Effect of heat treatment on the physico-chemical properties of starch from different botanical sources

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Abstract: Changes in the physicochemical properties of wheat, sago, tapioca and potato starches were studied after heating for 1 hour at 100°C, 110°C, and 120°C and for 2 hours at 120°C. These properties were characterised through the swelling behaviour of starch granules, amount of carbohydrate materials leached from the granules, starch paste retrogradation rate and gel strength. For all starches except wheat, the swelling ability, rate of retrogradation and gel strength decreased while solubility increased with increasing temperature and heating time. Wheat starch followed this pattern only when heated at 120°C for 1 and 2 hours. Gel strength correlated well with the ratio of amyllose to amylopectin (R) in the leachate. To produce fried crackers with good expansion properties, the granule has to be sufficiently degraded so as to allow more amylopectin to be leached out to achieve R value of 0.25-0.5. This can be achieved by heating wheat starch at 120°C for 1 hour or longer.

Keywords: Amylose-amylopectin leaching; Wheat, Sago, Tapioca, Potato

Introduction

One of the important properties of starch is its ability to swell and leach soluble materials when heated above its gelatinization temperature and this determines its specific functional property when utilized in food products. Starch pastes from different botanical sources exhibit different physical characteristics and notable amongst them is potato starch paste which is ‘stringy’ in nature and corn starch can be described as ‘clumpy’. When used in products such as fish crackers or ‘keropok’, only tapioca and sago starches are used, mainly due to the poor expansion properties of the fish crackers during frying when other starches such as wheat are used. The type of starch used plays a very important role in the expansion of fish crackers (Yu, 1991). Several experiments have been conducted to test the effects of various types of starch on the quality of keropok (Kyaw 1998; Kyaw et al., 1999; Kyaw et al., 2001; Kyaw et al., 2001). It was reported that keropok made from tapioca starch were preferred by sensory panellists (Norrakiah 1987; Yu 1993; Wang and White 1994). Wheat starch however, does not produce good quality keropok with the desired texture but after heating at 120°C, the expansion property is almost similar to that of tapioca and sago starch (Kyaw 1998; Kyaw et al., 1999). As wheat is generally not used in the production of keropok due to it’s poor expansion properties, this particular observation has wide ranging application since it opens up the possibility of wheat starch being used in the production of keropok. However, the reasons why wheat starch heated at 120°C should produce keropok with good expansion characteristics as compared to poor quality keropok from wheat steamed under normal conditions (100°C), are not fully understood. To assist in gaining a better understanding into this matter, this study was carried out where four starches obtained from different botanical sources: wheat (cereal), tapioca (tuber), sago (stem), and potato (root) were selected and the changes in their physicochemical characteristics during heating such as swelling, solubility and amylose leaching as well as the rate of retrogradation of the starch pastes and gel strengths were observed.
Materials and Methods

Raw materials
Tapioca starch (*Manihot esculentus*) was obtained from Yolimex Sdn. Bhd. (AAA brand, Thailand); and sago starch (*Metroxylon sagu*) from Gamex Enterprise, Malaysia. Wheat starch (*Triticum aestivum*) and potato starch (*Solanum tuberosum*) were imported from Roquette Starch Co., France and BDH Chemicals, Inc., respectively.

Sample preparation and heating conditions
Starch were solubilised with water in 15 ml centrifuge tubes and mixed thoroughly using a Vortex Shaker (IKA Vortex 3 Shaker, USA). The starch suspensions were heated for 5 minutes in boiling water bath and immediately subjected to various thermal treatments: (i) 100°C for 60 minutes in a water bath, (ii) autoclaved at 110°C for 60 minutes, (iii) autoclaved at 120°C for 60 minutes, and (iv) autoclaved at 120°C for 120 minutes. These heating conditions were chosen to cause leaching of starch polymers.

Swelling properties
Starch suspension (5 g of starches per 180 g water) in a 250 ml centrifuge bottle was heated according to the above mentioned treatments. Water was added to the mixture to reach a total volume of 200g and inverted a few times before mixing using a Vortex Shaker (IKA Vortex 3 Shaker, USA) It was then centrifuged (Heraeus Multifuge 3L, Thermo, Germany) at 2200 rpm for 15 minutes to allow a good separation. The swelling power of the starches was determined according to published method (Schoch, 1964).

