Dual-modification of sago starch by gamma irradiation and annealing

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Abstract. Similar to other native starch, native sago starch found limited industrial applications due to its poor functional properties. Various modification methods may be employed to improve the properties, however, owing to the health concerns over chemical modification, this study was designed to modify sago starch using combination of two physical techniques – gamma irradiation and annealing (ANN). Native sago starch was first subjected to four doses of gamma radiation (5, 10, 25, 50 kGy) followed by annealing treatment. Measurement of pH, FTIR (Fourier Transform Infrared) analysis, characterization of pasting properties and thermal properties of the samples were performed. Irradiated samples contained shorter starch chains with higher mobility to effectively participate in the molecular realignment during ANN, resulted higher crystalline perfection and hence higher gelatinization temperature. The interactions and re-association of starch chains during ANN exerted significant change to the pH and functional properties of irradiated sago starch. Combining gamma irradiation at 5 and 10 kGy with annealing could potentially improve the heat and shear stability of sago starch. Dual-modification was also found able to improve the heat stability of sago starch without affecting the gelatinization enthalpy. These improved features can be beneficial to the sago-based food products that require high temperature processing.

1. Introduction
Irrespective of the botanical origin, starches in their native forms are not suitable for industrial application due to their poor functional properties such as poor thermal and acid stability, low shear resistance and high retrogradation tendency. Various kinds of modification either by chemical, enzymatic, physical approaches are employed to tailor the starch properties for specific application in food and non-food industries. It is known that chemical modification is very efficient and promising; nonetheless, it creates concerns if the modified starches are to be used in food products due to the potential health hazards by chemical residuals left in the final products.

Gamma radiation is an ionizing radiation. Through the generation and transformation of free radicals [1], irradiation may alter the starch polymers by degradation and cross-linking of starch chains [2]. Modification of starch using radiation does not require any catalyst and the final product is free from toxic residues [3]. Annealing (ANN) refers to heating starch slurry in the presence of excess water at a temperature above the glass transition, but below the gelatinization of the starch. This modification leads to interactions and re-association of amylose and amyllopectin in the starch without affecting the granular structure [4]. Both of these modification methods are principally easier, faster, cheaper, safer and more environmentally friendly than chemical modification.
ANN is widely employed to improve the thermostability of starch; in which the extent of modification is influenced by the arrangement of the starch chains within the amorphous and crystalline domains of the native starch granules, apart from the starch composition and source [4]. Since gamma radiation may induce structural changes in starch molecules via formation of free radicals [5], combining gamma radiation with ANN in starch modification is hypothesized to result in unique properties as opposed to modification by ANN alone. Even though studies regarding ionizing irradiation and annealing of starch can be widely found in the available literatures, but the combination of these two techniques to modify the properties of sago starch has not been reported so far. The present study aimed to explore the effects of combining both gamma radiation and annealing on the physicochemical properties of sago (Metroxylon Sagu) starch.

2. Methodology

2.1 Sample preparation
Food grade sago starch was purchased from the market and used as received without further purification. Sago starch was first subjected to four doses (5, 10, 25, 50 kGy) of gamma radiation following the procedures of Chung et al. [6]. After that, the irradiated starches were subjected to annealing at 10 °C below the onset of gelatinization for 24 hours. The ratio of starch (dry basis) to water was fixed at 1:2 [7].

2.2 Determination of pH
Starch dispersion of 40% (w/v) was prepared by mixing 10 g of starch sample with 25 ml of distilled water and the pH was measured using a pH meter (Eutech pH 700). The pH meter was calibrated using standard buffer solutions at pH 4, pH 7 and pH 10 at room temperature.

2.3 FTIR analysis
Starch samples were characterized using a FTIR spectrometer (Spectrum 100 Spectrometer, Perkin Elmer). The starch powder was blended with KBr powder and pressed into tablets before measurement. Calibration was carried out using KBr as a blank. The spectra were recorded within the range 400-4000 cm\(^{-1}\) [8]. The intensity ratio of 1047 to 1022 cm\(^{-1}\) was calculated to evaluate the starch short-range orders [9].

