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Physico-chemical properties, amino acid profile and antinutritional factors in seeds of three Malaysian grown jackfruit cultivars

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Abstract

Jackfruit seed is an under-utilized crop with essential dietary nutrients gaining more attention in the recent years. The physico-chemical properties, amino acid profile, and some anti-nutritional factors in seeds (JFS) of three jackfruit cultivars were investigated. The moisture, protein, ash, fat, fibre, and starch content ranges were 14.26-24.08%, 7.62-8.46%, 3.19-3.70%, 1.09-1.48%, 2.80-7.19%, and 15.95- 62.04%, respectively. The pH ranged from 4.57 to 5.91. Essential amino acids histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and cysteine were present in the three cultivars analysed. The total amino acids for cultivars J29, J31 and J33 were 115.78, 116.60 and 103.86 mg/100 g, respectively. The anti-nutrients (tannin, phytate and trypsin inhibitor) were found in trace amounts. Glucose (0.18 - 0.22%), fructose (0.02 - 0.08%), and sucrose (0.02 - 0.13%) were the simple sugars found in JFS. Linoleic acid (C18:2) dominated the polyunsaturated fatty acid in JFS (4.72 - 22.34%). Others included linolenic (C18:3) (0.54 - 1.50%) and arachidonic (C20:4) (0.37 - 0.71%) acids. Oleic acid (C18:1) (0.47 - 1.18%) was the only monounsaturated fatty acid present, while the saturated fatty acid included palmitic (C16:0) (1.10 - 6.59%), stearic (C18:0) (0.42 - 1.44%), behenic (C22:0) (0.60 - 1.92%), and tetracosanoic acid (C24:0) (0.92%). With the presence of all the essential amino acids, fatty acids, and trace amount of sugar and anti-nutritional factors jackfruit seeds can therefore serve as a cheap source of dietary nutrients and healthy snack for over-weight people when processed.

Key words: Jackfruit seed, physico-chemical properties, dietary nutrients, amino acid profile, anti-nutritional factors.

Introduction

Jackfruit (*Artocarpus heterophyllus* L.) is a tropical fruit indigenous to India, and widely grown in Bangladesh, Burma, Sri Lanka, Brazil, and other countries [1]. It is locally known in Malaysia as "Nangka", and it is a high yielding crop which bears fruit all year round with peak period during the months of June and December [2]. Jackfruit contains high levels of protein, starch, calcium, and thiamine [3]. Unripe jackfruit is used in many culinary dishes such as vegetable, and eaten as fruit when ripened. Jackfruit seeds may be boiled, or roasted before eaten. Roasted ground seeds are often incorporated into wheat flour for baking [4]. Jackfruit seed flour contains 6.09% moisture, 2.70% ash and 1.27% fat (dry matter basis), while the protein content, fibre and carbohydrate contents are 13.50%, 3.19%, and 79.34%, respectively [5]. Although the seeds are good source of nutrients, the presence of anti-nutritional factors such as tannin, phytate, and trypsin inhibitors have been reported. Consuming of raw jackfruit seeds may result in digestive ailment [4, 5].

Most studies on jackfruit seeds have been focused on the physico-chemical, functional, and nutritive values of the raw, and processed jackfruit seed flour [5-8]. The amino acid composition, and toxicological evaluation of jackfruit seed in rat diet have also been reported [9]. This study aimed at investigating the chemical components, amino acid profile, and anti-nutritional factors in seeds of three jackfruit cultivars.

Materials and Methods

Sample: Three cultivars of jackfruit (*A. heterophyllus* L.) (varieties J29, J31, and J33) were obtained from a commercial farm in Mantin Kampong Cola, Negeri Sembilan, Malaysia. The harvested fruits were transported to the laboratory at the Universiti Putra Malaysia on the same day.