Carbohydrate leaching
The amount of carbohydrate leached from the granules was measured according to a method described by (Dubois, 1956). Starch (20 mg) was suspended in distilled water (6.25 ml) and given heat treatment. After centrifugation (3500 rpm, 10 min), 1 ml of the supernatant was pipetted and diluted (50X). One ml of the diluted supernatant was mixed with 1 ml 5% phenol solution in a screw-capped test tube and followed by rapid addition of 5 ml concentrated sulphuric acid (stream of acid directed against liquid surface rather than against the side of the test tube to get good mixing). The mixture was set aside for 10 minutes in a water bath at 20 – 30°C before readings were taken. It was then shaken with the Vortex Shaker (IKA Vortex 3 Shaker, USA) and the absorbance of the yellow-orange colour mixture was measured at 490 nm with distilled water as blank using a UV-VIS Spectrophotometer (Ultrospec 3000, Pharmacia Biotech, UK). The amount of sugar was determined with reference to a standard curve.

Amylose determination
Twenty mg of starch in a narrow-bottom 20 ml centrifuge tube was mixed with 5 ml 85% (v/v) methanol and heated in water bath at 60°C for 30 minutes. Following centrifugation, the supernatant was discarded and this extraction was repeated three times. Water (5 ml) was added to the sample and given heat treatment. The next steps were centrifugation, solubilisation and amylose determination as mentioned in the literature (Chрастil, 1987). The amount of amylose leached was determined with reference to a standard curve.

Rate of cloudiness
Starch pastes (3%, w/v) were produced when starch (0.5 g) was suspended in water (5 ml) in screw-capped tubes and placed in boiling water bath for 5 minutes. Following that, heat treatments were given and tubes were thoroughly shaken (lightly) every 5 minutes. After cooling to room temperature, the absorbance at 650 nm was determined against a blank (distilled water) using the UV-VIS spectrophotometer (Ultrospec 3000, Pharmacia Biotech, UK). Readings were taken at every half an hour for 10 hours. The rate of cloudiness was determined from the slope of a linear curve (natural log of absorbance versus time) as done by Miles *et al*. (1985a).

Gel strength
Aqueous suspensions of starch (10%, w/v) were given heat treatments as mentioned. For treatment (i), the suspensions were agitated slowly and constantly until the viscosity increases. This prevented sedimentation and disruption of the granules. The pastes were poured into a plastic container (5 cm diameter) to about 3 cm in height and cooled. Paraffin oil was poured onto the gel surface to prevent moisture loss and the gel was stored at room temperature (25 ± 2 °C) for 24 hours. The strength of the gel was measured using a Texture Analyser (TA-XT2i, Stable Micro Systems, UK). The gel with the container was placed on the compression platform. The 10 mm diameter cylindrical ebonite probe (P10) was then driven at a constant speed (2 mm/s) into the gel for a distance of 16 mm. The trigger force was set at 5g and the first peak was recorded as gel strength. The readings are expressed in unit of grams.
Granule morphology

The morphology of the heat-treated starch paste was studied using a light microscope with a phase-contrast mode. A thin layer of the paste was prepared on a slide and stained with iodine solution. The granules were photographed using a Nikon camera attached to the microscope.

Statistical analysis

Analysis of variance was done using the SAS method. Duncan’s Multiple Range test was used to determine significant differences among means. Linear regression was done to determine the line of best fit for standard curves and rate of cloudiness using Microsoft Excel.

