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EFFECT OF PRETREATMENT ON THE LEVEL OF ANTINUTRITIONAL FACTORS IN JACKFRUIT SEEDS

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Abstract

Jackfruit waste is attracting considerable widespread interest due to their good nutritious profiling. The seed of jackfruit comprises a good level of macronutrients such as dietary fiber, protein, fat as well as micronutrients like vitamins and essential acid amino. Few compounds in the seed were reported to have a beneficial role for the human health. Nevertheless, a trace level of antinutritional factors has been reported to be founded in the seeds. Tannin, phytic acid and saponin are few antinutritional factors that are regularly associated with agricultural waste. The existence of these compound in human diets may affect the overall nutritional value of the foods by averting nutrient bioavailability. The aim of this study was to investigate the effect of these two methods (germination and roasting) in minimizing the level of these antinutrients i.e. tannin and phytic acid before applying. Samples were subject to two pretreatment processes: germination and roasting. Quantifying of these two antinutritional factors was conducted via spectrophotometry method. Our results revealed that both pretreatment methods may reduce the level of tannin and phytic acid. In addition, roasted jackfruit seed contained the lowest level of tannin and phytic acid (0.1039 ±0.06 % DW and 0.0159± 0.1g/100g respectively).

Keywords: germination, jackfruit, phytic acid, roasting, tannin

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Introduction

Artocarpus heterophyllus and *Artocarpus integer* which are commonly known as jackfruit, have been widely cultivated around the world especially in India and Southeast Asia. The local production of this fruit in 2018 was estimated at 31 023 tons with the production value of RM 64 761 (Agrofood Statistics, 2018). This fruit is common for their fresh sweet and juicy flesh consumption. In addition, it is also served as a pickle with or without spices, cooked with coconut oil, made into ice cream, chutney, jam or canned in syrup. Its seed, which always considered as a waste, are believed to be a good source of nutritious food ingredient. In commercial scale of processing jackfruit, a huge amount of inedible parts such as peel and seed, are relinquished as a waste or being transform into animal feed (Ranasinghe et al., 2019). Jackfruit waste has high potential to be explored as a source of pectin and good quality of starch. By-product recovery from fruit wastes not only improves the overall economics of processing units, but also reduce the problem of environmental pollution considerably (Begum et al., 2014). More recent evidence from Waghmare et al., (2019), revealed that the seed of jackfruit comprises a good level of macronutrients such as dietary fiber, protein, fat and micronutrients like vitamins and essential acid amino. There are few compounds in the seed which were reported to have a beneficial role for the human health. The seeds are rich in dietary fiber and B-complex vitamins, and due to their high fiber content, they help to lower the risk of heart disease, prevent constipation and promote weight loss. A study done by Mushumbusi (2015), described that each part of this fruit including the seeds possess a medicinal and functional properties. It is believed that the seed may alleviate Vitamin A deficiency, cure diarrhea and also contain two important lectins namely jacalin and artocarpin that are useful for the well-being of human immune system. The reported values for the nutrients in jackfruit seed seem to vary owing to the differences in source, variety, environment, and ripeness of the fruit (Mahanta and

Kalita, 2015). It is notable that the moisture content of the seeds was around 55% and therefore does not hold well for long in ambient temperature. Tons of seeds were wasted during the processing of jackfruit due to this perishable nature. To extend its shelf life, the seeds need to be properly stored or turned into powder. Eke-Ejiofor et al., (2014) in their study highlighted that due to its high carbohydrate content and other nutrients, jackfruit seed can be added to baked products for value addition without affecting the functional and sensory properties of the final product. These remarkable features illustrate the promising traits of jackfruit seeds as sources of valuable compound and highlight their huge potential to be utilized as premium food ingredients.