Seed preparation: The fruits were allowed to ripen using ripening agent in a humidified atmosphere (85% RH) for 3 days. The fruits were cut open and the seeds were removed. The seeds were manually decorticated, and sliced into tiny slices (2.7 cm × 1.5 cm × 0.4 cm) using a food processor. Raw jackfruit seed slices were dried in an oven at 40°C for 24 h to reduce the moisture content. Dried seeds were packaged in flexible aluminium pouches, vacuum sealed and stored in the refrigerator (3 - 5°C) for further analysis.

Chemical analysis: Proximate analysis (moisture, crude fat, dietary fibre, crude ash, and crude protein) were determined using the AOAC [10] methods. Carbohydrate content was determined by difference. The starch was calculated after the determination of glucose (dextrose) using the Somogyi-Nelson method [11]. The pH of the jackfruit seeds (JFS) was determined using a pH meter (model 3505, Jenway, Bibby Scientific Ltd, Dunmow, Essex, UK).

Sugar determination: Simple sugars were determined as described by Hunt *et al.* [12]. Ten grams of ground JFS were heated with 100 ml of 85% methanol in a steam water bath for 30 min at 80°C. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted twice with 75 ml of 85% methanol. The filtrate was evaporated using a rotary vacuum evaporator at 50°C to concentrate the sample solution to about 10 ml. The evaporated sample was filtered through a C-18 Sep-Pak cartridge and a 0.45 µm membrane filter using a syringe and stored in vials until further analysis. The HPLC was equipped with a Water 410 differential refractometer detector, and a Waters PU-501 HPLC pump. The column used for the analysis was a 5 µm Purospher Star NH2 column, Lichrocart (250 mm × 4.6 mm) (Merck KGaA, Darmstadt, Germany). Degassed 80% acetonitrile was used as the mobile phase. Ten microlitres of the extracted jackfruit seed sample was manually injected. Sugars in the samples were quantified by comparing peak areas of the samples with standards. Glucose, fructose, and sucrose standards at concentrations of 0.5, 1.0, 1.5 and 2.0% (w/v) were used. A calibration curve was obtained for each of the sugars. Data were analysed using Borwin PDA software (version 1.5, JASCO CO. Ltd., Japan).

Amino acids profile determination: Amino acid composition was determined according to Pico-Tag method as described by Bidlingmeyer *et al.* [13]. Sample was hydrolyzed using 15 ml of 6 M HCl at 110°C for 24 h in vacuum-sealed tubes flushed with nitrogen gas for 1 min. The hydrolysate was used to determine the amino acid profile using reverse phase HPLC. Analysis was done in triplicate for each sample. The HPLC was equipped with a photodiode array detector (Model: MD-2010; JASCO, Tokyo, Japan) a JASCO PU-2080 HPLC pump, and a column oven JASCO CO-2065. The column used for analysis was a 5 µm Purospher Star RP-18 end capped column (250 mm × 4.6 mm). Data were analysed using Borwin PDA software (version 1.5, JASCO CO. Ltd., Japan).

Determination of fatty acid composition: Fatty acids were prepared into fatty methyl esters (FAME) upon derivitazation using sodium methoxide method [14]. The FAME was analysed as described by Christie [15] using gas liquid chromatography. The crude fat was first extracted from the JFS using Soxhlet apparatus, followed by trans-esterification of oil with hexane and sodium methoxide. FAME was analysed on a capillary gas chromatography using Hewlett-Packard HP6890 gas chromatography (now Agilent Technologies) equipped with split-splitless injector, and flame ionization detector. A DBwax-70 (30 m × 0.2 mm × 0.25 µm) column was used. The oven was programmed for 2 min at 100°C, increased at 5°C/min to 230°C, and maintained for 10 min. Helium was used as carrier gas with flow rate of 1.0 ml/min. Injector and detector temperatures were maintained at 25°C, at splitless command with 1 µl of sample injected. Identification, and quantification of FAME was accomplished by comparing the retention times of pure standard peaks analysed under same conditions. The results were expressed as percentage relative concentration of each fatty acid.

