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Changes in physicochemical properties and antioxidant activity of fermented *kepayang* (*Pangium edule* Reinw.) seeds

[1,2]Mustaffer, N.H., [1]Ramli, N.S., [2]Tongkhao, K., [2]Vangnai, K., [1]Rosni, N.K. and [1,3,*]Rukayadi, Y.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Rd, Lat Yao Chatuchak, Bangkok, 10900, Thailand*
[3]*Natural Medicines and Product Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

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Abstract

One method for processing *Pangium edule* Reinw. seeds for consumption is fermentation. The aim of this study was to determine the physicochemical characteristics of *P. edule* Reinw. seeds during fermentation and antioxidant activity of the extracts. *P. edule* Reinw. seeds were boiled for 2 hrs and subjected to spontaneous fermentation for 40 days. The physicochemical properties were evaluated according to AOAC methods. Raw and fermented *P. edule* Reinw. seeds (day 40) were dried and extracted using ethanol and distilled water. The antioxidant activity was measured using 2,2-diphenyl-1-dipicrylhydrazyl (DPPH) radical scavenging assay while total phenolic content was measured by following the Folin-Ciocalteu method. The results showed that there were no significant differences in water activity while the pH value was decreased along fermentation days. The TSS was significantly increased from day 0 (11.00) to day 30 (19.50), however, it drastically decreased to 11.50 after 40 days of fermentation. The value of lightness (L^*) parameter was decreased from 66.44 to 25.28 on day 40 but no significant differences for a^* and b^* parameter. For proximate analysis, the percentage of ash, crude protein, crude fat, and crude fibre were significantly increased while moisture content and carbohydrate were significantly decreased throughout the fermentation days. Besides, the highest DPPH activity was observed in the ferment-water extract at concentration 10 mg/mL which about 95.61% while the highest total phenolic content was obtained from the ferment-ethanol extract (173.79 mg GAE/100 mg). In conclusion, there were some changes in the physicochemical properties of *P. edule* Reinw. seeds during fermentation and potentially has antioxidant activities. The results of this study might be used as basic information to develop the fermented seeds as a functional food.

1. Introduction

The tropical rainforest offers excellent sources of indigenous fruits and vegetables that have a valuable effect and one of them is *kepayang* fruit or its scientific name *Pangium edule* Reinw. This *P. edule* Reinw. tree is mainly grown in the South-East Asia region including Malaysia, Indonesia, Papua New Guinea, Vanuatu, and the Philippines. The fruit is commonly consumed due to its taste and it is widely used as a preservative agent. *P. edule* Reinw. seeds have been used as an alternative approach to preserve and maintain the quality of raw fish, shrimp, and meat when the supply of ice and cooling supplies were limited (Heruwati *et al.*, 2007; Heruwati *et al.*, 2009; Kasim and David, 2013). Traditionally, this fruit can be used to treat infections and it also has an anthelmintic, antiseptic and antibacterial property.

Interestingly, *P. edule* Reinw. seeds are edible raw after undergoing some treatment such as boiling or soaking in water. These seeds usually called as "*dage*" which can be eaten and is commonly being utilized as a vegetable especially in West Java, Indonesia. Other than that, these *P. edule* Reinw. seeds also undergo fermentation processes and it is commercially called

*Corresponding author.
Email: *yaya_rukayadi@upm.edu.my*

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"*keluwak*" (Hoe and Siong, 1999). According to Andarwulan *et al.* (1999), the fermentation process can make these seeds edible as it rids them of their cyanide content and at the same time increases their nutritional value and flavour. Besides that, this process also changes the colour of the seeds from milky white to brown or black and softens the texture.

In general, the fermentation process a common method that can be used to process and preserve food. Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. It can convert complex compounds of carbohydrates, such as starch or sugar, into a simpler compound such as alcohol or acid (Chojnacka, 2006). This fermentation process enhances the free water molecules and indirectly produces edible food products by removing or destroying the undesirable or anti-nutritional factors that might be present in the food to ensure its food safety (Steinkraus, 2018). Other than that, fermentation also improves their taste, flavour and appearance thereby increasing their acceptability (Ejoh *et al.*, 2007). Based on previous studies, the fermentation process can enhance the nutrition content while increasing its food digestibility (Hasan *et al.*, 2014; Tamang *et al.*, 2016).

Traditionally, the process to ferment the *P. edule* Reinw. seeds were done manually using spontaneous fermentation. Hence, good hygiene practices should be emphasized during fermentation to lower or discard microorganisms that are dangerous for health. Generally, fermentation processes affect the chemistry of these foods as they change their physicochemical characteristics and biological activities. From a researcher's best knowledge, studies regarding physicochemical characteristics of *P. edule* Reinw. seeds during fermentation and their biological activities are still scarce. Therefore, this study intended to determine the physicochemical characteristics of *P. edule* Reinw. seeds during the fermentation process. Then, the raw and 40 days fermented *P. edule* Reinw. seeds undergo an extraction process and both extracts were used to determine their antioxidant activity and total phenolic content.

2. Materials and methods

2.1 Plant materials

P. edule Reinw. seeds samples were purchased from a traditional market in Bandung, Indonesia. The samples were deposited and identified in the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) and analyzed in the laboratory at the Faculty of Food Science and Technology, UPM.

