hydrogen bonds. In a previous study, it has been concluded that starch chains which has arrangement with numerous hydrogen bonds tend to result in relatively lower cold viscosity in initial stages of RVA test when the samples are oven-dried [21]. This outcome was strong enough to be correlated with the data of the WBC values. Correspondingly, final viscosities of oven-dried samples were also significantly ($p < 0.05$) lower than those of freeze-dried ones; which makes it safe to assume that it is a confirmation of the argument that suggests freeze-dried samples might form more stable gels.

Pasting temperatures of microwave-storing treated debranched starches were generally lower than that of native Hylon VII. The reduction of pasting temperature was caused by degradation of starch granule by debranching and heating. The differences in pasting temperature values between native and debranched starches were higher among oven-dried samples compared to freeze-dried ones. Diversity of decrease in pasting temperatures for three cycles of microwave application was more significant than that of the one cycle treatment. The highest breakdown value was obtained by debranching and three cycles of microwave-storing treatment. According to the results, it was thought that both pullulanase debranching and microwave irradiation served an effective function on degrading of granule and forming of new inter- and intra-molecular hydrogen bonds between amylose chains [5].

Conclusion

Combining microwave treatment and enzyme modification methods with RSM modelling helped to decide the optimum treatment points for higher resistant starch (RS) contents and lower in-vitro glycemic index values. According to the results, increases in RS contents and decreases in in-vitro GI values were obtained after enzyme modification by pullulanase debranching following with microwave irradiation-storing treatment regardless of drying method. The samples had an estimated GI values of 59.0–70.6, which could be classified as intermediate GI foods (GI value classification; low (<55), intermediate (55–70), and high (>70)). It was also estimated that freeze-drying increased the water binding capacity and solubility values of debranched and microwave irradiation treated Hylon VII samples compared to oven-drying. The RVA viscosity values of the samples decreased for all treatment conditions compared with the native Hylon VII. To face challenges for healthier diets with increased RS intakes, more combined modifications and methods should be improved and studied.

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Compliance with Ethical Standards

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