Results and Discussion

Swelling power

The swelling power of starches at different treatments is shown in Figure 1. The swelling power of sago, tapioca and potato starches showed much lower values for the temperatures 110°C and above. This indicates that the starch granules have been substantially degraded after undergoing maximum swelling around their respective gelatinization temperature. On the other hand, the swelling power of wheat starch was highest when heated at 120°C and registered a value which is similar to values of the other starches heated at 100°C. At 100°C sago, tapioca and potato starches were almost completely swollen but wheat starch swelled only to a limited extent. At this temperature, swelling of wheat starch was restricted due to the formation of amylose-lipid complexes in the granules. At temperatures above 100°C, some of these complexes were broken by heat and the granules were allowed to swell. Wheat starch granules imbibed increasing amounts of water and swelled freely at 110°C, resulting in significantly higher, (p < 0.05) swelling power. At this stage, the other starches were ruptured and started to disperse, allowing more leaching of soluble materials and resulting in decreased swelling power.

Solutions of sago, tapioca and potato starches heated at 120°C for 1 hour is assumed not to contain any swollen granules as the swelling power recorded was zero percent (Figure 1). This shows that total solubilization has occurred. For wheat starch, this point is almost its maximum swelling point. Upon extended heating at the same temperature, the swelling power decreased significantly, (p < 0.05) and ultimately reached zero percent. This shows that the wheat granules ruptured when heated at 120°C for duration of between 1 to 2 hours and by the end of 2 hours, it has achieved total solubilization. The swelling power of wheat at 120°C heated for 1 hour was almost the same as the swelling power of the

![Figure 1](image_url)
other starches heated at 100°C, which is similar to that reported in the literatures (Kyaw 1998; Kyaw et al., 1999; Kyaw et al., 2001).

The swelling pattern of starches has been used to explain the binding force existing within the molecules. Compared to sago, tapioca and potato starches, the swelling power of wheat starch indicated the existence of strong binding forces. Leach et al. (1959) reported that swelling behaviour of starches is a property of their amyllopectin content; amylose acts as diluent and inhibitor of swelling, especially in the presence of lipids which can form insoluble complexes with amylose during swelling. Amylose is believed to act as a restraint in swelling, and cereal starch granules do not exhibit complete swelling until amylose has leached out from the granules (Bowler, 1980). The amount of lipid and protein in native starches may affect their swelling behaviour. The mechanisms involved in maintaining starch content within the deformed heated granules could be affected by the minute amount of protein present in the granules; and the viscoelastic properties of the granule envelope that helps retaining the starch content (Han and Lim, 2004; Israkarn et al., 2007). They are different from storage proteins and are bound tightly on the surface and/or as integrated constituents within the granule structure. These proteins are mainly starch biosynthetic enzymes and have molecular weight around 5–149 kDa (Baldwin, 2001). According to (Morrison, 1988), complexes may have been formed between the starch lipids and residual amylose in the granules during swelling. Protein and starch interact due to the attraction of their opposite charges and form complexes during gelatinization and this inhibits swelling. The limited swelling power of legume starch is also influenced by the existence of peptide bridges in the starch granules that maintains the structure of the starch granule ghost (Oates, 1990). Ohmic heating reduced pasting temperature for commercial rice starch, resulting in a starch that swelled faster with weaker retrogradation properties than the conventionally heated sample (An and King, 2006).

**Solubility (carbohydrate leaching)**

The pattern observed in the solubility (carbohydrate leaching) characteristics of the four types of starches is not the same as observed from the swelling properties. All starches showed increasing solubility as the temperature and duration of heating was increased (Figure 2). However, solubility of wheat starch was relatively lower than the other starches, which is due to the fact that amylose-lipid complex existing in wheat inhibits swelling, cracking and dispersion of the granules (Leach et al., 1959). Therefore, there were limited means to promote leaching of soluble materials from the intact granules. Starch solutions of non-cereal starches heated at 120°C for 1 hour were almost completely solubilized due to complete dispersion of the granules. Wheat starch reached that particular level of solubilization only after 2 hours of heating at the same temperature. Similar to swelling pattern, solubilization of wheat starch heated at 120°C for 1 hour is almost the same as the solubility of other starches heated at 100°C. This explains that wheat starch, due to the presence of lipid complexes; requires a more severe heat treatment to allow leaching of carbohydrate polymer as indicated by the solubilisation values.

