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Thermal and chemical treatment of red kidney bean (*Phaseolus vulgaris* L.) starch: Effects on α-amylase digestion and resistant starch formation

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ABSTRACT

Starches from red kidney beans (*Phaseoulus vulgaris* L.) subjected to autoclave, microwave, and hydroxypropylation treatments, were studied for treatment effects on granule structural organization and relative rates of α-amylase digestibilities. Total Starch (TS), Digestible Starch (DS), and Resistant Starch (RS) contents were determined with the peridochrom oxidase/peroxidase (GOD-PAP) assay procedures. Microstructural properties, pre- and post-enzymatic hydrolysis, were also studied. All treated starches had significantly higher ($p \leq 0.05$) DS than the native starch. Among treated starches, DS was in the order Microwave > Autoclave > Hydroxypropylation. The RS production was highest in native starch (9.3 ± 1.4%) and least in microwaved starch (4.7 ± 1.6 %). Hydrolysis rate and starch granular structure were all significantly affected by the various treatments applied to the starches. The data from this study suggest that hydroxypropylation may induce resistance to enzyme digestion of starches leading to the production of resistance starch.

Keywords: kidney bean starch; microwave; autoclave; hydroxypropylation; resistant starch

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INTRODUCTION

The major storage carbohydrate of plants is starch which contains α-glycosidic linkages making it potentially digestible by the amylose-hydrolyzing enzymes secreted by the human digestive tract (1). However, structural arrangement, in addition to other factors, can affect the rate and extent of starch digestion, with subsequent effect on the absorption in humans (2). The rate at which a starchy food is digested in the small intestine depends largely on its physical form, hence, any inhibition of the process by which pancreatic amylase comes into contact with a starch substrate during enzymatic degradation of starch to glucose will also inhibit the rate of starch hydrolysis, or even prevent the rate of starch hydrolysis in the small intestine.

Plasma glucose and insulin responses in humans vary with the type and physical form of starchy foods digested, and this effect may be related to differences in starch enzyme digestion rates. Foods in which carbohydrates such as starch are digested and absorbed slowly may be useful in the control of diabetes and obesity, by reducing the increase in blood glucose levels after a meal (3). Studies previously conducted on the relationship between structure and digestibility of starches have focused on α-amylase digestibility of cereal starches after various treatments and processing methods, with little attention to legume starches which are rich and inexpensive source of dietary protein (4).

Legumes are high in fiber, protein, and antinutrients, and the starch content is only slowly digested. They also produce relatively small blood glucose rises after consumption by both normals and diabetics, and in the long term, result in improved diabetic control. Currently, strong efforts are being made to increase the legume intake in populations with high prevalence of diabetes, obesity and cardio-vascular diseases (5).

In view of the nutritional significance of legume starches, there is currently a growing interest in the exploration of legumes starches as a potential source of energy and nutrients. However, the emphasis has been on the protein and antinutritional factors, as well as on the effects of processing on protein digestibility and antinutrient activity (6). Studies on the effect of heat and chemical treatments on the structure of legume starch granules, and the subsequent effect on the enzymatic digestibility of the starch, are limited.

The objective of this study was to investigate the influence of heat and chemical treatments on starch granule structural organization in red kidney beans (*Phaseolus vulgaris* L), and the subsequent effect on the relative rate of *in vitro* α-amylase digestibility.

MATERIALS AND METHODS

Red kidney beans (*Phaseolus vulgaris* L.) used in this study was purchased from local commercial outlet. Porcine pancreatic α-amylase and amyloglucosidase were purchased from Sigma-Aldrich (St. Louis, MO, USA), and Peridochrom/Oxidase (GOD-PAP) reagent kit was obtained from Randox Laboratories (UK).

Starch Extraction

The raw (dry) kidney beans were milled into flour in a Perten Laboratory Mill 3100 (Perten, Sweden) to pass a 250 µm mesh. Flour was suspended in 0.5% (w/v) NaOH solution and stirred for 5 hrs at 25^{O}C, followed by centrifugation at 1600 x g for 30 min. Supernatant was discarded, and the sediment re-suspended in distilled water and centrifuged as before. The process was repeated four times, and sedimented starch was dried at 60^{O}C for 12 hrs in a conventional hot air oven, and then ground into starch powder using a mortar and pestle to pass through 250 µm mesh.