The functional properties of jackfruit seeds can be improved by various processing technique including physical or chemical techniques. These process improve characteristic of the seeds from the point of gelatinization, temperature, water solubility, solution viscosity, swelling ability and water uptake as well as resistance to enzymatic degradation (Kittipongpatana and Kittipongpatana, 2011). In the next few years, jackfruit seed has the potential to become a premium food ingredient. Food manufacturers have shown interest to incorporate modified jackfruit seeds in food products like chili sauce and prebiotic. Experiment on incorporating modified jackfruit seed starch in production of chili sauce was performed by Rengsutthi and Charoenrein (2011). They pointed out that jackfruit seed starch incorporated chili sauce exhibited superior quality compared to other starch. In their experiment, starch from jackfruit shown the best consistency and lowest serum separation during storage. The significant amount of non-reducing sugar in jackfruit seed also makes it a good source to be utilized in prebiotics (Bhornsmithikun et al., 2010).

Regardless of considerable studies highlighting the benefits of jackfruit seeds to human body, this waste may also contain antinutritional factor (ANF) which could limit their consumption. The presence of tannin, trypsin inhibitor and phytate have been reported in jackfruit seeds (Azeez et al., 2015). ANF are substances that are generated by the secondary metabolism of plants to protect themselves from predators (Torres-León et al., 2018). Consumption of these compounds could limit the functional properties of foods in which are they present due to its ability to form strong bonds with carbohydrates or proteins, making them unavailable for digestion (Ndyomugyenyi et al., 2014). A recent review on this topic by Mahanta and Kalita (2015), found that 50% of the protein jackfruit seeds contain jacalin and lectin, a form of ANF as they bind with carbohydrates during ingestion. Hitherto, numerous investigations have been carried out to investigate the characteristic of ANF that promote negative aspects of this seeds. Tannin are complex, astringent and water soluble phenolic compounds known to reduce the bioavailability of nutrients in gut (Sharma et al., 2019). In a major advance in 2010, Kumar et al. (2010) deduced that phytic acid has become the center of concern for human nutrition and health management. The unique structure of this compound can strongly chelate with cations like calcium, magnesium, zinc, and iron to form insoluble salts and adversely affect the absorption and digestion of these important minerals.

Recent finding regarding ANF has led to the application of various processing technologies for minimizing its level in agricultural wastes. By reducing the level of ANF in agricultural waste like jackfruit seeds, a higher value product may be developed via effective modification. Common practice for reducing ANF include physical and chemical methods such as soaking, cooking, germination, fermentation, selective extraction, irradiation and enzyme treatment. Application of single treatment is periodically inadequate for effective treatment, so combinations are commonly practiced (Bora, 2014). Mahanta and Kalita, (2015) affirms that practice of processing involving heat treatment prior to consumption could reduce the ANF in the seed. Apart from heat processing, fermentation and enzyme treatment were among effective approaches to minimize the presence of ANF in plant (Lai et al., 2017). As mentioned by Jezierny et al., (2010) and Torres-Leon et al., (2018), the choice of treatment to be applied depends on several factors such as availability of equipment, facility, cost and mainly on the type of ANF that is to be eliminated. A recent review on germination by Megat Rusydi and Azrina (2012) ascertained that it is a natural process that occurred during growth of seed. This process gave beneficial impact to the chemical profiling of the seeds. Heat processing or thermal treatment was widely recognized as the most common practice to eliminate these substances (Lai et al., 2017). This treatment was used widely in food processing sector due to the low cost and simplicity for the technique amongst other. According to Sandhu et al., (2015), heat treatment is the most extensively used method for improving texture, nutritive value, denaturation of proteins, increased nutrient availability, inactivation of heat labile toxic compounds and other enzymes.

Therefore, this study was designed to evaluate the efficacy of two pretreatment processes i.e., germination and roasting, in reducing tannin and phytic acid in jackfruit seeds. These two processes were chosen because both treatments are low cost techniques that could be applied by the industry to diminish the presence of this undesired compounds from plant materials before its utilization in food production.

