Phytochemical Contents of Rambutan (*Nephelium lappaceum* L.) Seed Influenced by Soaking and Germination

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Rambutan (*Nephelium lappaceum* L.) seeds are normally discarded as waste product during fruit consumption. Consumption of germinated product is being associated with improvement in human health due to wide range of biological properties. This study was aimed at monitoring the modification of bioactive constituents (polyphenol, tannin, ascorbic acid and antioxidant activity) in rambutan seed during soaking and germination. Soaking was performed for 8, 12, 16, 20, 24 hours at the ratio of 1:4 (seed: water). Germination was conducted for 48, 60, 72, 84, 96 hours at 30°C. The raw, soaked and germinated rambutan seeds were exposed to a temperature of 50°C for drying till the constant moisture 14% was obtained and analysed for various chemical attributes such as polyphenol, tannin, ascorbic acid and antioxidant activity. Our results revealed that polyphenol, ascorbic acid and antioxidant activity were increased while tannin was decreased during soaking and germination. The optimal soaking and germination time were recorded at 20 and 84 hours respectively.

Keywords: Rambutan seed, soaking, germination, polyphenol, tannin, ascorbic acid, antioxidant activity

INTRODUCTION

Soaking and germination are economical processing technologies. Soaking may improve the quality of legumes due to the removal of some anti-nutritional factors (Singh et al. 2014). During germination, hydrolytic enzymes are activated to decompose the large molecules such as starch, non-starch polysaccharides and proteins for the respiration, which results in the synthesis of new cell constituents of germinated seeds (Maninder et al. 2017). Germination not only caused significant changes in the biochemical, nutritional and sensory characteristics, but also showed a significant accumulation of bioactive components (Gan et al. 2016). The nutritional value of seeds is also improved through an increase in amino acids (Komatsuzaki et al. 2007), and increase in amino acid bioavailability (Sangronis and Machado, 2007) and a decrease in some antinutrients such as phytic acid (Albarracin et al., 2013). The effects of germination on antinutritional factors and antioxidants activity in legumes were assessed (Singh et al. 2014). Effect of germination on antioxidant activity, total phenolics, β-carotene, ascorbic acid and α-tocopherol contents of lead tree sprouts was determined (Suryantiet al. 2016). There was significant reduction in tannin during germination of amaranth seed (Babatunde and Saka, 2017). Soaking and germination has a positive impact on the nutritional, sensorial and phytochemical attributes of Nigella sativa (Suri et al. 2019). The rambutan (*Nephelium lappaceum* L.) is one of the most important tropical fruit grown in Vietnam. Rambutan seeds are normally discarded as waste product during fruit processing (Solís-Fuentes et al. 2010). However, these seeds contain a considerable amount of crude protein,
crude fat and carbohydrate (Kong et al. 2018). The disposal of this agricultural waste can lead to a serious environmental issue. Utilization of these seed would be encouraged to reduce wastage. Objective of our study focused on the effect of soaking and germination to bioactive constituents of rambutan seed.

MATERIALS AND METHODS

2.1 Material
Rambutan seeds as waste product were manually collected from local market in SocTrang province, Vietnam. All solvents and chemicals were of analytical grade.

2.2 Researching method
Rambutan seeds were primarily sterilized by peracetic acid 25ppm in 30 seconds. These seeds were then soaked in distilled water at 8, 12, 16, 20, 24 hours at the ratio of 1:4 (seed: water). Water was drained and the soaked seeds were incubated at 30°C for 48, 60, 72, 84, 96 hours. The raw, soaked and germinated rambutan seeds were exposed to a temperature of 50°C for drying till the constant moisture 14% was obtained and analysed for various chemical attributes such as polyphenol, tannin, ascorbic acid and antioxidant activity.

2.3 Chemical analysis
Total phenolic content (mg GAE/100g) was determined using Folin-Ciocalteu reagent (Hassan and Bakar, 2013). Tannin content (µg GAE/100g) was estimated by Folin–CiocalteauColorimetry as per Afify et al. (2012). Ascorbic acid content (mg/100g) was determined as a method described by AOAC (2005). Free radical-scavenging activity or DPPH (%) was evaluated according to Bakar et al. (2017).

2.4 Statistical analysis
The experiments were run in triplicate with three different lots of samples. The data were presented as mean ± standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS AND DISCUSSION

3.1 Soaking
Soaking is the process of softening and saturating the raw sample by immersing in water thereby decreasing the amount of anti-nutrients and increasing the enzymatic activity which will further initiate germination (Suri et al. 2019). In our research, total phenolic, ascorbic acid and antioxidant activity were increased while tannin was decreased during 20 hours of soaking (see table 1). Our results were similar to data presented by Ojha et al. (2018) in soaking of fenugreek seeds. Reduction of tannins might be due to the leaching of tannins into water (Shimelis and Rakshit, 2007; Sorour et al. 2017). Binding of polyphenols with other organic substances such as carbohydrate or protein makes tannin unavailable (Deshpande et al. 1982; Bravo et al. 1998). Sorour et al. (2017) observed changes of total phenolic, tannin, and antioxidant activity of sorghum as affected by soaking. The results indicated that soaking had significant reduction of total phenolic, tannin and antioxidant activity. During soaking, various complex compounds undergo biological breakdown into simpler compounds as proposed by Narsih and Harijono (2012).