Proximate Analysis

Moisture, protein, fat, and ash content of starch were determined on dry basis (db) using Approved Methods (7).

Starch treatments

Autoclaving

Starch was thinly spread in aluminum tray, covered with aluminum foil and pressure-cooked in an autoclave (Astell, Model AMA270, UK) for 15 min at 121^{O}C. Autoclaved starch was cooled and placed in ziploc bags and desiccated until further use.

Microwave treatment

Moisture content of starch was adjusted to 30% by the addition of appropriate amount of water and placed in glass beakers, and sealed with a perforated polyethylene foil. The moisture-adjusted starch was subjected to microwave-heating for 10 min at a power setting of 700 W in a household microwave oven (LG Model MS-543XD). After heating, starch was placed in desiccator to cool and transferred into ziploc bags for further use.

Chemical treatment

Chemical treatment of starch was achieved through hydroxypropylation using the method described in Gunaratne and Corke (8). Fifty gram (50 g) of starch (db) was suspended in 100 ml distilled water containing 10 g Na_2SO_4 in a centrifuge bottle. The pH was adjusted to 11.3 with 1 M NaOH, 5 ml of propylene oxide was added and the bottle was shaken vigorously. Sample was then placed at 35^{O}C in shaking water bath with continuous shaking for 24 h. The reaction was terminated by adjusting pH to 5.3 with 1 M HCl. Slurry was then centrifuged at 3000 x g for 10 min and recovered starch cake was washed with distilled water and dried at 35^{O}C.

Total Starch (TS), Digestible Starch (DS), and Resistant Starch (RS)

Native (N), Microwaved (M), Autoclaved (A), and Hydroxypropylated (H) starches were subjected to determination of Total Starch (TS), Digestible Starch (DS), and Resistant Starch (RS). Total starch content was determined on triplicate samples as the glucose released by enzyme hydrolysis following gelatinization in boiling water and treatment with KOH, as previously described (4,9). Samples (200 mg, db) each were dispersed in 6 ml of 2M KOH followed by the addition of 3 ml of 0.4 sodium acetate buffer (pH 4.7) and 60 µl of amyloglucosidase to hydrolyze starch to glucose. The mixtures were incubated at 60^{O}C for 45 min in a shaking water bath. The glucose liberated was determined with the peridochrom oxidase/peroxidase (GOD-PAP) reagent kit and converted to total starch (TS).

Digestible starch (DS) in the various samples was determined using the method described in Tovar and Melito (10). Starch samples were incubated with Termamyl (a heat-stable α-amylase) at boiling temperature, and further digested with amyloglucosidase at 60^{O}C, followed by measurement of glucose liberated with the peridochrom oxidase/peroxidase (GOD-PAP) reagent kit. DS was calculated from glucose values as before, and resistant starch (RS) was calculated as the difference between Total Starch (TS) and Digestible Starch (DS) (11,12).

***In Vitro* Starch Digestion**

Alpha-amylolysis of starches was conducted as described in O'Brien and Wang (13). Approximately 5 g samples (db) were incubated with constant shaking in 20 mM phosphate buffer, pH 6.9, at 50^{O}C in a water bath. An equivalent of 200 U α-amylase enzyme (35.7 U/mg) was added to each slurry to initiate hydrolysis, and aliquots of 5 ml were taken after 30 min and frequently thereafter for a total period of 180 min. The aliquots were centrifuged at 1500 x g for 10 min, and the supernatant was subjected to the peridochrom oxidase/peroxidase (GOD-PAP) test to determine glucose released after hydrolysis as described before. The degree of starch hydrolysis was calculated as the percentage of TS hydrolyzed at the different incubation times (4,13). The residues left after α-amylase hydrolysis at each incubation time were dried in a conventional oven at 40^{O}C for 24 hr, powdered through a 200 µm mesh, and used to determine the effect of α-amylolysis on thermal and morphological characteristics of the starch granules.