2.2 Preparation for fermentation of Pangium edule Reinw. seeds

The *P. edule* Reinw. fruits were cut open to obtain their seeds and were rinsed through running water and boiled for 2 hrs. The seeds were cooled on a tray and the shell of the seed was allowed to dry. The *P. edule* Reinw. seeds were selected for the fermentation process, where the spoiled and germinated beans were discarded. The spontaneous fermentation process of *P. edule* Reinw. seeds was conducted by placing the seeds in a tight lid container which was filled and covered with charcoal. The covered *P. edule* Reinw. seeds were allowed to ferment at a temperature of 35±2°C and the fermented seeds were collected for 0, 10, 20, 30 and 40-days fermentation. The nuts were taken out from the shell of the *P. edule* Reinw. seed for further analysis.

2.3 Physicochemical analysis

2.3.1 pH value, total soluble solid, water activity and colour

The pH value of *P. edule* Reinw. seeds samples was determined by a pH meter (3505 Jenway, Staffordshire, United Kingdom). The samples were blended with distilled water at a ratio of 1:5 (w/v) before the determination of pH (Wongwiwat and Wattanachant, 2015). The total soluble solid (°Brix value) of each sample from its fermentation days were determined by using a hand refractometer (Model N-300E, ATAGO CO., LTD, USA) of 0~10 scale. The samples were diluted with distilled water with a dilution factor of 10. The homogenate samples were pipetted using a Pasteur pipette and a drop was put on the glass surface of the refractometer. °Brix value was calculated as follows:

$$\text{Total soluble solid (°Brix)} = \text{Reading value (°Brix)} \times \text{dilution factors}$$

Besides that, the water activity (a_w) of *P. edule* Reinw. seeds samples were measured using a water activity meter (Aqualab model series 3TE, Decagon, USA). Samples were cut to small and tiny sizes in order to fill the surface of the cups, and the water activity of the sample was measured at 25±0.1°C. Lastly, the colour analysis was measured using Chroma Meter CR-410 (Konica Minolta Inc, Japan). The results were expressed according to the colourimetric parameters of the CIELAB system and the rates of L*, a*, and b* parameter. L* parameter was measured for lightness which has a range from 0 for black until 100 for white, respectively a* parameter range from −60 for greenness to +60 for redness, and b* parameter from −60 for blueness until +60 yellowness values.

2.3.2 Proximate analysis

The nutritional parameters including moisture, ash,

crude protein, crude fats, crude fibre, and carbohydrate were determined using proximate analysis by following the standard official procedures in the Association of Official Analytical Chemist methods (AOAC, 2005). The moisture content was determined by oven drying 3 g of sample in an oven at 105°C until a constant weight was obtained and the loss of weight was used for calculation, method No. 952.45 (AOAC, 2005). The ash content was attained by incinerating all organic matter of 3 g samples at 550°C in a muffle furnace for at least 7 hrs until it turns white without any black particles. The crucibles that contain ash was cooled in desiccators and weighed, method No. 945.46 (AOAC, 2005). The determination of crude proteins in terms of nitrogen content was referred to the Micro Kjeldahl method. The nitrogen value was converted to protein content by multiplying by a factor of 6.25, method No. 981.10 (AOAC, 2005). Crude fat content was determined by using the Soxhlet apparatus where petroleum ether was used as the solvent, method No. 945.16 (AOAC, 2005). The weight of crude fibre was calculated by the differences between the weight of dried sample residue to the weight of ash after the digestion of a known weight sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions according to method No. 926.09 (AOAC, 2012). Lastly, the percentage of carbohydrate was calculated using 'by difference' method which 100% was subtracted with the sum percentage of moisture content, ash content, crude fat, crude protein and fibre. The percentage of the proximate compositions were calculated based on the formula as follows:

$$\text{Moisture content (\%)} = \frac{(\text{Weight sample before drying} - \text{Weight sample after drying})}{\text{Weight (g) of sample before drying}} \times 100$$

$$\text{Ash Content (\%)} = \frac{(\text{Weight of ash} + \text{Weight of crucible}) - (\text{Weight of crucible})}{\text{Weight of sample}} \times 100$$

$$\text{Nitrogen (\%)} = \frac{(\text{Vol.H2SO4 to titrate boric acid} - \text{Vol.H2SO4 to titrate blank}) \times \text{Normality H2SO4}}{\text{Weight of sample}} \times 1.4$$

Therefore, Protein content (%) = % nitrogen × conversion factor

$$\text{Crude fat content (\%)} = \frac{(\text{Weight of flask} + \text{oil}) - (\text{Weight of flask})}{\text{Weight of sample}} \times 100$$

$$\text{Crue fibre content (\%)} = \frac{(\text{Weight crucible+filter paper+dried residue} - \text{Weight filter paper without ash}) - \text{Weight crucible+ash}}{\text{Weight of sample}} \times 100$$

Total carbohydrate by difference = 100% - [moisture + ash + protein + fat + fibre] (%)

2.4 Preparation of extracts

A total of 100 g of raw and 40 days fermented *P. edule* Reinw. seeds were dried using an oven at 40°C for 8 hrs and ground into powder form by using a dry blender. Then, each dried raw and 40 days fermented *P. edule* Reinw. seeds powder was soaked and extracted into 400 mL absolute ethanol and distilled water for 48 hrs at room temperature using a shaker (Rukayadi *et al*., 2008). The mixture was filtered using Whatman filter paper No. 1 and then allowed to evaporate from the extracts using the rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at temperature 60°C and speed of 150 rpm until all solvents were dried out. All the crude extracts samples were kept at -4°C until further analysis.