Materials and methods

Samples collection

Jackfruit seeds were acquired from few sources as stock of jackfruit seeds was limited at the time of study. Waste was obtained from minimally processed jackfruit industry and whole fresh fruits were procured from a jackfruit farm in Lanchang, Pahang. These two locations were selected as they conformed to the variety of jackfruit chosen for this study – the *Honey* jackfruit variety. The separation of the seed from the pulp were carried out manually. The seeds were washed using filtered tap water and were sliced. Samples were then divided for the germination and roasting process.

Pretreatment process

For the germination process, the seeds were washed with filtered tap water approximately three times. Samples were then soaked in filtered tap water and the process of germination started from 0 hour (no germination process) until 96 hours. The germinated seeds were then sliced into thin layers (Hallde, Sweden), dried in a forced draft oven (Memmert, Büchenbach, Germany) at 60°C for 24 hours. The dried samples were finally milled into flour using a grinder (Toshiba, Tokyo, Japan) prior analysis.

Jackfruit seed that was subject for roasting treatment were weighed at 150g, then homogenized with distilled water. Next, it was heated to 60-80°C in the forced draft oven to carried out the roasting process (Memmert, Büchenbach, Germany) for 30 to 60 mins. Muslin cloth filter were applied to separate the solution. The mixture was then allowed to settle for 2 hours in room temperature before being dried in an oven dryer (Memmert, Büchenbach, Germany) at 60ºC. The dried samples were milled into flour using a grinder (Toshiba, Tokyo, Japan) and used for further analysis.

Quantification of tannin and phytic acid using spectrophotometer

Determination of tannin

The experimental set up on determination of tannic acid was based on the work done by Azeez et al. (2015) which was proposed by Makkar (2003),with slight modification. Dried (finely ground sample, 0.2 g) was soaked in 25 ml of 70% acetone in 25 ml beaker and suspended in iced ultrasonic water bath for 20 mins (2 X 10 min with 5 min break in between). The sample were kept in low temperature to avoid the degradation of phenolics as tannin is naturally polyphenolic. The contents of the beaker were then centrifuged for 10 min at approximately 3000 rpm at 4°C (Sigma 2-16K Sartorius Centrifuge, Göttingen, Germany). The supernatant was filtered using Whatman No 1 filter paper and continuedly be kept in ice. Three concentration of filtrate was taken, made up to 0.5 ml with distilled water and put on 500 µl of Folin-Ciocalteau reagent. The mixture was then added to 0.5 ml distilled water and 1.5 ml of Na_2CO_3. It was then vortexed, incubated for 40 mins at room temperature. Absorbance of sample and tannin standards was read against blank at 725 nm. Result was expressed as % tannic acid.

Determination of phytic acid

The procedure used was described by Latta and Erskin (1980). A 0.5 g of dried sample was weighed into 250 ml conical flask. The initial extraction was done with 2.4% 25 ml HCl, whereas sample was shake in orbital shaker (Labwit ZHWX-304 Orbital Shaker) for an hour at room temperature and then centrifuged at 3000 rmp for 30 mins (Sigma 2-16K Sartorius Centrifuge, Göttingen, Germany). A 3 ml clear supernatant was transferred into test tube for the phytate analysis. A 1 ml of Wade reagent (0.03% solution of $FeCl_6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to the test tube and vortexed for a minute. Sample was read at 500 nm absorbance (Microplate Reader EON, Biotek, Vermont, USA) and phytate was quantified from a standard calibration curve of phytic acid (2 to 10 mg/ml). Result was expressed as phytic acid, g/100 g.

Statistical analysis

Statistical analyses were performed in Statistical Analysis Software (SAS) package (version 9.1.4 of SAS Institute, Inc. Cary, NC, 2008). In case of significant effects, means were determined by one way Analysis of Variance (ANOVA) then compared using Duncan Multiple Range Test (DMRT) to determine significances between the means. Effects were considered significant at $p < 0.05$.