2.4 Pasting profile characterization
The pasting profile for starch samples was determined using a Rapid Visco Analyser (RVA-4, Newport Scientific) according to the method of Ng et al. [10].

2.5 Differential scanning calorimetry (DSC)
2.0 mg of starch corrected by dry matter was loaded into a hermetic aluminum pan and distilled water was added (1:1 for starch to water ratio). The sample was left overnight for equilibrium before subjecting to DSC measurement (PYRIS™ Diamond, Perkin-Elmer). The DSC analyser was calibrated using indium and an empty aluminum pan was used as reference. The sample was heated from 30 to 100 °C at 10 °C/min\(^{1}\).

2.6 Statistical analysis
The data reported are the average of triplicate measurements and expressed as means ± standard deviations (SD) or standard error of the mean (SEM) when adequate. Mean comparison was performed using one-way ANOVA with Tukey’s HSD test (p < 0.05) using SPSS (Statistical Package for the Social Sciences) ver 20.
3. Results and Discussions

3.1 pH and FTIR analysis

Figure 1 shows the pH and the intensity ratio of 1047 to 1022 cm\(^{-1}\) for the starch samples. The pH of sago starch was remarkably dropped after subjecting to gamma irradiation (p < 0.05). The acidity of the samples increased with increasing radiation dose. This is expected as during irradiation, the degradation of starch chains formed carboxylic acids [11] and hence brought about increment in the acidity of the irradiation sago starch. Increase of radiation dose caused more severe degradation and formation of more acids in the samples.

FTIR analysis was carried out to confirm the breakdown of glycosidic bonds and decrease of the short-range crystalline order (double helices) in the starches [12]. As the bands at 1047 and 1022 cm\(^{-1}\) are sensitive to changes in the crystalline and amorphous regions of starch [13], the ratio between these bands has been frequently used to quantify the degree of short-range ordering in starch [5]. It is apparently seen that \(R_{1047/1022}\) decreased along with increase of radiation dose, indicating higher extent of double helices damage was experienced by starches irradiated at higher doses. After ANN, the \(R_{1047/1022}\) intensity ratios were found increased in contrast to that of irradiated counterparts. ANN causes interaction between amylose-amylose chains and amylose-amylopectin chains to promote crystalline perfection [4]. The improved crystalline perfection in the dual-modified samples suggests that the degraded starch chains by gamma irradiation facilitated the reorganization of starch chains during ANN. This is because shorter starch chains have higher mobility that makes the rearrangement to be more effective. The total increase in the crystalline perfection in turn reduced the amount of free acids and thus the acidity in the irradiated samples as shown by the higher pH obtained.

3.2 Pasting properties

As shown in Table 1, native sago starch exhibited the highest pasting profile (p < 0.05), indicating its high swelling power but poor heat and shear stability, as well as high retrogradation tendency upon
cooling. Irradiation significantly reduced the pasting parameters with 50 kGy showed the lowest values (p < 0.05). Starch degradation by gamma radiation is responsible for this observation [12]. Pasting temperature is the temperature indicates the onset of granular swelling, the radiation-induced destruction of molecular structure eased the expansion of starch granules [14] to take place at lower temperature. Reduction of peak viscosity with increasing radiation dose was related to the reduction of the molecular weight of the starch macromolecules by the breakdown of the glucosidic bonds [15]. Decreased swelling ability of the irradiated starch [6] could also be one of the contributing factors. The lowering of other viscosity parameters (breakdown, final viscosity and setback) of the irradiated starches is the consequence of the low peak viscosity attainment. After irradiation with 50 kGy, sago starch lost almost 86.5% of its peak viscosity, hence the subsequent change of viscosity along the RVA pasting analysis conditions was less pronounced as compared to other samples. Comparing to other irradiated samples, this sample (irradiated at 50 kGy) can be cooked at lower temperature, imparts low thickening property, with less viscosity change upon heating and shearing.