Determination of phytic acid: The phytic acid was determined using the procedure described by Latta and Eskin [16], which was later modified by Vaintraub and Lapteva [17]. A 0.5 g of dried sample was weighed into a 250 ml conical flask. A 25 ml of 2.4% HCl was added, allowed to stand for 1 h at ambient temperature, and centrifuged at 3000 rmp for 30 min. The clear supernatant was used for phytate analysis. A 1 ml of Wade reagent (0.03% solution of $FeCl{\cdot}6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the supernatant, and vortexed for a minute. The absorbance was read at 500 nm using a spectrophotometer. Concentration of phytate was calculated from a standard calibration curve of phytic acid (2 to 10 mg/ml), and result was expressed as phytic acid g/100 g.

Determination of tannin: Tannic acid was determined according to Markkar and Goodchild [18] with slight modification. Approximately 0.5 g of sample was soaked in 25 ml of 70% acetone and placed in ice water bath for 12 min. The solution was filtered through Whatman No. 1 filter paper. Three concentrations of filtrate was taken, made up to 0.5 ml with distilled water and 500 µl of Folin-Ciocalteu reagent was added, followed by 1.5 ml of Na_2CO_3. The mixture was vortexed, and incubated for 40 min at room temperature. Absorbance of sample and tannin standards was read against blank at 725 nm. Results were expressed as % tannic acid.

Determination of trypsin inhibitor: Trypsin inhibitor was determined as described by Kakade *et al.* [19]. This involves weighing of 0.2 g of sample into centrifuge tubes and addition of 10 ml of 0.1 M phosphate buffer (pH 7.2). The mixture was shaken for 1 h and centrifuged at 5000 rpm for 5 min. A 20 µl to 100 µl of clear supernatant was pipetted into test tubes, and made up to 1 ml with 0.1 M phosphate buffer. A 6 ml of 5% tri-acetic acid was added to one of the test tubes to serve as blank in each set. All the test tubes were placed in water bath at 37°C for 20 min. Casein solution (2 ml) was added to the other test tubes. The reaction was stopped by adding 6 ml of 5% tri-acetic acid to the remaining test tubes. The mixture was vortexed for 1 min and allowed to stand for 1 h, filtered through Whatman No 4, and the absorbance was read at 280 nm against trypsin standard. Calibration curve was used to calculate the amount of trypsin inhibitor in the JFS.

Statistical analysis: All data obtained were subjected to analysis of variance (ANOVA), and expressed as mean ± standard deviations. Significant means were separated using Fisher's least significant difference test at acceptable significance level of 5% probability.

Results and Discussion

Proximate composition: The proximate composition, starch content, and the pH of the three cultivars of JFS are presented in Table 1. The moisture contents for the three cultivars were significantly different ($P \leq 0.05$) ranging from 14.26% to 24.08% (dwb). The values are higher than those earlier reported [5, 20] for jackfruit seed and flour. The moisture value reported in this study is in close agreement with the value (12.34%) earlier reported for jackfruit seed cake [9]. Cultivar J29 had the lowest protein content (7.62%) compared to J31 (8.46%) and J33 (8.24%). The results obtained in this study are comparable to the values (6.34 to 8.57%) earlier reported by Mukprasit and Sajjaanantakul [21]. Considerable biochemical differences between seeds of the two jackfruit varieties have been found [20]. Percentage ash content of the seed ranged from 3.19 to 3.70%. The J33 and J31 were not significantly different ($P \geq 0.05$) from each other, but there was a significant difference (P

Table 1. Proximate composition, starch, sugar and pH in seeds of jackfruit cultivars.