Result and Discussion

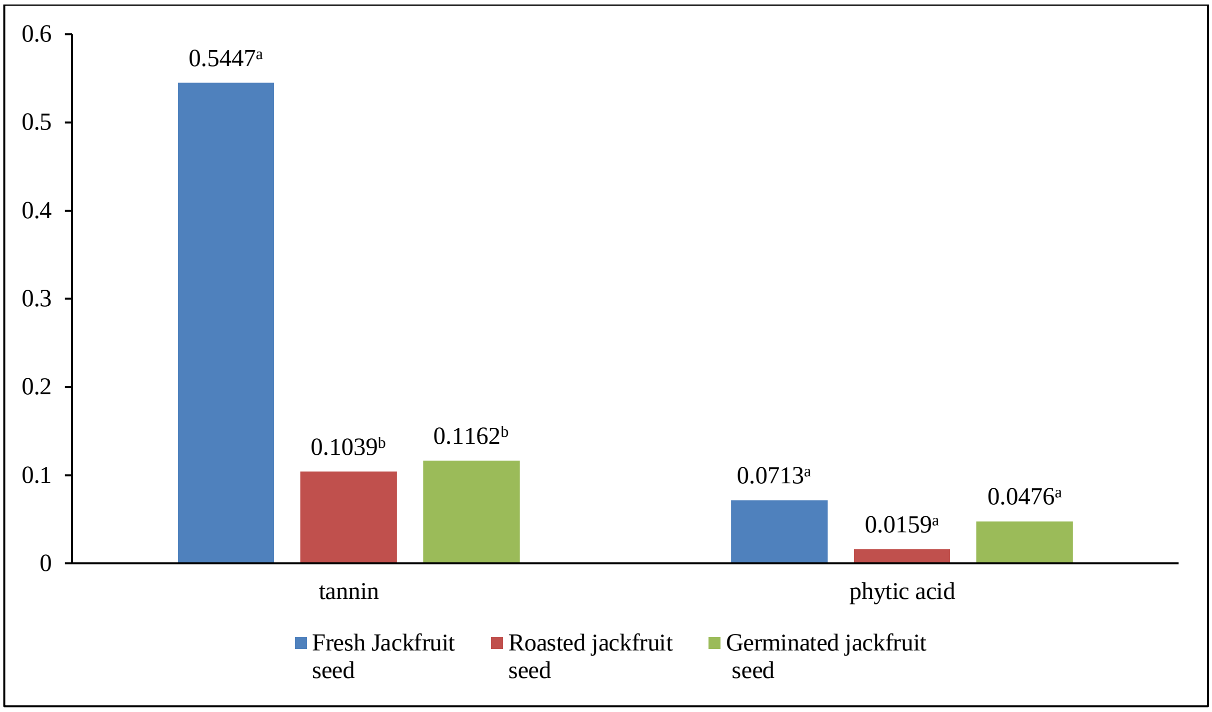
Germination and roasting treatment may have different impact on chemical properties of the seed. An increasing numbers of studies have found that both treatment does not only give benefit for the nutritional properties, it could also reduce antinutritional factor such as phytic acid (de Farias Leite et al., 2020 and Sokrab et al., 2012).

Figure 1 illustrates the difference in jackfruit seed between 0 hour and 96 hours germination. Experiment on the chemical composition and acid amino profile done by Zuwariah et al.,(2018), demonstrated that acid profile in jackfruit seeds were reduced during the first twelve hours of germination but started to increase at the next eighteen hours and reached the optimum germination period at 24 hours. However, during the last two periods (72 and 96 hours), the amino acid profile started to decrease. The decreasing effect could be seen in figure 1 (96 hours germination). During germination, the enzymatic activity promotes the hydrolysis of the starch into simple sugars and other changes in the nutritional components (Charoenthaikij et al., 2012).

Figure 1. Jackfruit seeds at 0 hour germination and 96 hours germination.