| Sample  | Pasting Temperature (°C) | Peak Viscosity (cP) | Breakdown (cP) | Final Viscosity (cP) | Setback (cP) |
|---------|--------------------------|--------------------|----------------|---------------------|-------------|
| Native  | 74.38 ± 0.08              | 397.92 ± 2.17      | 256.17 ± 1.64  | 193.14 ± 4.51       | 51.39 ± 4.84 |
| 5 kGy   | 73.88 ± 0.20              | 179.00 ± 2.49      | 131.42 ± 0.55  | 60.64 ± 1.48        | 13.05 ± 0.86 |
| 10 kGy  | 73.60 ± 0.05              | 171.69 ± 0.79      | 158.89 ± 0.85  | 16.78 ± 0.90        | 3.97 ± 0.57  |
| 25 kGy  | 73.63 ± 0.03              | 128.14 ± 0.56      | 128.06 ± 0.70  | 2.39 ± 0.26         | 2.31 ± 0.32  |
| 50 kGy  | 72.37 ± 0.03              | 53.77 ± 0.42       | 54.89 ± 0.54   | 0.29 ± 0.04         | 1.42 ± 0.09  |
| 5kGyANN | 77.93 ± 0.03              | 251.65 ± 1.40      | 193.45 ± 0.40  | 78.43 ± 0.25        | 20.23 ± 1.44 |
| 10kGyANN| 78.70 ± 0.05              | 207.91 ± 0.09      | 189.55 ± 0.04  | 22.41 ± 0.36        | 4.04 ± 0.41  |
| 25kGyANN| 79.57 ± 0.03              | 127.39 ± 0.25      | 126.89 ± 0.25  | 2.53 ± 0.46         | 2.03 ± 0.46  |
| 50kGyANN| 79.93 ± 0.03              | 34.34 ± 0.86       | 35.39 ± 0.97   | 0.22 ± 0.08         | 1.12 ± 0.11  |

Means with identical alphabet within the same column denote insignificant difference (p > 0.05).

Combination of gamma irradiation with ANN elevated the pasting temperature significantly in a dose-dependent manner (p < 0.05). Increasing radiation dose promoted more perfect crystals in the annealed starch as evident by the results of R₁₀₄₇/₁₀₂ (Figure 1). Therefore, higher temperature was required to completely melt the more heat stable crystals in the samples. ANN is known to reduce granular swelling (hence peak viscosity) and retrogradation. Both crystalline perfection and amylose chain interactions decrease the hydration of the amorphous regions of starch, thereby decreasing granular swelling. An increase in molecular organization is also responsible for the reduction in solubility of starch [4]. Since both 25kGyANN and 50kGyANN experienced higher degree of depolymerization by irradiation and also crystalline perfection by ANN, their hot paste and cold paste viscosities were generally lower than the irradiated counterparts (p < 0.05). This in turn implies that these two samples had poor thickening properties. It is interesting to note that 5kGyANN and 10kGyANN had higher peak viscosity and breakdown than the irradiated counterparts (p < 0.05). Even though the peak viscosities for these samples were not as high as native sago, the remarkable reduction in setback (indicator for retrogradation) was reported when compared to native counterpart. It is therefore worth mentioning that with appropriate modification conditions, dual-modification with gamma irradiation and ANN has potential to improve the heat and shear stability of sago starch.

3.3 Thermal properties

All the starch samples displayed a single broad endotherm over the range of temperature 30-100 °C (data not shown). Similar to the effect on pasting temperature (Table 1), the influence of gamma radiation on the gelatinization temperatures was insignificant up to 25 kGy, significant reduction was observed at 50 kGy (p < 0.05) as seen in Table 2. Irradiation did not affect the enthalpy (ΔH) of the gelatinization.
Atrous et al. [5] found that gamma irradiation until 50 kGy did not affect the gelatinization temperatures and enthalpy of wheat starch. Since the starch crystallinity also did not change after irradiation treatment until 50 kGy, they suggested gamma irradiation did not cause considerable macromolecular rearrangements and less stable macromolecular interactions within wheat starch granules.