2.5 Antioxidant activity

2.5.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was conducted to analyze the antioxidant activity of the raw and 40 days fermented *P. edule* Reinw. seed extracts based on their capability to scavenge the DPPH free radical. The assay was performed as described by Prieto (2012) with some modifications. The assay was conducted using a 96-well microplate added with 50 µL aliquots of sample extracts at 5000 ppm and Trolox which acts as a positive control was transferred in the well. Then, 100 µL of DPPH was added to each well prepared and incubated in the dark for 30 mins. After 30 mins of reaction, the absorbance was measured at 517 nm using a microplate reader (SPECTRAmax PLUS). The percentage of the inhibition scavenging was calculated using the formula as follows:

$$\text{DPPH SC (\%)} = \frac{(\text{Ao} - \text{As})}{\text{Ao}} \times 100$$

Where, Ao is the absorbance of the reagent blank while As is the absorbance of the test samples. All tests above were performed in triplicate.

2.5.2 Total phenolic content (TPC)

The total phenolic content (TPC) was quantified according to the procedure reported by Zhang *et al*. (2006) using the Folin-Ciocalteu method with some modifications. A volume of 20 µL of standards or test samples were mixed with 100 µL of Folin-Ciocalteu reagent in 96-well plates and incubated for 5 mins. After that, 80 µL of 7.5% sodium carbonates (Na_2CO_3) was added to each well. Then, the plate was covered and kept in the dark for 90 mins. After 90 mins of reaction, the absorbance was measured at 750 nm using a microplate reader (SPECTRAmax PLUS). The standard calibration curve was obtained with gallic acid and the results of total phenolic content in the sample were expressed in mg gallic acid equivalent (GAE)/100 mg extract of the sample.

2.6 Statistical analysis

Data were analysed using MINITAB (Version 17, Minitab Pennsylvania, USA) statistical application software for the one-way analysis of variance (ANOVA). Besides, Tukey's test was used to determine the significance of differences ($P \leq 0.05$) between those samples. The data was analysed and expressed as mean value±standard deviation.

3. Results and discussion

3.1 Physicochemical characteristics

Pangium edule Reinw. seeds had undergone the spontaneous fermentation process for 40 days and the changes had been assessed based on the physicochemical properties of the seeds throughout the process which highlights its pH value, water activity (a_w), total soluble solids (TSS) and colour determination with *L**, *a** and *b** value and also proximate analysis. Table 1 shows the results of pH value, TSS and water activity while Figure 1 shows the colour determination (*L**, *a** and *b**) results. In addition, Table 2 shows the results of the proximate composition of raw *P. edule* Reinw. seeds and the seed after fermentation.

3.1.1 pH value

The first parameter of the physical characteristic is pH value. Table 1 shows that there are significant differences ($p < 0.05$) in the pH values of *P. edule* Reinw. seeds throughout the fermentation process. The pH value of the *P. edule* Reinw. seeds at the start of fermentation at day 0 were 5.73, and the pH value decreased at day 10 with 5.30 and day 20 with pH value 5.15, pH value 4.83 at day 30 and lastly, the lowest pH value was at day 40 which is 4.47. In general, the pH of *P. edule* Reinw. seeds decreased as the duration of fermentation increased. Santoso *et al.* (2014) also conducted a study on *P. edule* Reinw. seeds and found that the pH value of the non-oil fraction at concentration 0.1% solution (in water) decreased from 5.38 (day 0) to 2.95 after 40 days fermentation. Liptáková *et al.* (2017) reported that the decrease of the pH value was due to the production of organic acids by the microflora of Lactic acid bacteria (LAB) which is a heterofermentor, reported converting glucose into an equimolar mixture of lactic acid, ethanol and carbon dioxide. This reduction of pH value was also crucial to prevent the growth of unwanted microorganisms, such as pathogenic bacteria, moulds and yeasts which also helps to ensure food safety (FAO, 2002).

3.1.2 Total soluble solid (TSS)

The results of total soluble solids (TSS) that is demonstrated in Table 1 shows a significant difference ($p < 0.05$) throughout the 40 days of fermentation. Besides that, the results also show the trend of the TSS value changes in which the value continuously increased from day 0 with TSS value 11.00 to 13.50 on day 10, followed by 17.00 on day 20 and the highest after 30-day fermentation which is 19.50. Interestingly, the TSS value suddenly decreased to 11.50 on fermentation day 40. The TSS values indicate the total amount of soluble constituents foods. These soluble constituents were mainly sugar content, with smaller amounts of organic acids, free amino acids, proteins, vitamins, essential oils, and glucosides (Fernández *et al.*, 2014). According to Chai *et al.* (2018), the increase of the TSS value at the early fermentation process is most likely related to the increase of soluble solids released mainly fructose and glucose. However, it can be seen that there was a drastic decline in the TSS value after 40 days of fermentation. This finding is supported by the evidence from Dzogbefia *et al.* (1999) that mentioned the decrease of the sugar content during fermentation is mainly due to the utilization of free sugars by the microorganisms to sustain microbial metabolic activity.