The bar chart below (Figure 2) depicts the content of tannin and phytic acid in the sample for 3 different treatments. Our finding confirmed that there was a trace amount of ANF present in the control sample (fresh jackfruit seeds). Tannin level in our fresh sample were observed at 0.5447%. This level is higher than those reported in Eyoh G.D (2020), (0.01%) and Azeez et al., (2015) which are 0.01% to 0.04%. Level of phytic acid in our sample (0.0713g/100g) was scarcely distinguishable from study done by

Okudu et al. (2015) and Eyoh, G.D (2020) which were 0.19% and 109 mg/100g respectively. Our finding was higher than Azeez et al. (2015) in which they discovered that level of phytic acid in their study samples were in the range between 0.06 to 0.3 mg/100g. This result extended our knowledge on different levels of ANF in jackfruit seeds. In their cutting-edge paper, Ranasinghe et al. (2019) reported that chemical composition of jackfruit varies depending on the variety. Their studies discovered that there were at least 30 strains of jackfruit in subcontinent India and 30 more types in Malaysia. These different types of jackfruits are diverse, not only in chemical composition, but also in morphological, phenotypic, and organoleptic characteristic.



*Value are expressed as mean± standard deviation (n=3)
*Values followed by the same letter within the same types of antinutritional factor are not significantly different at p>0.05
*y axis = level of antinutritional factor (%)
*x axis = types of antinutritional factor

Figure 2. Level of antinutritional factors (tannin and phytic acid) in jackfruit seeds

From the study, we noted that there was a significant difference of tannin level between fresh samples and samples that were subjected to roasted treatment. Roasted treatment samples showed the lowest level of tannin (0.1039%). Tannin as describe by Sharma et al., (2019) is a plant based polyphenol. It possess water soluble characteristic, which could easily leach into liquid medium during heat process. Our experiment for reduction of tannin corroborates with previous work done by Olanipekun et al., (2015). It was observed that roasting reduced the anti-nutrients in their sample to a very low level (0.10%). This discovery was also consistent with Ndyomugyenyi et al.,(2014) which found that jackfruit beans which has undergone roasting treatment removed more than 84% tannins from the raw sample. Level of tannin in the mentioned study was reported in the range of 0.125 to 0.174%. As put forward by Eyoh G.D (2020), sample with heat treatment gave no detected level of tannin (0.0%) compared to raw sample with level of 0.04%. The author deduced that heating treatment is an efficient mechanical process to detoxify this compound. Similar finding were also reported by Olawepo et al., (2014) when kenaf seeds were roasted. Tannin in the raw sample was quantitated at level of 1.18 in raw sample to and 0.98 dry weight basis in roasting sample. In their study, cooking and roasting significantly ($p<0.05$) reduced level of tannin.

The significant decrease of tannin content in our germinated sample is worth mentioning because it was consistent with the result reported by de Farias Leite et al., (2020). Level of tannins in their germinated sample (0.017g/100g) was lower compared to natural levels (0.23g/100g). Okpala (2010) observed the reduction of tannin from 6.02mg/100g in raw jackfruit seed to 2.47mg/100g in germinated sample (96 hour sprouted jackfruit seed). This discovery support the concept that significant decrease of the tannin content in the seeds after germination, was attributed to the oxidation of polyphenols, which is activated during germination and causes tannins enzymatic hydrolysis. Reduction of tannin was also detected in the study done by Khandelwal et al., (2010) on different types of Indian pulses. The authors stated that during germination, enzymes were activated and resulted in the hydrolysis of various component including the polyphenol. Medugu et al., (2012) emphasized that germination is an effective process to remove or reduce anti-nutritional factor in feedstuff. It has now been demonstrated that tannins could be diminished at a significant level by several domestic processing treatments such as cooking, germination, soaking and etc (Sharma et al., 2019).