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Starch Granule Morphology

The surface morphology of starches was observed with a Field Emission - Scanning Electron Microscopy (FE-SEM). Freshly prepared powdered sample were mounted rigidly on specimen stub by sprinkler and kept in desiccator for few hours to remove moisture. The samples were then sputter-coated and examined in a Zeiss Supra-50 VP Field Emission Scanning Electron Microscope (Zeiss, Germany).

Thermal Properties

Gelatinization and dissociation characteristics of starches were determined using a Netzsch DSC 204-F1 (Netzsch, Selb, Germany) differential scanning calorimeter equipped with a thermal analysis data station. The heating atmosphere consisted of nitrogen at the flow rate of 50.0 ml/min. About 2 mg of each starch was placed in coated aluminum hermetic pans and sealed in the sample press. Samples were scanned from 20^{O}C to 200^{O}C at a rate of 10^{O}C/min. Onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH) of gelatinization were automatically obtained from the instrument's data station.

Statistical Analysis

Data were reported as means ± standard deviations of at least three determinations (where necessary), and statistical analysis was performed with SYSTAT 11 (SYSTAT Software, Inc. Richmond, CA, USA). The data were subjected to analysis of variance (ANOVA) procedures, and significant differences among means were determined using Tukey's pairwise comparison test at the 5% level.

RESULTS AND DISCUSSION

The moisture content of the extracted red kidney bean starch was 8.3 ± 1.8% and total starch content was 89.2 ± 0.9% on dry basis. The determined values were similar to values determined in previous studies (14,15), indicating that the starch extracting procedure in this study yielded starch of good purity.

TS, DS, and RS

Table 1 shows the enzymatically accessed TS and DS contents of native, autoclaved, microwaved, and hydroxypropylated red kidney bean starches, as determined through the GOD-PAP assay procedures, and the RS content as determined by difference. Both microwaved and autoclaved starches had a significantly ($p \leq 0.05$) higher TS (44.1 % and 43.4 %, respectively) compared with native starch (37.5 %), even though the difference in TS content between microwaved and autoclaved starches was not significant.

Table 1. Total Starch (TS), Digestible Starch (DS), and Resistant Starch (RS) in Differently Processed Red Kidney Beans*

Sample	TS	DS	RS[1]
Raw	37.5 ± 1.1^{c}	28.2 ± 2.1^{d}	9.3 ± 1.4^{a}
Autoclave	43.4 ± 0.9^{a}	36.8 ± 1.1^{b}	6.6 ± 0.8^{c}
Microwave	44.1 ± 0.7^{a}	39.3 ± 0.9^{a}	4.7 ± 1.6^{d}
Hydroxypropylation	39.8 ± 0.3^{b}	31.5 ± 0.2^{c}	8.3 ± 0.2^{b}

*Means ± SD, % Dry Matter. Means with different superscripts in the same column are significantly different ($p \leq 0.05$).
[1]RS = TS - DS

Hydroxypropylation produced higher TS than native starch but was significantly lower than in microwaved and autoclaved starches ($p \leq 0.05$). A similar pattern was observed in DS values, where microwaved starch had significantly higher DS values (39.3%) than autoclaved starch (36.8%) and native (28.2%) starch. This resulted in a reverse order of highest apparent RS level of 9.3 % in native starch and the

least RS value of 4.7 % in microwaved starch. This suggests that the greater amount of heating and pressure in microwaving and autoclaving processes may lead to gelatinization of starches, thereby increasing their starch digestibility rate and decreasing RS content. This effect was less pronounced in hydroxypropylation, which is a chemical process not involving heat and pressure. Consequently, native starch showed significantly higher RS than microwaved, autoclaved, and hydroxypropylated starches in the absence of any meaningful gelatinization process in the native starch. Bravo et al. (4) reported similar findings in which processing resulted in an apparent increase of TS and decrease in RS in grain legumes, with the TS content of cooked pulses being significantly higher than in the raw ones. In their study, raw legumes were found to contain very high amounts of RS, resulting in low apparent starch digestibilities and low DS content. However, contrary results had earlier been reported by Tovar and Melito (10) in which RS measured in conventionally and high-pressure steam beans was higher than in raw pulses, and attributed this results to the retrogradation in the steamed beans. Retrograded amylose, ungelatinized starch granules, and physically inaccessible starch fractions are the three major types of indigestible starches (12), and it is possible these processes account for the differences in whether heat processing would decrease or increase RS in various starches.

