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Effect of Heat-Moisture Treatment on the Formation and Properties of Resistant Starches From Mung Bean (*Phaseolus radiatus*) Starches

Su-Ling Li, Qun-Yu Gao

***Abstract*—**Mung bean starches were subjected to heat-moisture treatment (HMT) by different moisture contents (15%, 20%, 25%, 30% and 35%) at 120℃ for 12h. The impact on the yields of resistant starch (RS), microstructure, physicochemical and functional properties was investigated. Compared to native starch, the RS content of heat-moisture treated starches increased significantly. The RS level of HMT-20 was the highest of all the starches. Birefringence was displayed clear at the center of native starch. For HMT starches, pronounced birefringence was exhibited on the periphery of starch granules; however, birefringence disappeared at the centre of some starch granules. The shape of HMT starches hadn't been changed and the integrity of starch granules was preserved for all the conditions. Concavity could be observed on HMT starches under scanning electronic microscopy. After HMT, apparent amylose contents were increased and starch macromolecule was degraded in comparison with those of native starch. There was a reduction in swelling power on HMT starches, but the solubility of HMT starches was higher than that of native starch. Both of native and HMT starches showed A-type X-ray diffraction pattern. Furthermore, there is a higher intensity at the peak of 15.0 and 22.9 Å than those of native starch.

***Keywords*—**resistant starch, mung bean (*Phaseolus radiatus*) starch, heat-moisture treatment, physicochemical properties

I. Introduction

THE advent of novel and innovative products is a strategic field in the food industry. As an increasing trend towards health and nutrition, consumers are not merely interested in traditional nutritional aspects of the food, but also are concerned with supplementary health merits derived from its regular ingestion [1]. Since starch is the most important carbohydrate in the human diet and serves as an energy source, its digestion and additional health value should be taken into account. In food industry, many manufactures sought to find an alternative way to provide carbohydrate foods that can be competed with fibre stuff. Therefore, resistant starch (RS) is called in to existence and becoming increasingly interested by a large number of food researchers and producers. Resistant starch is the sum of starch and fraction of starch degradation that escapes digestion in the small intestine of healthy people [2], but may be fermented by the colonic microorganism. Potential physiological value of the resistant starches was reported have some relationship with colonic health for the effects on fecal bulk and SCFA metabolism [3].The beneficial effect of RS has been summarized by several authors, which serving as impediment of colon cancer, reduction of the glycemic and insulinemic responds to food, substrate for the microflora in the large intestine, protective against gall stone formation, hypocholesterolemic effects, control of fat accumulation, and advanced digestion of minerals [2], [4], [5].Therefore, RS, as a functional fiber, is likely to have potential for an innovative food additive for its physiological effects. RS could be parallel with traditional dietary fibers regarding to consumer acceptability and greater palatability.

Heat-moisture treatment (HMT) is one of hydrothermal modifications in low moisture contents (<35% W/W), and commonly exposure at a temperature above the glass transition temperature (Tg) but below the onset (To) temperature of gelatinization for a certain period of time [6]. Currently, the growing popularity for physical modifications and are also referred to be safer than chemical possess. The physical method of heat-moisture method is particularly favorable for the food applications with novel functional properties [7]. Known reports on the HMT change of several starches are corn, cassava, potato, wheat, waxy corn, amylomaize, oat, and barley and legumes starches. The significance of this impact was related to the moisture levels during heat treatment and starch source [6].

Great effort has been made to demonstrate that HMT might enhance the yields of RS. For example, Shi and Trazasko have put forward one patent about the preparation of granular resistant starch by HMT with amylomaize starches [8]**.** Sievert and Pomeranz prepared RS from normal and waxy starches by HMT at 18% moisture and demonstrated HMT reduced enzyme susceptibility [9]. Chung et al. showed that the RS levels of corn, pea, and lentil starches had increased from 4.6 %, 10.0%, 9.1.% to 12.3%, 14.5%, 14.7% respectively after HMT [5]. Luo, Gao and Yang had attempted to prepare boiling-stable resistant starch through using the method HMT [10].