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**Figure 2.** Solubility of starches at different treatments

**Figure 3.** Amylose leaching of starches at different treatments
**Amylose leaching**

Generally, all starches had increasing amounts of amylose leaching as the temperature and heating period were increased (Figure 3). This is similar to the pattern observed for solubility. The difference between the leached amylose of the sample heated at 100°C and 120°C heated for 2 hours was small for all starches except for wheat starch. The small difference occurred because at 100°C, most of the amylose has leached through the cracks. For wheat starch granules, amount of amylose leached at 100°C was only 66% compared to 85% or more for other starches. At 110°C, amylose from wheat starch leached significantly with 24% increment while other starches have leached more than 90% of the total amylose. At 120°C, after heating for 2 hours, all of the amylose has been leached out for all starches. Wheat starch displayed a slightly different pattern compared to the other starches at 120°C for 1 and 2 hours of heating. It was believed that longer heating at higher temperatures caused the level of dispersion and solubilization of the wheat starch to be higher; resulting in higher amylose leaching. Taster and Morrison (1990) showed that the amount of leached amylose correlated very highly (p < 0.01) with the extent of starch swelling. The bonding forces within the starch granules influenced the extent of swelling. Thus, highly associated starch granules should be relatively resistant to swelling and amylose leaching. Complexes may have been formed between natural starch lipids and residual amylose in the granules during swelling (Morrison, 1988). Since lipid and protein contents of wheat starch are higher (Pomeranz, 1991) formation of lipid-amylose and protein-amylose complexes may be increased upon heating. This inhibits leaching of amylose from the granules; resulting in low swelling power and solubility. The higher temperature and longer heating time used in this experiment, however, have managed to break these complexes and allow amylose to leak from the wheat granules. As a result, higher swelling power and solubility in wheat starch were obtained, following dispersion of these granules.

**Turbidity changes**

The rates of cloudiness for starches from various botanical sources at different heat treatments are shown in Table 1. For all starches, the rate of cloudiness (rate of amylose retrogradation) decreases as the temperature and duration of heating are increased. When starch suspensions are heated with high temperature, the granules rupture and disperse, causing leaching of amylose. The chain of amylose molecules breaks to shorter chains as the temperature and time of heating is increased. Upon cooling, the amylose molecules re-associate to form a network during retrogradation. This causes cloudiness in the paste and increase in absorbance.

Among the starches examined, the rate of cloudiness is highest in wheat starch (Table 1). The absorbance value of wheat starch pasted at 100°C for 1 hour was almost constant after only 6 hours of standing. The rate decreased as the temperature and time of heating is increased. Wheat starch pasted at 110°C (1 hour), 120°C (1 hour) and 120°C (2 hours) were almost constant after 6.5 hours, 9.0 hours and 10 hours, respectively. Sago and tapioca starches had rate of cloudiness value of approximately 0.3 to 0.4. These values were observed in wheat starch samples only after treatment at 120°C (1 hour) and 120°C (2 hours), but were not observed in potato starches. Similar to wheat starch, the rate of cloudiness for sago, tapioca and potato starches pasted at 100°C for 1 hour were the highest compared to pastes treated at higher temperatures and longer time. This is probably because the pastes are more concentrated due to less leaching of soluble materials, and the existence of bigger remnants and longer amylose chains. According to Wang (1997), the breakdown of starch granules causes it to lose its water-holding capacity. The rate of development of opacity depends on polymer concentration and molecular weight. The rate at which samples become turbid decreases with decreasing polymer concentration and molecular weight (Miles, 1985). Lipids contribute to paste opacity, perhaps by restricting granular swelling (Craig, 1989; Wang and White, 1994). Amylose content may also affect the absorbance value of these pastes. The ratio of amylose to amylopectin molecules for different starches with a small proportion of amylose were dispersed easily, therefore decreasing their absorbance value (Swinkles, 1985). The granule size and amylose/amylopectin level may play an important role in starch paste clarity (Bello-Perez and Paredes-Lopez, 1996). Potato starch paste presented the lowest absorbance value and lowest rate of cloudiness. This indicates that remnant structures of granules in starch pastes provoke major light reflection and minor clarity. The high clarity of potato paste is due to the phosphate groups covalently bound preventing association by intra-or-intermolecular bonds (Banks and Greenwood 1975).