However, in our experiment, phytic acid level between treated and fresh samples was insignificant ($p>0.05$). The apparent lack of correlation could be attributed to the technique in sample preparation and quantification of phytic acid. Apart from this slight non-alignment, the result confirmed that both treatments had a positive influenced in lessened the phytic acid level in jackfruit seeds. Roasted sample gave the lowest reading which is 0.0159 g/100g. Our experiments substantiated previous finding in the literature done by Haileslassie et al., (2016) in which traditional cooking or boiling did not result in significant reduction in phytate content. Prior research generally agreed with this data as highlighted by Olawepo et al., (2014), cooking and roasting had no significant ($p>0.05$) effect on the phytic content of kenaf seeds (0.14 in raw sample to 0.3 in dry weight basis for roasted sample). The author drew a conclusion that heat treatment alone is relatively unproductive in bring down level of phytate in plant material. Ndyomugyenyi et al., (2014) described phytate reduction was lower than 32% in all their treatments study, where level of phytate in roasted sample was 0.565% and the raw sample was 0.754%. In a research done by Eyoh, G.D (2020), the cooking and toasting sample ranged from 0.19% in raw sample to 0.14% to 0.09% respectively. This discovery led to their conclusion that processing techniques employed could minimize array of antinutritional factors in jackfruit seeds with toasted being the best. Experiment on effect of legume processing treatments on their phytic acid content were conducted by Rasha Mohamed et al. in 2011. The researcher discovered low level of phytic acid in legumes during cooking at significantly level ($p<0.05$) and this may partly be due to leeching into the cooking medium, deterioration by heat or formation of insoluble complexes between phytate and other components. The authors underlined that phytic acid is heat-stable, hence significant and prolonged inputs of energy are required for its destruction. This fact was also supported by Kumar et al. (2010) who accentuated that the intrinsic plant phytase which is thermolabile, prolonged exposure to high temperature may lead to the inactivation of endogenous enzyme.

Determination of phytic acid in our germinated sample was lower (33%) from the raw sample. Germinated sample contained 0.0576% of phytic acid level. This finding was in line with work done by Sokrab et al., (2012), where they found level of phytic acid decreased significantly during 4 to 6 days of germination (exceed more than 80%). The same authors also highlighted antinutritional factor i.e. phytic acid could be lessened after undergoing germination process. Work done by Okpala, (2010) also showed that sprouting significantly reduced the phytate to almost 60% from raw sample (48.08 mg/100g) to 12.08mg/100g in 96 hours sprouting. The author noted that the reduction attributed to the increased phytase activity during sprouting. Further test carried out by Rasha Mohamed et al., (2011), concurred with this finding by reported notable significant reduction in phytic acid content tested beans for 5 days germination. This treatment was also effective in reducing phytic acid in soy bean and peanut (Megat Rusydi and Azrina, 2012). Phytic acid content significantly reduced (21.74%) in soybean and (38.11%) in nut after germination. From the work done, they pointed out that depending on types of legumes, reduction was not only due to the phytase activities, but also because of the diffusion into the soaking medium which is known as leeching. Kumar et al., (2010) made a statement in his review paper that phytic acid in legumes was reduced during germination due to intrinsic phytase. Plant seed utilised phytate as a source of inorganic phosphate during germination and thus tend to improve palatability and nutritive value. As put forward by Albarracín et al., (2013), effort on reducing antinutritional factor like phytic acid could be achieved via soaking and germination treatment leading to protein digestibility and mineral disability.

Despite that we could not confirm the effectiveness of both treatment (at $p<0.05$) to reduce the presence of these two ANF significantly, it still interesting to discuss the improvement of these method in nutritive value of jackfruit seeds. Our earlier research illustrated in Table 1 below showed the differences of nutrition composition of jackfruit seeds after go through both treatments. The overall value showed that these two processing parameters affected the chemical compositional of our jackfruit seed flour samples. We believe that the pre-treatment process had increased this particular nutrient in jackfruit seeds.