Mung bean *(Phaseolus radiatus)* is an under-utilized and promising legume, primarily cultivated in China and also exported to other countries of the world for its high national production. Mung bean had high carbohydrate content and is a valuable source of starch. Mung bean starch is referred to be the best raw material to make transparent cellophane noodles, and includes a potential resistant starch ascribing to its high amylose content and strong interplay of the granule starch [11]. However, in China, modification research regarding improving

quality of mung bean starch is limited and mung bean starch is still in the dilemma of poor economic value. A great many studies have been focused on common by HMT, but reports of mung bean RS through hydrothermal treatment have not been carried out yet. It is necessary for China to identify this promising starch for the sake of supplementing the available starches. Since mung bean plays a significant part in various kinds of legumes in China, there is a necessity to exploit added-value products from mung bean starch. Consequently, it is considered worthwhile to investigate appropriately modified mung bean starch and might also develop an alternative way to avoid excessively utilizing of common starches in the long term.

The objective of the present study was to prepare mung bean resistant starch by HMT, and evaluate its physicochemical and functional properties. This would be of importance both for the development of novel starches for food application and for growing understanding the effect of moisture content on properties of mung bean RS.

II. Materials and Method

A. Materials

Mung bean starches (Harbin Hada Company, China) were used as raw materials to produce the resistant starch (RS) samples.

Enzymes and Chemicals. Resistant starch assay kit was bought from Megazyme International Ireland Ltd., Co.Wicklow, Ireland. Standards amylose and amylopectin were obtained from Sigma Chemical Company (St. Louis, MO, USA). Standard dextrans was purchased from Pharmacia Ltd., Germany. Chemicals and solvents in this work were of analytical grade.

B. Heat-moisture Treatment

The moisture levels of starches samples were adjusted to 15, 20, 25, 30, 35% (the moisture level of the raw starch was pre-determined) by dispersing in appropriate amount of distilled water. The samples were sealed into different stainless steel containers and kept for 24h at ambient temperature. The sealed samples were heated in a thermostatically controlled convection oven at 120℃ for 12h and cooled to room temperature. The contents were removed from the containers and dried at 45℃ to uniform moisture content (~8%). All the samples were ground and screened through a 70 mesh sieve. Based on the treatment moisture content, the resulting HMT starches will be referred to as HMT-15, HMT-20, HMT-25, HMT-30 and HMT-35.

C. Resistant Starch Determination

The method for determining resistant starch (RS) contents was according to the analysis procedure provided by the Resistant Starch Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). In short, starch (100mg) and 4 ml of mixture (pancreatic a-amylase, 10mg/ml, and amyloglucosidase, 3 U/ml) was added to each test tube, and then incubated in a shaking water bath (Wisebath@, Feedback Control Digital Timer Function, Sweden) for 16 h (37□, 200 strokes/min) to hydrolyze digestible starch. The resistant portion was deposited with 95% ethanol and residue obtained was washed with 50% ethanol twice, and then treated with KOH solution (4 M, 2 mL) to solubilize RS. RS solution obtained was adjusted to pH 4.75 with 1.2 M sodium acetate buffer (8 ml, pH 3.8), incubated with amyloglucosidase (0.1 ml, 3300 U/ml) at 50 ℃ for 30 min. Samples were centrifuged at 1500g for 10 min. Aliquots (0.1ml) of the supernatant, 3 ml of GOPOD was added and the mixture was incubated at 50 □ for 20 min. Absorbance was measured using a spectrophotometer (Model 722, Shanghai No.3 Analytical Instrument Company, Shanghai, China) at 510 nm.

D. Scanning Electron Microscopy

Starch samples were prepared by sprinkling the starch on double-sided adhesive tape attached to a circular aluminum stub, and then coated with 20nm gold under vacuum. The samples were viewed and photographed in a scanning electron microscope (model S-3700N, Hitachi, Japan) at an accelerating potential of 20 kV.

E. Polarized Light Microscopy

Birefringence of native and modified mung bean starch granules was observed under an optical microscope (model BH-2, Olympus, Japan). All samples were dispersed in solution (glycerin/ deionized water; 1:1 v/v) and the images were recorded at the same magnification (400×).

F. Apparent Amylose Contents

Apparent amylose contents of sample were assessed according to the colorimetric procedure of Juliano and Yeon [12].

G. Hight-performance Gel Fraction Chromatography

The sample was dissolved in 90% DMSO and then centrifuged at 3000 g for 20 min. The sample solution was filtrated through cellulose–acetate membrane filters with 0.45 mm pore size. The solution was then injected into a Waters 600 HPLC system (Waters Corp., Milford, MA) with two analytical columns (Ultrahydrogel™ Linear 300mm×7.8mm) and a differential refractive index detector (Model 2410, Waters Corp., Milford, MA). The column temperature was maintained at 45 ℃. The mobile phase of 100mM $NaNO_3$ containing 0.02% NaN_3 was circulated at a flow rate of 0.9 mL/ min. Standard dextrans with different molecular weights (MW) were used for MW calibration.