3.1.3 Water activity

For the parameter of water activity, the data in Table 1 clearly shows that there are no significant differences throughout the process where the a_w value was 0.94 except for day 20 fermentation when the a_w was 0.93. Water activity can be considered as the quantity of free water bound inside the food. It recognizes how much water in food is bound water and this water is not free to support microbial growth or to participate in or support chemical or enzyme reactions and spoilage processes inside the food. Hence, this parameter of water activity was associated with the prediction of the shelf-life of the food product and can be used to determine the potential of spoilage caused by microorganisms.

3.1.4 Colour determination

Colour determination also an essential parameter in the fermentation of *P. edule* Reinw. seeds and the result was presented in Figure 1. The result of colour was expressed in three rates, L* value which represents lightness (0 = black, 100 = white), a* value represents greenness (−a) and redness (+a) while b* value represents blueness (−b) and yellowness (+b). In general, the colour of *P. edule* Reinw. seeds endosperm

Table 1. Physicochemical properties of *Pangium edule* Reinw. seeds during the fermentation process

Physicochemical properties	Duration of fermentation (day)				
	Day 0	Day 10	Day 20	Day 30	Day 40
pH value	5.73±0.02^{a}	5.30±0.03^{b}	5.15±0.02^{c}	4.83±0.01^{d}	4.47±0.03^{e}
Total soluble solid (°Brix)	11.00±1.15^{d}	13.50±1.00^{c}	17.00±1.15^{b}	19.50±1.00^{a}	11.50±1.00^{cd}
Water Activity	0.94±0.01^{ab}	0.94±0.01^{ab}	0.93±0.00^{b}	0.94±0.00^{a}	0.94±0.00^{a}

Values are mean±standard deviation of replications ($n = 3$). Values with different superscript within same rows are significantly different ($P \leq 0.05$).

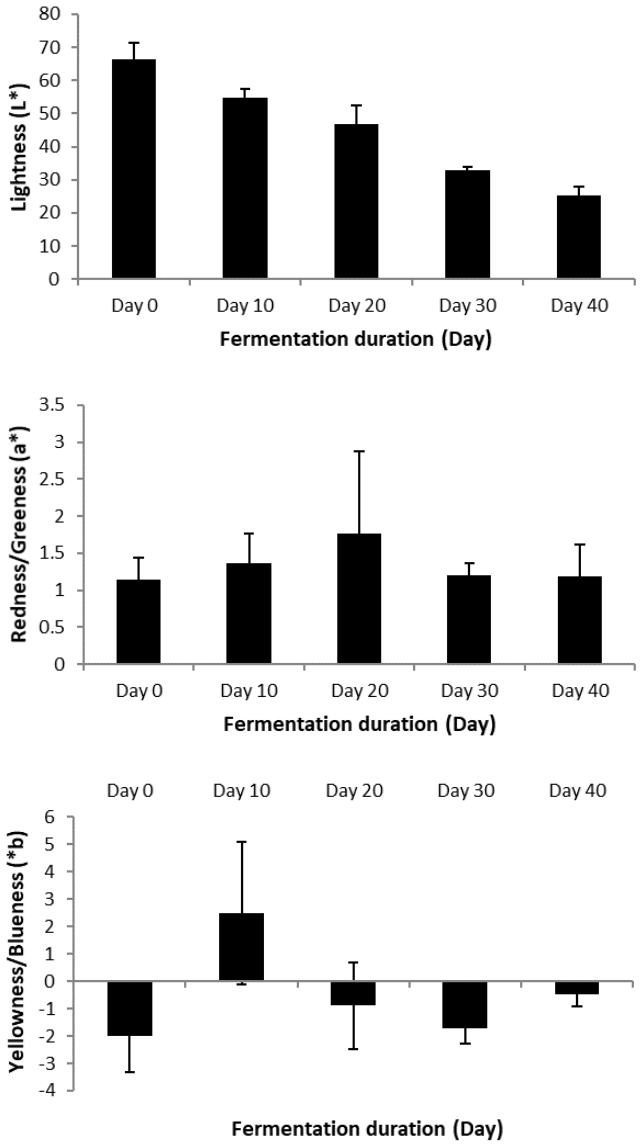

Figure 1. Colour determination in term of Lightness (L*), Redness/Greenness (a*) and Yellowness/Blueness (b*) of fermented *Pangium edule* Reinw. seeds during fermentation duration

experienced drastic changes when the colour changed from light brown to black throughout that 40 days fermentation process. The darkening of the endosperm colour was related to L* value which indicated the lightness of the *P. edule* Reinw. seeds where the results show a significant decrease from 66.44 on 0-day to 25.28 after 40 days fermentation. However, the result shows that there was no significant difference for a* and b* where the highest a* value was 1.76 at fermentation day 20 and the lowest value was 1.14 at fermentation day 0. For b* value, the results were represented in negative value which indicated blueness except for seeds at fermentation day 10 which *b value was 0.49 which yellowness as the value was positive. This can be concluded that the result of a* and b* value that related to reddish, greenish, yellowish and bluish colour did not affect the colour changes throughout the fermentation process.