Once subjected to ANN, the gelatinization endotherms for all the irradiated starches were shifted to higher temperatures with more prominent influence on onset temperature ($T_o$) and peak temperature ($T_p$) as compared to the native sago. $T_o$ is the temperature where starches begin to gelatinize, while $T_p$ is the temperature taken at the maximum intensity of gelatinization [16]. The short-range structural alteration by ANN was seen in these dual-modified samples, because it is well established that ANN commonly causes an increase in $T_o$ and $T_p$, as well as a decrease in the gelatinization range ($\Delta T$). The narrower melting endotherm after ANN indicates greater homogeneity during melting of the crystallites, as well as swelling and hydration of the starch granules [4]. Even though most of the $\Delta T$ values for dual-modification seemed lower than the irradiated counterparts, but they were found statistically insignificant ($p > 0.05$). This can be ascribed to the high standard deviation for $\Delta T$ found in 25kGyANN. Therefore, the effect of dual modification on the melting temperature range is less conclusive in this study. The enthalpy (\(\Delta H\)) during gelatinization determines the energy input and reflects the loss of double helical order within the granule [16]. Insignificant difference was reported for the enthalpy (\(\Delta H\)) of all starches ($p > 0.05$). This implies the molecular destabilization and rearrangement that happened is the modified starch could most probably involve the non-crystalline areas of the granule [17].

| Sample       | Onset Temperature, $T_o$ (°C) | Peak Temperature, $T_p$ (°C) | Conclusion Temperature, $T_c$ (°C) | Melting Temperature Range, $\Delta T$ (°C) | Enthalpy, $\Delta H$ (J/g) |
|--------------|-------------------------------|-------------------------------|-----------------------------------|-----------------------------------|------------------|
| Native       | 69.06 ± 0.02b                 | 74.69 ± 0.39b                 | 79.96 ± 0.41bc                     | 10.90 ± 0.43ab                    | 5.43 ± 0.73a     |
| 5 kGy        | 69.39 ± 0.33b                 | 74.08 ± 0.39b                 | 78.80 ± 0.93b                      | 9.41 ± 0.91ab                     | 4.54 ± 1.29a     |
| 10 kGy       | 69.50 ± 0.51b                 | 74.17 ± 0.18b                 | 78.39 ± 0.20b                      | 8.89 ± 0.57ab                     | 4.21 ± 0.61a     |
| 25 kGy       | 69.23 ± 0.19b                 | 73.99 ± 0.17b                 | 78.78 ± 0.40b                      | 9.55 ± 0.20ab                     | 6.20 ± 0.91a     |
| 50 kGy       | 66.12 ± 0.47a                 | 72.44 ± 0.10a                 | 77.03 ± 0.35a                      | 10.91 ± 0.24ab                    | 7.75 ± 1.96a     |
| 5kGyANN      | 76.07 ± 0.04c                 | 78.32 ± 0.10c                 | 81.99 ± 0.10c                      | 5.92 ± 0.08a                      | 5.85 ± 2.16a     |
| 10kGyANN     | 76.68 ± 0.51c                 | 79.38 ± 0.29d                 | 83.61 ± 0.74c                      | 6.93 ± 0.09ab                     | 6.03 ± 3.77a     |
| 25kGyANN     | 77.34 ± 0.55cd                | 80.39 ± 0.34d                 | 89.61 ± 5.96e                      | 12.27 ± 5.60b                    | 6.35 ± 4.81a     |
| 50kGyANN     | 77.75 ± 0.65de                | 81.02 ± 0.35e                 | 86.53 ± 4.45e                      | 8.78 ± 1.88ab                    | 8.22 ± 1.26c     |

Means with identical alphabet within the same column denote insignificant difference ($p > 0.05$).

4. Conclusions
The effects of dual-modification by gamma irradiation and ANN on the pasting and thermal properties of sago starch were determined in this study. Gamma irradiation prior to ANN promoted more extensive crystalline perfection in sago starch, the enhancement was found to increase with radiation dose. The interactions and re-association of starch chains during ANN exerted significant change to the functional properties of irradiated sago starch. Combining ANN with gamma irradiation at 5 and 10 kGy increased the hot and cold paste viscosities; whereas combining ANN with irradiation at 25 and 50 kGy could improve the thickening property and reduced the retrogradation tendency. Dual-modification also improved the heat stability of sago starch without affecting the energy requirement for melting.

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