H. Swelling Power and Starch Solubility

The swelling power and starch solubility were measured according to method of Adebowale and Olu-Owolabi[13], [14].

I. X-ray Diffraction (XRD)

X-ray patterns were obtained with D/Max-2200 X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan). The samples

were scanned through the 2θ range of 5 - 35°, with target voltage 40 kV, target current, 30mA, and scanning at the rate of 8 °/min.

J. Statistical Analysis

The test data were statistically analyzed using one-way ANOVA on SPSS version 13.0 software for Windows (USA). Duplicate determinations were performed for each test, and the results reported are average values. LSD (p<0.05) test was applied to determine differences between means for the treatments at the 5% significance level.

III. Results And Discussion

A. Resistant Starch Contents

The resistant starch (RS) contents of native and heat-moisture treated mung bean starches are presented in Table I.

TABLE I
RS LEVELS OF NATIVE AND HEAT-MOISTURE TREATED MUNG BEAN STARCHES

Samples	RS(%)
Native starch	5.16±0.02[a]
HMT-15	30.89±0.45[b]
HMT-20	45.15±0.06[c]
HMT-25	36.49±0.17[d]
HMT-30	30.81±0.82[b]
HMT-35	17.41±0.20[ab]

Means values with different letters within each column are significantly different (P<0.05)

The enzyme resistant portion of native starch was very small amount (5.16%), however, the RS values of heat-moisture treated starch increased greatly, 30.89% for HMT-15, 45.15% for HMT-20, 36.49% for HMT-25, 30.81% for HMT-30, 17.41% for HMT-35. During treatment, the difference of modified starch with various levels of moisture contents was pronounced with the exception of HMT-15 and HMT-30. The increment of modified starches was obvious and 2-8 times more than the counterpart of non-treated native starch. Among these modification conditions, when heat-moisture level reached 20%, the enzyme-resistant component arrived at the maximum peak of 45.14%. These results exhibits that moisture contents play an important role in RS formation during heat-moisture treatment. These changes are in accordance with the observation with corn starch [15]. The report pointed out that rearrangement of the starch molecule to the enzyme-resistant structure may the cause of increment of RS during HMT under non-gelatinized condition.

Moisture content is the key factor in heat-moisture treatment, the action of water on hydrogen bonds between molecular chains within the starch granular are formed [16]. At low water content, the motion of water amongst starch molecular chains is weakened and therefore water activity is discouraged. If treated with excessive water content, it is presumable that molecular chains become flexible and enzyme can attack them more easily. This could explain low RS levels in non-treated starch. As a result, controlling appropriate water content during heat-moisture treatment may help increasing resistant starch content.

B. Scanning Electron Microscopy

The shape and surface characteristics of native and modified starches are showed in Fig.1.

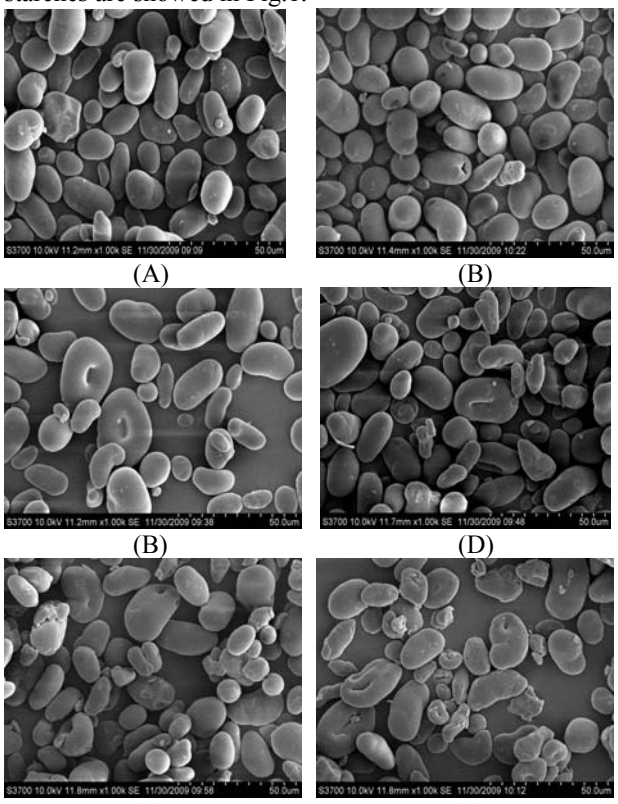

Fig.1 Scanning Electron Micrographs (SEM) of native and heat-moisture treated starches: (A)Native starch, (B) HMT-15, (C) HMT-20), (D) HMT-25, (E) HMT-30, (F) HMT-35. Scanning electron micrographs of all starches are recorded at 1000×magnification.