Autoclaving, microwaving, and hydroxypropylation processes applied to the starches in the current study significantly reduced RS values because native granules, responsible for the high RS content of native starches, are gelatinized upon processing (4). The apparent increase of the TS content in processed legume starches may also be attributed to the partial loss of soluble materials some of which may be soluble sugars, oligosaccharides, soluble polyphenols, or soluble dietary fiber components (4). Periago et al. (16,17) reported an increase of the TS content of cooked peas and chick peas in comparison with the raw materials.

It has been previously reported that starch in raw foods is contained in granules that are poorly affected by hydrolytic enzymes, and are therefore indigestible (18), hence the high RS content of raw legumes. The difference in the starch digestibility in the three differently treated starches in this study might also be due to differences in degree of crystallinity induced by these processes in the starch granules, factors which are known to affect starch digestibility (12,19). The presence of intact tissue/cell structures enclosing starch granules hinders the swelling and solubilization of starch, resulting in a reduced digestion rate *in vitro* (11). During cooking, starch granules are gelatinized and partly solubilized, becoming available to digestive enzymes, explaining the increase in starch digestibility after the various treatments, relative to the native starch, with a significant decrease in the corresponding RS values (4).

The TS values observed in the variously treated starches from red kidney beans in the current study generally fell within the ranges reported in other studies on legume starches (20,21). Tovar and Melito (10) reported a lower TS value in the starch of raw red kidney beans compared with the level in high-pressure steamed beans. However, their study also showed that the readily available starch content of common black beans prepared from 90-min-heated seeds was significantly lower than in those coming from raw seeds, and extensively steam-heating of the pulses for ≥ 120 min resulted in a significant drop in both available an total starch levels. Total starch content of pre-soaked red and white kidney beans has also been reported to have decreased significantly as a result of cooking (20).

***In Vitro* α-Amylase Digestion**

The profile of *in vitro* α-amylolysis of the native and treated starches is shown in Fig. 1. The degree of hydrolysis (DH), expressed as the % TS hydrolyzed, increased rapidly from 0 - 60 min, for all starches, followed by a gradual increase from 60 - 180 min of digestion. With the exception of microwaved and hydroxypropylated starches, it appeared that native and autoclaved starches slowly reached peak hydrolysis after about 120 min thereby reaching a plateau at the end of the digestion period. At all times during the digestion period, native starch exhibited lower hydrolysis than the other treated starches, while the microwaved starch underwent the highest hydrolysis after about 90 min of digestion. The hydrolysis data nearly mirrored that of the DS in the various starches (Table 1) where microwaved starch showed the highest DS content and native starch the lowest amount of DS. This is in line with the general observation that the reaction rate of starch hydrolysis is, among other factors, dependent on the concentration of digestible starch. The DH data also correspond to the higher RS values obtained in native starch compared with values for the other starches (Table 1), and further that the application of heat treatment and pressure during autoclaving and microwaving increased the accessibility of starch to α-amylase, as previously reported (20,22).

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As both microwaving and autoclaving involve the application of heat and pressure, several studies have shown that pressure cooking is more effective than other processing methods in increasing *in vitro* starch hydrolysis by α-amylase (23,24). Data reported in several previous studies have indicated that the *in vitro* legume starch hydrolysis increase on cooking (25-27), and on autoclaving (23,27,28). Further, pressure cooking was shown to be more effective than other processing methods in increasing the *in vitro* starch hydrolysis by porcine pancreatic α-amylase (23,28), similar to the findings in the current study in which starch availability to hydrolysis by amylolysis was highest in microwaved starch compared with the other treatments (Table 1).