Table 1. Nutritional composition in jackfruit seeds

Samples	**Raw jackfruit seeds**	**Germinated jackfruit seeds flour**	**Thermal jackfruit seeds flour (roasted)**
Moisture g/100g	$3.215±0.16^{b}$	$2.925±0.02^{b}$	$5.985±0.11^{a}$
Ash, g/100g	$2.75±0.04^{a}$	$2.56±0.04^{a}$	$2.43±0.04^{a}$
Protein, g/100g	$9.78±0.00^{b}$	$10.41±0.00^{b}$	$24.98±0.08^{a}$
Fat, g/100g	$0.56±0.01^{a}$	$0.48±0.03^{b}$	$0.22±0.03^{c}$
Dietary fibre, g/100g	$25.43±0.54^{a}$	$24.48±0.01^{a}$	$14.33±0.04^{b}$
Carbohydrate, g/100g	$83.68±0.13^{a}$	$83.63±0.08^{a}$	$76.03±0.04^{b}$
Starch, g/100g	$55.90±0.22^{b}$	$64.98±1.01^{a}$	$50.97±0.29^{c}$
Energy value, kcal/100g	$429.5±0.71^{b}$	$429.5±0.70^{a}$	$434.5±1.84^{b}$

*values followed by the same letter within the same row are not significantly different at $p>0.05$ (n=3)
Source: (Zuwariah et al., 2018).

In Table 1, we summarize the nutritional composition of jackfruit seeds in different treatments which obtained from proximate analysis. From the results, it appeared that both treatments improved few major nutrients like protein and starch. Jackfruit seed was reported as a good source of protein and starch (Noor et al., 2014). Moisture content is measured by quantifying its water content and it is an index of storage stability. The lower its moisture content, the better its shelf stability and hence its quality. Our finding for moisture content is in agreement with the finding by Eke-Ejiofor et al., (2014), which ranged from 3.2% to 6.6% with germinated jackfruit seed flour contain the lowest level of moisture content. This value was also hardly distinguishable from level reported by Ocloo et al., (2010). This finding supported the fact that germination treatment could longer the shelf life of jackfruit seeds. It is interesting to note that protein content in our experiment for roasting treatment is significantly different. This result supported the concept brought up by Eke-Ejiofor et al., (2014) in which roasting positively affected the protein availability, increased the water absorption capacity, high peak viscosity and amylose content which are important factors with regard to the end use of properties of product. Study done by Cornejo et al., (2015) also proved that germination treatment improved the nutritive value of protein content. The outcome of the study was dependent on the germination time. The same authors also highlighted that germination process is a low cost technology that provides better adsorption of nutrients linked to antioxidants-functional compounds.

Fat content in our experiment control showed significant differences for both treatment with roasting sample showing the lowest value at 0.22g/100g. This value correlate fairly well with Eke-Ejiofor et al., (2014) and Chowdhury et al., (2014). This pretreatment also showed positive impact on elevation of starch content in jackfruit seed. Jackfruit seed was reported to have considerable amount of starch content which can be industrially exploited (Mahanta and Kalita, 2015). Germination increased 16% starch content in our sample with the level of 64.98 g/100g in germinated sample, compared 55.90 g/100g in raw samples. This discovery confirmed previous investigation done by Eke-Ejiofor et al., (2014). In their work, germination sample gave the highest reading compared to other treatments.

As put forward by Khandelwal et al., (2010), raw pulses are subjected to processing technique prior consumption which include milling, dehulling, germination, fermentation, and cooking. These processing techniques saves time, energy and fuel as well as yielding edible products with higher nutritional value and reduce the level of antinutritional. This confirmed previous finding in the literature done by Sandhu et al., (2017) that thermal processing is the most extensively used method applied to cereals for improving their texture, palatability, nutritive value by gelatinization of starch, denaturation of protein, increased nutrient availability and inactivation of heat labile toxic compounds.

Conclusion

From this study, we found that germination and roasting pretreatments could lower the presence of antinutritional factors, i.e. tannin and phytic acid in jackfruit seeds. Our finding implies that both treatments are effective to decrease the presence of tannin and phytic acid in jackfruit seeds leading to the possibility of utilizing jackfruit seed as a food ingredient. Additional study needs to be conducted extensively, in order to further reduce the level of these antinutrients in jackfruit seeds. Further advanced techniques and parameters need to be explored as part of the process to utilize jackfruit seed as a food premium ingredient.

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