The results revealed mung bean starch granules were rounded, kidney-shaped or oval irregular shapes. The smaller starch granules were rounded, while the larger starch granules seemed elliptical and overwhelmed majority part of granules. The surface of native starch granules was smooth and showed no evident fissures under the SEM. The shape of granules hadn't been altered during HMT and the integrity of starch granules was preserved for all the conditions. This concurred with the results presented for heat-moisture treated with finger millet starch [13], canna starch [17], cocoyam starch[18].

However, heat moisture treatment caused surface change and there was cavity in the starch particles of MHT-15, MHT-30, MHT-35 (Fig.1 (B), (E) and (F)). It was evident that pitting and indentation was placed favorably at the center of heat-moisture treated starches granules (Fig.1 (C), (D), (G) and (F)), which was seemingly caused by re-association of the molecular structure at the center of the starch granule where the tissue structure was weak [19]. It was possible that rearrangement of

starch molecular and tightly molecular structure led less susceptible to enzymatic digestion of starch through channels and amorphous regions. Based on starch granule size, increments in surface area per unit weight were observed in HMT than that of native starch. This result agrees with the observations of new cocoyam starch [18]. It was hypothesized that expansion of starch surface areas would limit enzymatic erosion mainly on the surface of starch granules and decrease the accessibility between substrate and enzyme [20].

C. Polarized Light Microscopy

Polarized light micrographs of native and heat-moisture treated mung bean starch are shown in Fig.2.

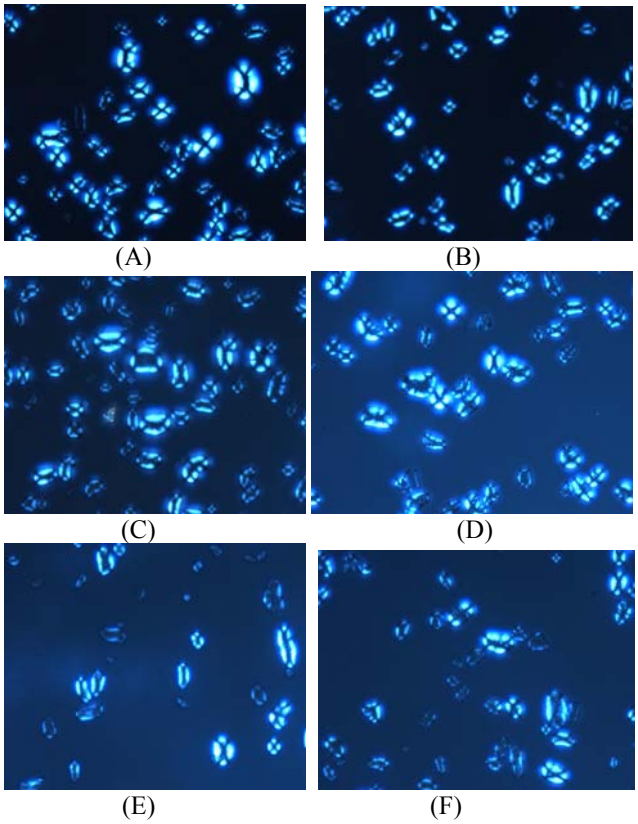

Fig.2. Photomicrographs of native and heat-moisture treated starches.(A) Native starch, (B) HMT-15, (C) HMT-20, (D) HMT-25, (E) HMT-30, (F) HMT-35. Photomicrographs of all starches are recorded are at 400×magnification.

A pronounced birefringence cross were exhibited at the center of native starch, which is a symbol of the average radial orientation of helical structures (Fig.2(A)). Birefringence was also found to remain at the periphery of HMT starch granules.