A relevant study by Santoso *et al.* (2014) is clearly supported by the current findings. This study also identified that the colour of *P. edule* Reinw. seeds have significantly changed during the fermentation process and indicated that the darkening was due to the browning reaction that occurred. Other than that, Belitz *et al*. (2004) also mentioned that the development of a darker colour change over time is most likely due to the reaction of the Maillard reaction as the seeds were treated by boiling prior to fermentation. According to Verzelloni *et al.* (2007), this Maillard reaction brings about dark-coloured and produces a high amount of melanoidins that functions not only as aroma and colour to the food but also contributes to antioxidant activity. In general, this parameter of colour was very important as it can be used to determine the quality of the seeds during their fermentation process and is also relevant in consumer perception of the quality of certain food products.

3.1.5 Proximate analysis

The nutritional composition which can be determined using proximate analysis was the relative amount of moisture, ash, protein, lipid, fibre, and carbohydrates in food. It was a necessary task to determine the proximate composition of a food sample as the total energy content of a food is provided by protein, lipid, and carbohydrates, while water and ash only contribute to the mass. These chemical properties of the food products are crucial in grading, processing, preservation and storage (Parvin *et al.*, 2015). All of the methods presented were according to AOAC methods (2005) and the results for proximate analysis including moisture content, ash content, crude protein, crude fat, crude fibre and carbohydrate in *P. edule* Reinw. seeds during the fermentation process were tabulated in Table 2.

The moisture of the food product was very important, and it can be considered as a significant factor in the prevention from deterioration and acceptability (Parvin *et al.,* 2015). Based on Table 2, the results of the moisture content of *P. edule* Reinw. seeds decreased throughout the fermentation process. The highest amount of moisture content was at day 0 at 57.46% and continued to decreased to 55.88% and 50.62% for day 10 and day 20 fermentation. However, the moisture content increased on day 30 at 52.35% and decreased again at the end of the fermentation process which is day 40 at 49.38%. Schwan and Wheals (2004) suggested that the decrease in moisture content was due to enzymatic degradation of mucilaginous pulp. Yeast breaks down cocoa pulp resulting in a liquid that drains away as sweating. In general, this moisture was used to determine the quality of foods as lower moisture content indicates a

Table 2. Proximate analysis of *Pangium edule* Reinw. seeds during the fermentation process

Composition (%)	Duration of fermentation (day)				
	Day 0	Day 10	Day 20	Day 30	Day 40
Moisture	57.46±1.51^{a}	55.88±1.17^{ab}	50.62±2.11^{bc}	52.35±3.20^{abc}	49.38±1.27^{c}
Ash	1.12±0.08^{ab}	1.06±0.19^{b}	1.23±0.08 ^{ab}	0.94±0.12^{b}	1.44±0.11^{a}
Crude Protein	5.43±0.07^{c}	6.56±0.44^{b}	5.01±0.62^{c}	7.17±0.11^{b}	8.45±0.16^{a}
Crude Fat	1.14±0.08^{c}	1.06±0.48^{c}	2.09±0.71^{bc}	3.29±0.65^{b}	5.07±0.67^{a}
Crude Fibre	21.97±1.70^{b}	28.19±2.79^{a}	27.59±1.60^{a}	27.99±2.20^{a}	28.94±1.15^{a}
Carbohydrate	12.88±1.09^{a}	7.26±2.20^{b}	13.46±0.83^{a}	8.26±1.48^{b}	6.72±1.56^{b}

Values are mean±standard deviation of replications (n = 3). Values with different superscript within same rows are significantly different ($P \leq 0.05$).

long shelf life.

Ash content is referring to the inorganic residue that remains in food after the burning process of organic compounds at temperature 500°C to 700°C (Nielsen, 2010). The percentage of ash content in *P. edule* Reinw. seeds throughout the fermentation process were around 0.94% at day 30 and 1.44% after 40 days. The higher amount of ash content acts as an indicator for a higher amount of the total inorganic mineral (Uba *et al.*, 2015). These results were supported by Oseni and Akindahunsi (2011) which mentioned that the ash content of 40 days of fermented cocoa bean seeds was higher than the raw seeds sample which is 4.75% and 4.43%, respectively. According to Oseni and Ekperigin (2007), the slight increase of ash content may be contributed by the fermentation of microorganisms.

Table 2 shows the crude protein content that fluctuated in percentage throughout the 40 days fermentation process. The percentage of crude protein content increased from 5.43% on day 0 to 6.56% on day 10 and it decreased to 5.01% after 20 days of fermentation. However, the percentage of crude protein suddenly increased to 7.17% on day 30 and 8.45% on day 40. The reduction of protein content indicates that the protein was degraded into peptides and amino acids by microbial activities responsible for fermentation and enzymatic activity (Makinde and Akinosa, 2014). A decrease in protein content as fermentation continues is attributed to amino acids being metabolized to ammonia and flavour compounds due to the accumulation of lactic acid activities (Pranoto, 2013). According to Onyango (2005), the increasing percentage of protein content after the fermentation process is associated with the release of nitrogen content which increased when the microorganisms had utilized carbohydrates as their source of energy.