**Gel strength**

The gel strength of starches at 10% (w/v) concentration is shown in Table 2. For all types of starch, starches heated for 2 hours at 120°C had the lowest gel strength while starches heated at 100°C
Table 2. Amylose to amylopectin ratio of starches at different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amylose: Amylopectin</th>
<th>Gel Strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Sago</td>
</tr>
<tr>
<td>100°C, 1 hour</td>
<td>49.4</td>
<td>1.14</td>
</tr>
<tr>
<td>110°C, 1 hour</td>
<td>3.29</td>
<td>0.58</td>
</tr>
<tr>
<td>120°C, 1 hour</td>
<td>1.10</td>
<td>0.49</td>
</tr>
<tr>
<td>120°C, 2 hours</td>
<td>0.46</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*Means of triplicates
a-d: Means within a column with different letters are significantly different, Significance level of p < 0.05 by Duncan’s analysis
ND: Not determined

Table 3. Absorbance* of starch pastes (24 hours) at different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absorbance (650 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
</tr>
<tr>
<td>100°C, 1 hour</td>
<td>2.11 ± 0.01</td>
</tr>
<tr>
<td>110°C, 1 hour</td>
<td>2.04 ± 0.02</td>
</tr>
<tr>
<td>120°C, 1 hour</td>
<td>1.86 ± 0.01</td>
</tr>
<tr>
<td>120°C, 2 hours</td>
<td>1.71 ± 0.01</td>
</tr>
</tbody>
</table>

*Means of triplicates
a-d: Means within a column with different letters are significantly different, Significance level of p < 0.05 by Duncan’s analysis
ND: Not determined

had the highest. Sago gel had the highest strength, followed by wheat, tapioca and potato gels. This is because it contains higher amounts of amylose and the degree of retrogradation is relatively greater (Kim & Lee, 1987). All starches excluding wheat starch showed a decreasing pattern as the heating conditions were increased. The strength of wheat gel increases from 100°C to 110°C and 120°C when heated for 1 hour. This possibly, is due to the amylose-lipid complex in the granules being broken by heating at higher temperatures. At 120°C and 1 hour of heating, wheat granules were almost completely swollen. These granules absorb more water, making the gel more rigid upon cooling. However, when heating was prolonged to 2 hours, gel strength decreased. This was similar to the decreasing patterns of the sago, tapioca and potato gels treated at 100°C, 110°C, 120°C for 1 hour respectively and 120°C for 2 hours. The reasons were probably because more soluble materials came out from the granules including water. This resulted in the formation of a dilute gel that was less rigid, compared to pastes treated under lower temperatures and for a shorter period of time. Wang (1997) reported that breakdown of granules cause it to lose its water-holding capacity.

Granule morphology

Photomicrographs of the heat-treated starches were produced using a light microscope. Figure 4a shows the round-shaped wheat granules with a circular outline after heating at 100°C for 1 hour. The granules were still intact but lines of cracks have appeared. Lines of cracks extended from centre outwards. When the starch was heated for the same duration of time at 110°C, it appeared that the granules were swollen and the shape became oval, but still intact. For starch paste heated for 1 hour at 120°C as in Figure 4c, the granules ruptured and were surrounded by the leached soluble materials. When heating was prolonged to 2 hours (Figure 4d), the granules were totally dispersed. For the non-cereal starches, they were swollen at 100°C (Figures 5a, 6a, and 7a) and at 110°C they ruptured as in Figures 5b, 6b, and 7b. Dispersion of granules was reached at 120°C within 1 hour (Figures 5c, 6c, and 7c) and total dispersion was reached after another hour of heating (Figures 5d, 6d, and 7d). At this stage, however, there were still some remnants present.
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When gel strength is plotted against the absorbance value of starch pastes at 24 hours of standing, it can be generally concluded that the absorbance value and gel strength are low for starches treated at higher temperatures and longer time. This indicates that pastes with lower clarity produce weaker gels. The absorbance values of the heat-treated starches at 24 hours of standing are shown in Table 3. High clarity pastes have less amount of bonded amylose. As more amylose leach out, more bonds are formed between the amylose molecules during retrogradation. As more bonds exist, gels of these starches become stronger (Wang, 1997; Kyaw, 1998).