However, there was a reduction in birefringence on HMT starch granules. For heat-moisture treated starches, some of them lost birefringence pattern at the granule center while others remained highly birefringent. Similar observations have been depicted on pea and navy bean after HMT [21], indicating that loss of birefringence at the granule center and retention of birefringence at the granule periphery. This result was also in agreement with the investigations on HMT of potato starches [22]. The study showed that birefringence had disappeared at the centre of the granules at the same time with the development of the voids. The authors indicated that the loss in radial orientation resulting from increasing mobility of starch chains at the granule center driven by thermal energy. It is probable that birefringence were maintained at the granule periphery owing to tightly cluster of starch chains at the periphery, which demands for a higher driving thermal energy to chain mobility.

D. Apparent Amylose Contents

In Table II, with heat-moisture treatment, the apparent amylose content of heat-moisture treated starch was increased compared to that of native starch.

TABLE II
APPARENT AMY LOSE CONTENT OF NATIVE AND HEAT-MOISTURE TREATED STARCHES

Samples	Apparent amylose contents
Native starch	27.73±0.24[a]
HMT-15	31.99±0.79[b]
HMT-20	35.00±0.55[c]
HMT-25	32.29±0.61[b]
HMT-30	31.99±0.55[b]
HMT-35	31.47±0.06[b]

Means values with different letters within each column are significantly different ($P<0.05$)

The difference between native and HMT starches was significant, and the change of apparent amylase content of HMT starch was not pronounced with the exception of HMT-20. As shown in Table 1 and 2, with enhancement of resistant starch levels, the apparent amylose content was increased in general. The apparent amylose contents of HMT-20 was the largest of all samples, meanwhile, its RS level was at the maximum of 45.15% correspondingly. This result was resembled with reporting that high resistant starch levels had some conjunction with high levels of amylose. It is likely that augmentation in amylose formation was susceptible to form longer or more ordered helical segments by amylose–amylose (AM–AM) and/or amylose–amylopectin (AM–AMP) interactions formed on HMT. Thus, it might be suggested that the rise on amylase contents could increase the color intensity of the amylose–iodine complex through this feasible colorimetric method [23].

E. High-performance Gel Fraction Chromatography

The MW distribution of native and heat-moisture treated starches were determined by high-performance gel fraction chromatography (HPGFC) is shown in Table 3. The calculated area and percentage of peaks were also shown in the table. The

TABLE III
MOLECULAR WEIGHT DISTRIBUTIONS OF NATIVE AND HEAT-MOISTURE TREATED STARCHES
A VALUE OF WEIGHT-AVERAGE. B VALUE OF NUMBER-AVERAGE MOLECULE.MOLECULE. C VALUE OF POLYDISPERSITY.

Samples	Peak 1				Peak 2			
	Mw[a]	Mn[b]	D[c]	Peak area (%)	Mw[a]	Mn[b]	D[c]	Peak area (%)
Native	854983	394360	2.17	100%	——	——	——	——
HMT-15	211545	81305	2.60	72.40	7406	6010	1.23	27.60
HMT-20	164908	53301	3.09	67.79	4404	3430	1.28	32.21
HMT-25	199362	67647	2.95	70.51	5070	3952	1.28	29.49
HMT-30	256058	83440	3.07	72.96	6358	5096	1.25	27.04
HMT-35	269116	77427	3.48	64.77	5291	4356	1.21	35.23

native mung bean starch displayed only one peak. However, after the heat-moisture treatment, the HMT starch fractions were composed of two MW peaks, labeled Peak 1 and Peak 2, respectively, which indicated the high and low molecular weight from elution profiles. The result showed that the unprocessed starch with one-peak was mainly composed of the high MW, that is to say, the native starch was formed of complex macromolecule. However, HMT starches had a large amount of the high MW fraction and a small proportion of the low MW fraction. The chromatograms of HMT starches were similar and the ration of area of Peak 1 appeared in the range of 64.77% to 72.40%.