Other than that, the composition of crude fat in *P. edule* Reinw. seeds was shown in Table 2. Fat is also an important source of energy to the body as it supplies 9 calories per grams. The percentage crude fat in *P. edule* Reinw. seeds were decreased from 1.14% on day 0 to 1.06% on day 10. However, after its 10th fermentation day, the percentage continuously increased at 2.09% at 20 days, 3.29% at 30 days and the highest crude fat content was at 40-day of fermentation with the percentage of 5.07%. Afoakwa *et al.* (2013) highlighted that the decrease of crude fat content was due to the action of lipases, which break down the triacylglycerols in the seeds into their separate groups of fatty acids. However, it increased at the end of fermentation because some microorganisms have the potential of producing oil during growth on a substrate (Akindumila and Glatz, 1998). Besides, Oboh *et al.* (2008) also highlighted that there were certain fungi capable to synthesize fat during a fermentation process.

The composition of crude fibre in *P. edule* Reinw. seeds were presented in Table 2 and the results indicate that the lowest fibre content was in the early fermentation process (day 0) with a percentage of 21.97%. However, this percentage continuously increased and shows that there were no significant differences in the crude fibre content throughout the fermentation process from day 10 until day 40. The highest percentage of crude fibre content was on fermentation day 40 whereby the percentage was 28.94%. Griffin (1994) also mentioned that fungi can produce several polysaccharides that commonly cellulose and chitin. These compounds may be exocellular associated with the membrane and cell wall or intracellular.

The percentage of carbohydrate can be obtained by calculating the difference, which is deducting the percentage of moisture content, ash, crude protein, crude fat, and crude fibre. From Table 2, it can be seen that the highest carbohydrate composition was on day 20 of fermentation (13.46%), followed by day 0 which is 12.88%. However, there are no significant differences in the percentage of carbohydrates between day 10 (7.26%), day 30 (8.26%) and day 40 with 6.72% which is considered as the lowest carbohydrate content. According to Makinde and Akinosa (2014), microbial activity requires energy and nutrient during the

fermentation process hence it decreased in carbohydrate content, which acts as the main source of energy (Makinde and Akinosa, 2014).

3.2 Yield of Pangium edule Reinw. seeds extract

Extraction is an important process involved to recover and isolate the phytochemicals compounds from plant materials. The main principle of solvent extraction is the mass transfer of soluble active component in the solid material move towards solvent when that solid material is exposed to a suitable solvent. In general, the yield of extracts is highly influenced by broad factors such as the type of extraction technique, the types of soaking solvent used, the ratio of solvent applied and the duration of the soaking period (Sultana *et al*., 2009; Sarker and Nahar, 2012). Besides that, Sarker and Nahar (2012) mentioned that the composition of the sample and the nature of the target compounds present in the extract such as the solubility of compound whether it is hydrophobicity or hydrophilicity, acid-base properties, charge, stability, and molecular size needs to be considered prior to the selection of method and solvent. However, this study only focused on the different types of solvent which are distilled water and ethanol that have different polarities.

There were two types of dried *P. edule* Reinw. seeds that have been used for the further extraction process, the raw and 40 days fermented seeds. Both raw and fermented seeds were extracted using solvent extraction (maceration) method and the solvents used were distilled water and absolute ethanol (99.9%) as described by Rukayadi *et al*. (2008), with slight modification. In general, solvent extraction is a simple, convenient, and less costly method. According to Azmir *et al*. (2013), the selection of soaking solvent during the plant extraction process plays a crucial role to determine the type of compound that can be extracted as different solvents can extract different compounds according to their varying polarities. Moreover, these two solvents were preferred because of their feasibility to recover the extracts as the solvents have boiling points below than or at 100°C which is safe to the thermolabile constituents.

The yield of raw and fermented *P. edule* Reinw. seeds extract was presented in Figure 2. The highest percentage of the extraction yield obtained per 100 g of dried weight of *P. edule* Reinw. seeds was observed in the fermented *P. edule* Reinw. seeds extracted using 400 mL of ethanol solvent at 27.90% and followed by the fermented seeds extracted using distilled water at 25.30%. For the raw *P. edule* Reinw. seeds sample, the percentage yield of extract using ethanol solvent was 18.08% while the lowest percentage of extraction yield was the raw seed extracted using distilled water which was only 17.67%. From the result, it can be concluded that the extraction using ethanol produce more percentage of yield extract compare to distilled water extract. According to Parekh *et al*. (2005), ethanol is one of the organic solvents that is classified as a polar solvent and is commonly used in the extraction of plant materials due to their ability to extract most of the polyphenolic compounds in plant-based materials such as alkaloids, phenolics, terpenoids and flavonoids compounds. It was able to extract ionic compounds in *P. edule* Reinw. seeds extract and is miscible in water. Ethanol solvent shows better dissolving capabilities compared to water due to its slightly low dipole and dielectric constant than water

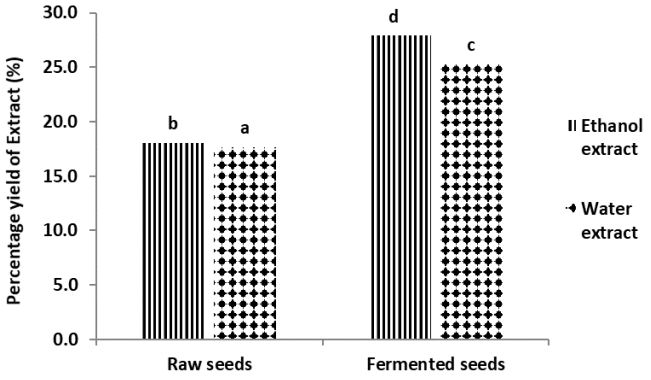

Figure 2. Percentage yield of raw and fermented *Pangium edule* Reinw. seeds extracts. Bars with different alphabet notations are significantly different ($P \leq 0.05$).