Table 2 exhibits the values for amylose to amylopectin ratio and gel strength of starches heated at various temperatures and time. Generally, the gel strength is high when amylose to amylopectin ratio is low (between 0.25 - 0.5). This shows that when the gel strength is high, the amount of amylose and amylopectin leaching are also high. The extent of puffing and the texture of snacks were reported to be influenced by the amylose-amylopectin ratio (Matz, 1984). Both amylose and amylopectin have significant effects on starch expansion (Wang, 1997). Kyaw et al. (2001) concluded that linear expansion of keropok made from different native starches increased with increasing amylose and amylopectin leaching. Therefore, lower ratio of the leached amylose to the leached amylopectin (0.25 - 0.5); and higher gel strength might possibly produce keropok with good linear expansion. From the results, starches that might have good expansion properties are wheat starch treated at 120°C for 2 hours, sago starch treated at and above 100°C for 1 hour or more and tapioca starch treated at and above 100°C for 1 hour. Such observation is supported by Mohd Adzahan et al. (2009) where the authors reported that the leaching ability of wheat starch was improved through irradiation by bringing its amylose to amylopectin leaching.
ratio closer to the ratio in native sago or tapioca starches. These modified wheat starches are expected to produce expanded fish-starch snacks with similar expansion properties compared to those made from the native sago and tapioca starches. For potato starch, although the ratio is low, its gel strength is not high due to its longer amylose chains. Therefore, it possibly will not produce keropok with good expansion properties.

The swelling of starch is due to hydration of water molecules (Leach et al., 1959). Entrapped water molecules inside the swollen granules may contribute to higher linear expansion of dried keropok upon frying in hot oil. The linear expansion and amylose leaching of the starches are positively correlated. Linear expansion is highest when starch granules are fully expanded (Kyaw et al., 2001). Therefore, starches with possibly good expansion properties are wheat starch heated at 120°C for 1 hour, sago, tapioca and potato starches heated at 100°C for 1 hour (Figure 1). Linear expansion of keropok was most likely due to the steam released from water in the granules during frying. The linear expansion is less when the granules breakdown. The fragmentation of starch granules is thought to be in the random chain splitting of the amylose network (Colonna, 1984) due to prolonged heating, which should weaken the network and lessen its ability to retain trapped water molecules.

Wheat starch releases substantial amylose but not substantial amounts of amyllopectin in the leachate during normal pasting process (100°C, 1 hour) but amylose and amyllopectin polymers in tapioca leached out together (i.e. substantial amounts of amyllopectin is released) under normal pasting properties. This can be related to the relative fragility of the tapioca and sago granules that allow the ‘branched’ molecules to leach out at a relatively lower heat treatment relative to the wheat starch granules. However, similar leaching behaviour (co-leaching) was also observed for wheat starch when it was subjected to a higher temperature regime (120°C for 1 or 2 hours). Critical AM/AP ratio is necessary to achieve and therefore contribute to gel strength (AM/AP ratio 0.25 – 0.5) in the soluble mass regardless of the botanical sources of starch (wheat, sago, tapioca) with the exception of potato starch. Application of this study will help in formulation of starch-based products such as fish crackers.

Conclusion

The pasting properties of starches from various botanical sources were affected by heat treatments. Swelling, solubility and amylose leaching of wheat starch pasted at 120°C for 1 hour or more were almost similar to the properties of sago, tapioca and potato starches pasted above 100°C for 1 hour. The lower the ratio, the higher the gel strength and samples with good expansion properties is produced when the amylose to amyllopectin ratio in the supernatant are low (0.25-0.5). It is expected that products with good expansion properties can be produced when wheat starch reaches maximum swelling, in this case at 120°C for 1 hour; and sago and tapioca starches are heated at and above 100°C for 1 hour.

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