Information obtained from Peak 1, the high MW fractions, indicated that MW distribution of HMT starches was lower than that of native starch. Lower MW distribution (Peak 2) of HMT could be found on HMT starches. These starch fragments indicated that the degradation of starch may result from thermal process during the periods of heat-moisture treatment. The amounts of molecular weight-average (Mw) followed the order: HMT-20 <HMT-25 <HMT-15 <HMT-30 < HMT-35< Native starch. It was also found that this order was identical to the order of resistant starch contents (Table 1) accordingly. HMT-20 had the lowest Mw of all the starches but had highest RS contents. It's probably assumed that double helices was unlocking or a macromolecule, basically amylopectin, was degraded of α-1,4 or α-1,6 linkages on starch during HMT. Furthermore, the result of molecular number-average (Mn) exhibited that reasonable reduction in Mn of macromolecule would help formation of resistant starch. In brief, the present of degradation of high molecule exerted a pronounced effect on forming enzyme-resistant portion. Polydispersity index is expressed as follow: $D=DP_w/DP_n$, DP_w is weight-average degree of polymerization; DP_n is number-average degree of polymerization. HMT starches had a higher polydispersity than the counterpart of original starch. However, the polydispersity of peak 1 and the resistant starch levels were not essentially linear. This was further supported by the sample of HMT-20 that had an intermediate polydispersity of 3.09 in the range of 2.17-3.48.

For peak 2, heat-moisture treated starches had similar trends in Mw and Mn with the change of different moisture contents. Resistant starch contents of samples could not only explained by polydispersity of starches. For example, polydispersity of HMT-20 and HMT-25 showed similar value of polydispersity (1.28), regardless of different RS levels (45.15% and 36.5%, separately). However, the low MW fraction should be taken into consideration because these fractions provide some short fragments for RS formation, such as amylase and short chain of amylopectin.

In the view of peak 1 and peak 2, it's naturally speculated that HMT might change the thermal movement and degradation of starch molecule. During HMT, short chains of amylopectin and amylose might come into being for the reduction of MW, which may help strengthening the interactions and formation of amylose -amylose chains or between amylose-amylopectin chains [15], [24]. This also could attribute to arrangement of starch chains into double helices in the amorphous regions [25]. This explanation was consistent with the enhancement of apparent amylose after HMT (Table 2). Obviously, increment in apparent amylose content offered more opportunities for participating in re-associations between amylose-amylose. Therefore, HMT is potential for suitable reduction in MW distribution of starch. The results can be suggested that the material with a large amount of high MW distribution and with relatively small amount of low MW distribution is favorable for RS preparation and HMT.

F. Swelling Power and Starch Solubility

The effect of temperature on swelling power of all the starches is presented in Fig.3. The effect of temperature on swelling power of all the starches is presented in Fig.3. With the increment of the assay temperature, the swelling power of the native and HMT starches were raised correspondingly. The swelling power of native starch was higher than those of HMT starches. The order for swelling power was as follows: Native starch> HMT-35> HMT-30> HMT-15> HMT-25 >HMT-20. During starch gelatinization in presence of water, the granules imbibe water, and swell. It is reasonable that reduction in

swelling capacity is attributing to rearrangement of molecular chains during HMT, strengthening its maintained force, which restricted in absorbing water within starch matrices and therefore a more rigid starch structure were formed after hydrothermal modifications [26], [27]. The swelling capacity of HMT-15, HMT-25, HMT-30, HMT-35 had a slight increment and exerted negative effect on RS compared with that of HMT-20.

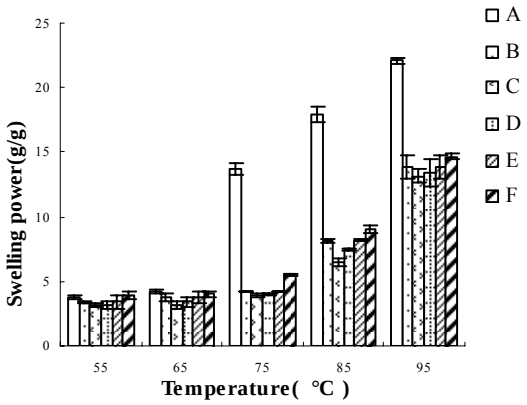

Fig. 3 Effect of native heat-moisture treatment (HMT) on the swelling power (on dry basis).(A) native starch; (B) HMT-15; (C) HMT-20; (D) HMT-25; (E) HMT-30; (F) HMT-35.

This was consistent with previous studies involving some starches, for instance, rice [28], millet [13], maize [16], mucuna starch [29], which reported the swelling capacity of starch were diminished by HMT accounting for the ordering rearrangement of starch molecule and restriction of starch hydration. It was reported that HMT could lead to crystallite growth or perfection and additional interaction of AM–AM and/or AM–AMP chains [30]. It was also presumable that strong interactions may have been formed between AM– AM chains during HMT. What's more, Adebowale et al. [29] has cited that the formation of amylose–lipid complexes within the starch granule might also be link to the reduction in the swelling capacity for restricting of granule swelling by amylose–lipid complexes.