(Fatoki and Onifade, 2013).

3.3 DPPH scavenging activity in Pangium edule Reinw. seeds extract

The 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was a general method used to determine the antioxidant activity of a sample in scavenging activity against free radicals (Prieto, 2012). Antioxidants agents have the capacity to stabilize or deactivate free radicals before oxidative damage takes place in the cellular structures. These antioxidants act as a free radical scavenger by donating hydrogen or transferring electrons to the free radicals (Ogunmoyole *et al.*, 2009). It combines with free radicals and reduces the DPPH free radical hence, neutralizing them to prevent a chain reaction of reactive oxygen species (ROS) from occurring which can cause oxidative damage to human cells (Nimse and Pal, 2015). Therefore, free radical scavenging activity can be used as an indicator in order to determine the antioxidant capacity of these extracts (Yang *et al.,* 2015). This will lead to the change of colour from purple to yellow and consequently the reduction in the absorbance reading.

The results for the percentage inhibition of DPPH free radical scavenging activity of the raw and fermented

P. edule Reinw. seeds that were extracted with water and ethanol are presented in Table 3. The concentration of the extract that have been evaluated were 0.31 mg/mL, 0.63 mg/mL, 1.25 mg/mL, 2.50 mg/mL, 5.00 mg/mL and 10.00 mg/mL extracts. Based on the results in Table 3, the percentage of inhibition of DPPH increased as the concentration of extract increased from 56.43% to 66.52% for raw-water extract, from 55.19% to 82.75% in raw-ethanol extract, from 52.12% to 95.61% in ferment-water extract and for ferment-ethanol extract, the percentage was increased from 54.24% to 91.01%. In general, it clearly showed that the percentage of radical scavenging activity of both raw and fermented seeds extracts increased with the increasing concentration of the sample extracts. Loganayaki *et al.* (2013) also supported the finding that antioxidant activity of extracts increased with the increasing concentration of extract to a certain extent and then levelled off with a further increase in concentrations.

The result in Table 3 shows that at the concentration of 10.00 mg/mL, the lowest percentage inhibition of DPPH was in raw *P. edule* Reinw. seeds extracted with water, followed by ethanol extract of raw seeds and the highest percentage of DPPH inhibition was in fermented *P. edule* Reinw. seeds extract that extracted with water. This indicated that the extracts tested exhibited varying degrees of radical scavenging activities as the percentage inhibition of DPPH scavenging activities between the raw and fermented *P. edule* Reinw. seeds extract. Previous studies mentioned that the biological activity of the active compound and nutritional component in the food ingredients increased after the fermentation process. This is due to during fermentation process, which induced the structural breakdown of plant cell walls which leads to the liberation or synthesis of various antioxidant compounds (Zhu *et al.,* 2007; Chelule *et al.*, 2010).

Trolox has been used in this study as a standard to compare their antioxidant activity with extract as it is one of the common standards used in DPPH assay. The result shows that the percentage of DPPH scavenging activities for all extracts were significantly different ($p < 0.05$) with Trolox as the percentage inhibition was as high as 96.46% at the concentration of 0.0313 mg/mL whereas the percentage of the other extract was 56.43%, 55.19%, 54.67% and 55.04% for the extract of raw-water, raw-ethanol, ferment-water and for ferment-ethanol extract, respectively. Trolox was used in this research due to reports that it has similarities in chemical structure with oil. In addition, according to Abramovič *et al.* (2018), there is a small impact on the amount of exchanged electron in Trolox that makes it more suitable as a standard compound than the other antioxidants such as gallic acid, caffeic acid and catechin.

3.4 Total phenolic content (TPC) in Pangium edule Reinw. seeds extract

The total phenolic content of the raw and 40 days fermented *P. edule* Reinw. seeds extracts that had been extracted with ethanol and water were determined using Folin-Ciocalteu's colorimetric method. Generally, through this method, the phenols change from yellow to form blue coloured phosphomolybdic–phosphotungstic–phenol complex in alkaline solution (Mohamed and Abdullah, 2016). In this study, the standard calibration curve of total phenolic content was obtained with gallic acid and the results were expressed in milligram of Gallic acid equivalent (GAE) per 100-milligram of samples extract. The total phenolic content (mg GAE/100 mg extract) of *P. edule* Reinw. seeds extract was shown in Figure 3.

Figure 3 shows that the highest total phenolic content was found in the ethanol extract of fermented *P. edule* Reinw. seeds which were 173.79±10.02 mg GAE/100 mg extract. This result indicated that there was a large significant difference ($p < 0.05$) between the ferment-ethanol extract and the other extract. The figure clearly showed that the total phenolic content for ethanol extract of raw *P. edule* Reinw. seeds were 48.64±3.88 mg GAE/100 mg extract did not have significantly different from the fermented *P. edule* Reinw. seeds that were extracted with ethanol with the total phenolic content value was 51.15±1.80 mg GAE/100 mg extract.