It has been suggested that cooking and autoclaving of legumes reduce the level of tannins, phytate, and amylase inhibitors which may to some extent increase starch hydrolysis of processed and cooked legume grains (28), and this observation of increase in starch hydrolysis during different heat treatments has also been attributed to granular swelling, gelatinization, and granular rupture (4,28,29).

The amount of starch left unhydrolyzed after α-amylase hydrolysis (RS) is of particular interest in this study. As shown in Table 1, the RS fraction of starch left unhydrolyzed by α-amylase was highest in native starch than in the other starches. Thus, while processing methods may lead to increase digestibility in starches in general, these processing methods produce the least amount of starch resistant to amylolysis. For diabetics, starch from raw legumes may therefore, provide an avenue to have a reduced blood glucose rise after a meal.

Table 2. Thermal parameters of native, autoclaved, microwaved, and hydroxypropylated starches pre- and post-enzymatic hydrolysis[a,b]

Treatment	T_o (^oC)	T_p (^oC)	T_c (^oC)	ΔH (J/g)
		60 min incubation		
Native	87.2 ± 0.3	91.1 ± 0.1	98.5 ± 0.4	22.9 ± 0.9
Autoclave	81.9 ± 0.1	88.6 ± 0.3	95. 0 ± 0.6	15.9 ± 0.3
Microwave	69.9 ± 0.2	76.3 ± 0.2	88.3 ± 0.2	14.8 ± 0.3
Hydroxypropylation	71.1 ± 0.1	82.2 ± 0.4	89.2 ± 0.9	10.3 ± 0.2
120 min incubation				
Native	83.2 ± 0.3	88.5 ± 0.3	91.3 ± 0.3	19.8 ± 0.1
Autoclave	76.3 ± 0.4	88.2 ± 0.6	93.4 ± 0.3	10.9 ± 0.5
Microwave	61.7 ± 0.1	72.8 ± 0.2	84.8 ± 0.8	11.1 ± 0.2
Hydroxypropylation	68.2 ± 0.2	77.6 ± 0.4	83.6 ± 0.2	8.5 ± 0.6
180 min incubation				
Native	80.8 ± 0.2	89.2 ± 0.6	93.2 ± 0.3	16.0 ± 0.7
Autoclave	72.5 ± 0.4	77.1 ± 0.7	82.0 ± 0.2	8.1 ± 0.3
Microwave	55.1 ± 0.2	60.0 ± 0.7	69.3 ± 0.4	8.9 ± 0.4
Hydroxypropylation	63.2 ± 0.3	69.1 ± 0.2	78.2 ± 0.2	6.8 ± 0.7

[a]*To*, onset temperature; *T*p, peak temperature; *Tc*, conclusion temperature; Δ*H*, gelatinization temperature.

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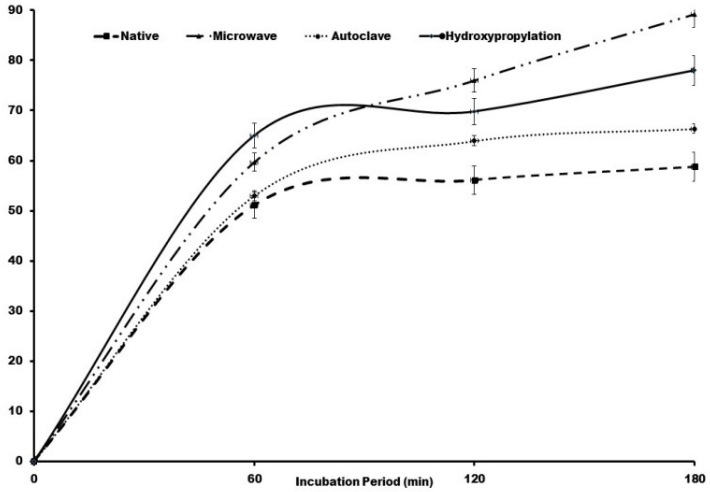

Figure 1. Degree of enzyme hydrolysis (DH) of native and treated starches. DH = % of Total Starch Hydrolyzed.