3.2 Germination
Consumption of germinated product is being associated with improvement in human health due to wide range of biological properties such as antibacterial, anti-viral, anti-inflammatory, anti-allergic present in phenolics (Sutharut and Sudarat, 2012). In our research, total phenolic, ascorbic acid and antioxidant activity were increased while tannin was decreased during 84 hours of germination (see table 2). From 84 to 96 hours of germination, total phenolic, ascorbic acid and antioxidant activity were gradually decreased. Ascorbic acid content increased on germination which was in accordance with the findings of Shah et al. (2011) in mung bean. It might be due to increased activity of L-galactono-Y-lactone dehydrogenase (Moriyama and Oba, 2008). Tannin content gradually decreased as the seeds developed into sprouts. Tannin reduction might be due to the result of the formation of a hydrophobic association of tannins with seed proteins and enzymes (Ojha et al. 2018). Duenas et al. (2009) revealed that germination increased total phenolics content in lupin seeds. Lopez-Amoros et al. (2006) proved that change of phenolic components during germination affected the antioxidant capacity significantly. Gujral et al (2011) indicated that the increase in the amount of free form phenolics in germinated brown rice is due to the decomposition of the cell wall during germination. Sorour et al. (2017) observed changes of total phenolic, tannin, and antioxidant activity of sorghum as affected by germination.
Table 1: Effect of soaking to total phenolic, tannin, ascorbic acid, free radical-scavenging activity of raw, soaked rambutan seed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw seed</th>
<th>Soaking time (hours)</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic (mg GAE/100g)</td>
<td>16.32±0.01c</td>
<td>19.75±0.01bc</td>
<td>24.63±0.02b</td>
<td>29.31±0.03ab</td>
<td>33.70±0.01a</td>
<td>34.05±0.02a</td>
<td></td>
</tr>
<tr>
<td>Tannin (µg GAE/100g)</td>
<td>6.79±0.02a</td>
<td>6.63±0.00a</td>
<td>6.01±0.01ab</td>
<td>5.70±0.02ab</td>
<td>5.21±0.01b</td>
<td>5.17±0.01b</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/100g)</td>
<td>6.30±0.00c</td>
<td>6.78±0.03bc</td>
<td>7.05±0.00b</td>
<td>7.66±0.01ab</td>
<td>7.95±0.03a</td>
<td>8.06±0.00a</td>
<td></td>
</tr>
<tr>
<td>%DPPH</td>
<td>33.71±0.03c</td>
<td>34.52±0.01bc</td>
<td>36.83±0.00b</td>
<td>40.27±0.02ab</td>
<td>43.08±0.00a</td>
<td>43.15±0.03a</td>
<td></td>
</tr>
</tbody>
</table>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above) the difference between them was not significant (α = 5%).

Table 2: Effect of germination to total phenolic, tannin, ascorbic acid, free radical-scavenging activity of soaked, germinated rambutan seed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Soaked 20 hours</th>
<th>Germination time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>Total phenolic (mg GAE/100g)</td>
<td>33.70±0.01d</td>
<td>39.55±0.02c</td>
</tr>
<tr>
<td>Tannin (µg GAE/100g)</td>
<td>5.21±0.01a</td>
<td>3.06±0.03b</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100g)</td>
<td>7.95±0.03c</td>
<td>10.66±0.01bc</td>
</tr>
<tr>
<td>%DPPH</td>
<td>43.08±0.00d</td>
<td>47.86±0.02cd</td>
</tr>
</tbody>
</table>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above) the difference between them was not significant (α = 5%).

The results indicated that germination had significant reduction of total phenolic, tannin and antioxidant activity. Ramadan et al. (2012) explained the decrease in antioxidant activity after germination as a cause of activation of some enzymes and other metabolic processes during germination craking the antioxidant components. Megat and Azrina (2012) found that total phenolic contents were decreased significantly in germinated peanut and soy bean while significant decreased of tannin content was found in germinated peanut. They explained that the decreased in total phenolic, tannin content was due to enzymatic changes during germination period in seeds. Suryanti et al. (2016) showed that the antioxidant activities of germinated seeds were affected by germination time. Maninder et al. (2017) highlighted the accumulation of high level of antioxidants, bioactive compounds and antioxidant activity after rice germination. Strong positive correlation was observed among total phenolics and the antioxidant activity. According to Renata et al. (2017), the best temperature for rambutan germination was 25°C and the storage could be realized with the seeds inside the fruit until the time of sowing, in up tol six days.

Renata et al. (2017) verified if the germination temperature, storage condition and period could influence rambutan seed germination.

CONCLUSION
During germination, enzyme synthesis could enhance the intrinsic phytochemical compounds and antioxidant activity. Components of germinated rambutan seed extracts are electron donors to free radicals, which help to terminate or stabilize radical chain reactions. After soaking and specially germination, there was significant variations in the content of phenolic, ascrobic acid, bioactive compounds and hence antioxidant activity. Meanwhile, tannin was decreased through these processes. The optimized conditions i.e. 20 hours of soaking and 84 days of germination can be successfully used for soaking and germination which will definitely initiate the utilization of rambutan seeds.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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