Table 3. The percentage inhibition of DPPH scavenging activities (%) of raw and fermented *Pangium edule* Reinw. seed extracts

Concentration of extracts (mg/mL)	Percentage inhibition of DPPH scavenging activities (%)			
	Raw – water	Raw - ethanol	Fermented - water	Fermented - ethanol
0.31	56.43±2.24^{a}	55.19±1.21^{a}	52.12±3.28^{a}	54.24±1.59^{a}
0.63	59.65±2.13^{a}	55.78±0.83^{a}	56.51±0.71^{a}	58.04±4.12^{a}
1.25	60.82±2.43^{a}	58.33±3.45^{a}	60.45±4.19^{a}	61.40±2.09^{a}
2.50	61.70±1.92^{b}	66.81±5.38^{ab}	73.03±3.09^{a}	64.55±1.29^{ab}
5.00	63.38±1.91^{c}	72.81±1.54^{b}	85.82±2.04^{a}	73.54±0.77^{b}
10.00	66.52±3.95^{c}	82.75±5.12^{b}	95.61±0.96^{a}	91.01±1.52^{ab}

Values are mean±standard deviation of replications ($n = 3$). Values with different superscript within same rows are significantly different ($P \leq 0.05$).

Apart from that, the water extract of raw *P. edule* Reinw. seeds had a smaller total phenolic content value which is

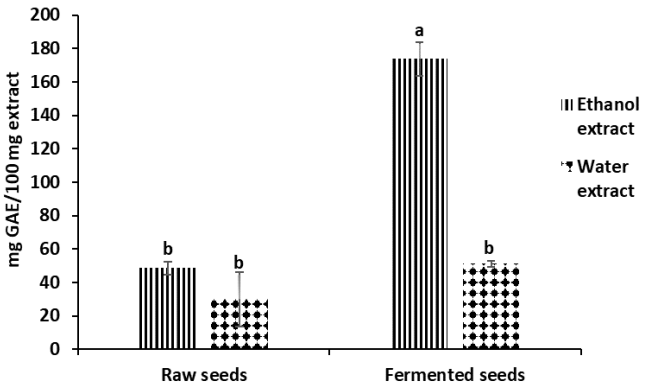

Figure 3. Total phenolic content (mg GAE/100 mg extract) of water and ethanol of raw and fermented *Pangium edule* Reinw. seed extracts. Bars with different alphabet notations are significantly different ($P \leq 0.05$).

29.89±16.45 mg GAE/100 mg extract.

Overall, it was clearly shown that the fermented *P. edule* Reinw. seed extract had a higher total phenolic content than the raw *P. edule* Reinw. seed extract. This shows that the phenolic contents were increased during the fermentation process. According to Messens and Vuyst (2002), the fermentation process will cause the release of the microbial enzyme which can increase freely available from chemicals such as flavonoid, tannin, alkaloid, and phenylpropanoids. Besides, the fermentation process induced the structural breakdown of plant cell walls which leads to the liberation or synthesis of various antioxidant compounds. This may contribute to the conversion to simple phenolic and the depolymerization of high molecular weight phenolic compounds (Othman *et al.*, 2009). Hur *et al.* (2014) also reported that some parts of the plant will increase their total phenols after the fermentation process. Other than that, the differences in the phenolic content in the plant extracts may be due to various factors, such as types of variety, growing conditions, maturation stage, and environmental conditions during fruit development, since extreme temperatures and high exposure to sunlight have been shown to increase phenolic components biosynthesis as an adaptive response of the plant (Al-Farsi *et al*., 2005).

4. Conclusion

In conclusion, this study found that fermentation of *P. edule* Reinw. seeds had improved the physicochemical properties, a significant increase in nutritional composition, total phenolic content and also the antioxidant activity of *P. edule* Reinw. seeds. There were some changes in the physicochemical properties of *P. edule* Reinw. seeds during the fermentation process as pH value decreased from 5.73 to 4.47. The total soluble solid was in the range from 11.00 (day 0) to 19.50 (day 30) while there were no significant differences in the water activity. However, a drastic change in colour of the seeds from white to brown colour is strongly contributed to the lightness (L^*) parameter. Generally, the proximate composition showed a significant ($p < 0.05$) increase in ash content, crude protein, crude fat and crude fibre content while the moisture and carbohydrate content decreased after 40 days fermentation day. For extraction, a higher yield was observed from the ethanol extract of fermented seeds and followed by fermented seeds extracted with water. In antioxidant analysis, the highest DPPH activity was observed from the ferment-water extract which was 95.61% while the highest total phenolic content was obtained in ferment-ethanol extract about 158.51±10.02 mg GAE/100 mg. These results show that the antioxidant activity and total phenolic content were enhanced after the fermentation process. However, there is some limitation for the current study. Recommendations for future research are to study the bioactive compounds present in both raw and fermented *P. edule* Reinw. seeds extract including the volatile and non-volatile compounds that are responsible for the antioxidant activities and other biological activities.

Conflict of interest

The authors declare no conflict interest. The authors alone are responsible for the content of the paper.

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