DSC

Gelatinization properties of starch residues from native, microwaved, autoclaved, and hydroxypropylated starches after α-amylase hydrolysis are shown in Table 2. Gelatinization enthalpy (*ΔH*) decreased in all starches as hydrolysis progressed, suggesting that amylolysis progressively imparted changes in starch granule structure, reducing the amount of energy needed to gelatinize the respective starches. Enthalpy was highest in native starch (22.9 J/g) at the beginning of enzyme hydrolysis, and decreased to 16.0 J/g at the end of incubation period, a reduction in gelatinization enthalpy of about 30%. For the treated starches, autoclaved produced the largest reduction in *ΔH* of 49% between the beginning of hydrolysis and the end of the incubation period, while hydroxypropylation resulted in the lowest reduction of 34%. At all incubation periods, native starch exhibited the highest gelatinization enthalpies compared with the treated starches. Comparison among the differently treated samples indicates that at longer hydrolysis times of 120 and 180 min, microwaved starches exhibited higher *ΔH* (11.1 J/g and 8.9 J/g, respectively) than autoclaved (10.9 and 8.1 J/g, respectively) and hydroxypropylated starches (8.5 and 6.8 J/g, respectively). The decrease in *ΔH* in native and treated starches could support the theory of hydrolysis of the crystalline and helical structures in starch, indicating a simultaneous hydrolysis of both amorphous and crystalline structures of native and treated starches (13).

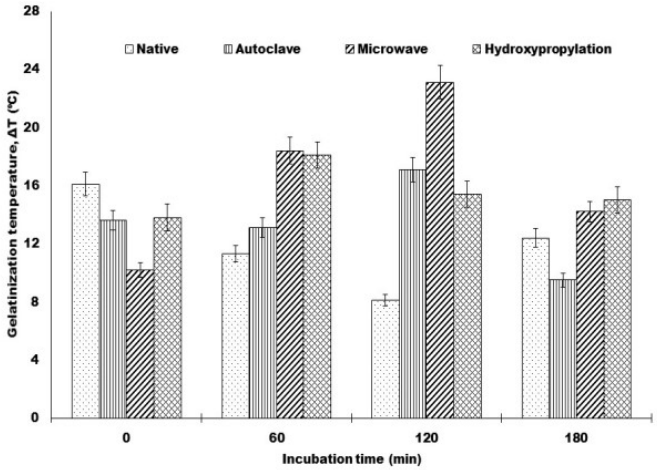

Figure 2. Gelatinization temperature (T_c - T_o) of native and treated starches after enzyme hydrolysis.

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The clear pattern of reduction in *αH* of the starches in the order: autoclave > microwave > hydroxypropylation > native, however, was not replicated in the gelatinization temperatures *(ΔT)* of the starches (Fig. 2). At the beginning of hydrolysis, native starch had the highest *ΔT*. However, no clear pattern was observed in all the treated starches. Microwave heating has been reported to have increasing effect on gelation temperatures (*ΔT*), and decreasing effect on process enthalpy *ΔH* due to the structural changes of the starch macromolecules (30). This effect was observed in the microwaved starches hydrolyzed for 120 min. Slade and Levine (31) attributed the gelatinization endotherm to the melting of microcrystallites in the presence of plasticizing water, in which crystalline melting is indirectly controlled by the kinetically constrained continuous amorphous region.