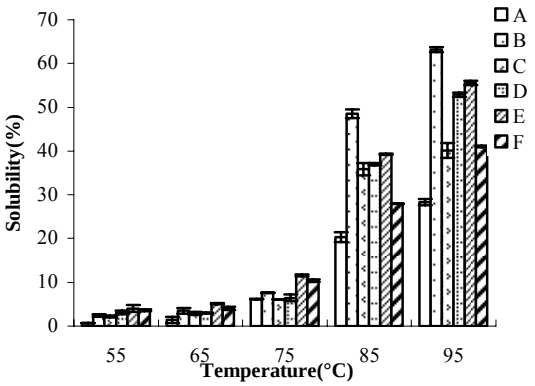

Fig. 4. Effect of native and heat-moisture treatment (HMT) on the solubility (on dry basis). (A) native starch; (B) HMT-15; (C) HMT-20; (D) HMT-25; (E) HMT-30; (F) HMT-35.

The solubility profile of the native and HMT starches at different temperature is shown in Fig.4. Compared to the native starch, solubility of heat-moisture treated starch was increased. There was a growing increase in solubility with the temperature increased (50–90℃). Solubility of HMT starches increased sharply when the temperature was increased above 70. However, the change in starch solubility of heat moisture treated mixture did not increase as the moisture content increased over the range of 15-35%. Similar results for increment in solubility after HMT were confirmed to the studies involving maize starch [16], finger millet starch [13], indicating that HMT starches had a higher solubility than that of native starch.

G.X-ray Diffraction (XRD)

The X-ray diffraction pattern and intensities are demonstrated in Fig. 5. The native mung bean starch has the characteristic 'A' pattern, which showed strong intensity at the peaks of 16.9, 19.7 and 21.88 Å. After treatment, the HMT starches (MHT-15, MHT-20, MHT-25, MHT-25 and MHT-30) displayed relatively strong diffraction intensity at the peak of 15.0, 16.9, 20.0, 21.88, 22.9 Å. The A type pattern remained unchanged at different moisture conditions after modifications. The intensity of the peaks of HMT starches was slightly higher than original starch at the peak of 15.0 and 22.9 Å. This result indicated that moisture content played an important role in starch crystalline.

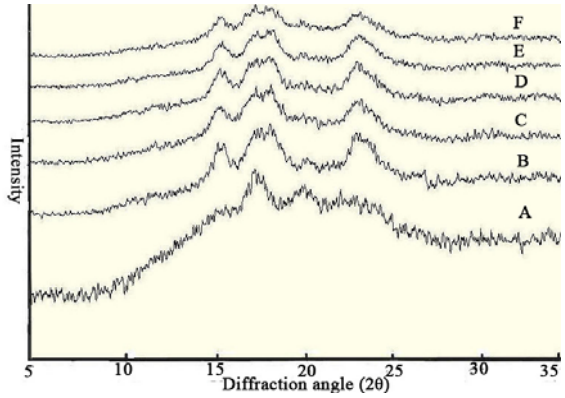

Fig. 5 X-ray diffraction pattern of native and heat-moisture treated mung bean starch. (A) native starch; (B) HMT-15; (C) HMT-20; (D) HMT-25; (E) HMT-30; (F) HMT-35.

The intensity of starch granule was not always increased with moisture conditions of HMT. Franco et al. perceived that there was a slight decrease in intensity of waxy corn starch treated at 18 and 27% moisture, with the increment of moisture level [26]. A lower water-bind capacity and percentage of enzymatic hydrolysis were found at 18% moisture level because increment of moisture level would disrupt the previous crystalline order. Differences in X-ray intensity were linked to the manner in which the double helices are arranged within the crystalline domains of the granules. This could explain the fact that HMT-20 had the greatest intensity of all the starches. Appropriate moisture content will enable structural rearrangement regarding to realignment of bonding forces in starch granules and formation of ordered double helical amylopectin side chain clusters [18]. This result was further demonstrated the outcome of the limitation of starch swelling (Fig. 3).

Overall, this finding appears to suggest that heat-moisture treatment is considered to be a potential and effective physical processing to prepare mung bean resistant starch. Mung bean starch can be deemed as a supplemental source for common starch, which improving the value of mung bean starch in China.

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