Parameters	J29	J31	J33
Proximate			
Moisture (%)	24.08±0.33^a	14.26±0.08^c	14.26±0.08^c
Protein (%)	7.62±0.07^a	8.46±0.38^a	8.24±0.54^a
Ash (%)	3.70±0.16^a	3.39±0.00^b	3.19±0.00^b
Fat (%)	1.09±0.00^b	1.48±0.04^a	1.17±0.17^{ab}
Carbohydrate (%)	60.71±0.00^c	65.40±0.00^b	66.20±0.00^a
Dietary fibre (%)	2.80±0.00^c	7.19±0.00^a	5.70±0.00^b
Starch (%)	62.04±0.76^a	15.95±0.64^c	29.55±0.78^b
Sugar			
Fructose (%)	0.02±0.00^b	0.08±0.00^a	0.03±0.00^b
Glucose (%)	0.22±0.07^a	0.18±0.02^a	0.18±0.02^a
Sucrose (%)	0.04±0.00^b	0.13±0.01^a	0.02±0.00^b
PH	5.91±0.02^a	4.57±0.02^c	4.86±0.01^b

Values are means and standard deviations of duplicate analyses; values followed by different superscript letters in a row are significantly ($p < 0.05$) different from each other; J29, J31, and J33 are JFS cultivars.

Table 2. Amino acid composition (mg/100 g) in seeds of jackfruit cultivars.

Amino acid	J29	J31	J33
Histidine	3.49±0.15^a	3.51±0.11^a	2.76±0.75^a
Arginine	4.24±0.19^a	4.84±0.19^a	3.19±0.42^b
Threonine	6.50±0.00^a	7.29±0.21^a	6.39±0.95^a
Valine	7.16±0.00^a	7.06±0.16^a	7.15±0.05^a
Methionine	0.43±0.03^b	1.03±0.10^a	0.39±0.00^b
Isoleucine	6.48±0.12^a	6.11±0.08^a	6.61±0.03^a
Leucine	4.58±0.30^b	5.87±0.47^a	3.51±0.27^b
Phenylalanine	5.97±0.71^{ab}	4.30±0.05^b	6.76±0.00^a
Lysine	1.19±0.18^a	0.84±0.03^b	1.05±0.02^{ab}
Cysteine	13.55±0.06^a	13.42±0.12^a	13.59±0.36^a
EAA*	53.59	54.27	51.4
Aspartic	24.06±0.26^a	22.25±0.82^a	15.53±0.21^b
Glutamic	12.31±0.10^a	12.43±0.01^a	12.56±0.60^a
Serine	9.45±0.07^a	10.30±0.06^a	10.28±0.86^a
Glycine	0.86±0.01^a	0.89±0.07^a	0.76±0.09^b
Alanine	4.33±0.22^b	5.41±0.08^a	3.47±0.54^b
Proline	5.23±0.00^a	5.29±0.07^a	4.80±0.74^a
Tyrosine	5.95±0.03^a	5.76±0.01^a	5.06±0.76^a
NEAA**	62.19	62.33	52.46
Total amino acid	115.78	116.60	103.86

Values are means and standard deviations of duplicate analyses; values followed by different superscript letters in a row are significantly ($p < 0.05$) different from each other; EAA* – Essential amino acid; NEAA** – Non-essential amino acid; J29, J31, and J33 are JFS cultivars.