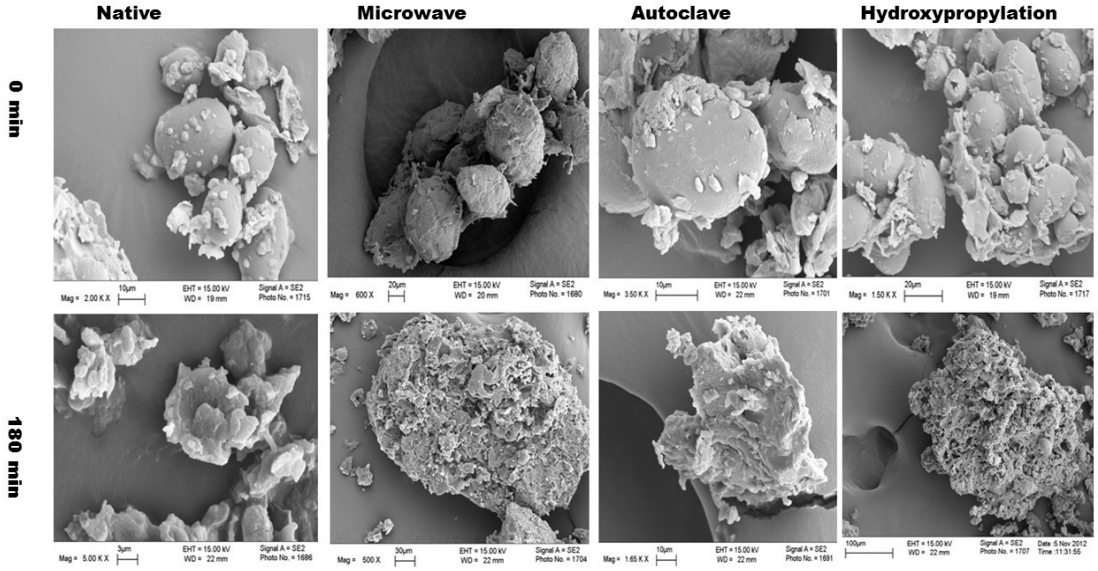

Figure 3. Representative micrograms of red kidney beans starches subjected to different treatments followed by hydrolysis. N = Native, A = Autoclave, M = Microwave, H = Hydroxypropylation.

Starch Morphology

Representative FE-SEM micrographs of native and treated starches before and after hydrolysis is shown in Fig. 3. It is evident that starch granules were intact in native, microwaved, autoclaved, and hydroxypropylated starches before incubation, as the regular elliptical shape and smooth surfaces were present. However, within each sample, enzyme digestion appeared to have altered the granule surfaces differently. Observed changes ranged from small defects for native starch, to surface crack and deformation for the treated starches after hydrolysis. Granule structures at the beginning of incubation was much different after 180 min of hydrolysis, and similar effect could be seen in all the treated starches, It is evident (Fig. 3) that the smooth surfaces of the granules were completely fragmented, albeit in different manner, after 180 min of hydrolysis. After 180 min of digestion, treated starch granules appeared to have been extensively degraded resulting in the disappearance of the smooth, rounded surface hitherto present in the native granule. The seeming inability of the hydrolyzing enzyme to digest native starch may be related to the limited degree of gelatinization of native starch in the raw beans.

The most dramatic effect of α-amylolysis could be seen in the granules of the microwaved and hydroxypropylated starches. After 180 min of hydrolysis, there was an extensive degradation and surface erosion of granules, similar to the findings reported by Bertoft et al. (32) on pea starches. There were also several perforations on the surfaces of the granules, with further total destruction after 180 min digestion. The multiple attacks of localized digging, resulting in small pits into the granule of microwaved and

hydroxypropylated starches after digestion is similar to the one reported for α-amylase hydrolysis of waxy and corn starches (13).

CONCLUSION

The formation of RS in processed starches is an important consideration for the design of legume-based foods products for diabetics in view of the nutritional importance of legumes. This study looked at the effect of different treatments on the granular structure of legume starch and the subsequent effect on α-amylase digestibility and the formation of resistant starch. Starch granules remained mostly intact in both the native and treated starches. Native starch from red kidney beans contained lower TS and DS, but higher RS values than starch from microwaved, autoclaved, and hydroxypropylated starches. Enzyme digestion appeared to have altered the granule surfaces differently for the differently treated starches, with microwaved and hydroxypropylated starches showing the most dramatic effect of α-amylolysis. The data obtained from this study suggest that different heat and chemical treatment of legume starches could be a potential avenue to produce resistance starches that can be applied in the manufacture of diabetic food products.

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