≤ 0.05) between these two cultivars, and J29 cultivar. The ash content is comparable to the value of 3.6% reported by Singh *et al.*[7] and 3.97% reported by Tulyathan *et al.* [22]. The high ash content may be attributed to the higher mineral composition in jackfruit seed. There was a significant difference ($P \leq 0.05$) in the fat content of the three cultivars. However, the fat contents of JFS obtained in this study are higher than the values reported by some authors[7, 22]. Jackfruit seed, an underutilized crop, was observed to be moderately high in dietary fibre content (2.80 - 7.19%). Dietary fibre has been reported to be very effective in the treatment, and prevention of many diseases such as colon cancer, coronary diseases, obesity, and gastro intestinal disorders [23]. Although there was significant difference in total dietary fibre among the seeds, J29 cultivar has the lowest content of total dietary fibre (2.8%), while the J31 has the highest one (7.19%). Jackfruit seed is majorly composed of carbohydrate. The carbohydrate contents of the three cultivars significantly differed ($P \leq 0.05$) from each other. The carbohydrate compares favourably with the values reported in literature [5, 21]. Starch content of JFS cultivars ranged from 15.95 to 62.04%. The J31 had the lowest starch content, while the J29 had the highest starch value. Jackfruit seeds have been reported to be good sources of starch (22%), and dietary fibre (3.19%) [24]. Starch content of soft and hard jackfruit seeds were given as 92.5%, and 94.5%, respectively [25]. The starch content of jackfruit seed was influenced by maturity time and differences in geographical locations [26]. This probably accounted for the variations observed in the starch contents among the JFS studied. The pH of J29, J31, and J33 were 5.91, 4.57, and 4.86, respectively (Table 1). The pH of jackfruit cultivars were significantly different ($P \leq 0.05$). Jackfruit seed can be categorized as a medium to low acid food. Previous studies reported the pH values of 5.68 and 5.78, respectively for JFS [5, 22].

Amino acid composition: The results of the amino acid profile of JFS are presented in Table 2. The three cultivars comprise of all the essential amino acids in appreciable amount with the exception of methionine (0.3 - 1.03 mg/100 g) and glycine (0.76 - 0.89 mg/100 g). Amino acids are the building blocks of protein. Essential amino acids are suggested to contribute to the amino acid stimulation of muscle protein synthesis [27, 28]. Total essential amino acid content is lower than the total non-essential amino acid content for all the cultivars. This implies that JFS may be considered a moderately good source of essential nutrients for human. The total amino acid content for J29, J31, and J33 were 115.78, 116.60, and 103.86 mg/100 g, respectively. Aspartic acid (15.53 - 24.06 mg/100 g) was highest for all the cultivars, followed by cysteine (13.42 - 13.59 mg/100 g), glutamic acid (12.31 - 12.56 mg/100 g), and serine (9.45 - 10.30 mg/100 g). The amino acid contents of JFS are higher than that reported for *Terminalia catappa* seed except for glutamic acid [29]. Also, the amino acid composition of selected edible nut seeds when compared to the values obtained in this study tends to be much lower with the exception of glutamic acid, arginine, and leucine [30].

Sugar composition: The sugar content of JFS was detected in trace amount (Table 1). The three simple sugars found were fructose, glucose, and sucrose. Glucose has the highest concentration (0.18 to 0.22%) in all the cultivars, with no significant difference ($P \geq 0.05$) among them. Similar values have been reported in *T. catappa* seed [29]. Fructose has the lowest concentration (0.02 to 0.08%) for all the cultivars, though comparable to the fructose content found in pecan, macadamia, and almond cultivars [31]. The low sugar content of jackfruit seed is an indication of its potential as a snack for diabetic patients.

Fatty acid composition of jackfruit seeds: A total of eight fatty acids were found in JFS with variation in the three cultivars (Table 3). Jackfruit seed is composed of both saturated and unsaturated fatty acids, of which all the essential fatty acids were present. The major polyunsaturated fatty acid (PUFA) was linoleic acid (C18:2) with values ranging from 4.72 to 22.34% relative concentration. Linoleic acid has been recognized to favour oxidative modification of low density lipoprotein (LDL) cholesterol [32, 33], increase platelet response to aggregation [34], and suppress the immune system [35]. Value obtained for J29 cultivar in this study was within the range reported in pecan (23.68%) and almond nut (29.21%) [30]. Also, other PUFAs, such as linolenic (C18:3) and arachidonic (C20:4) were present in little amounts. Arachidonic acid (C20:4) is an essential

Table 3. Fatty acid composition and anti-nutritional factors in seeds of jackfruit cultivars.

Parameters	J29	J31	J33
Fatty acid (%)			
C16:0 Palmitic	1.90±0.46^{b}	6.59±0.90^{a}	1.01±0.12^{b}
C18:0 Stearic	0.58±0.13^{b}	1.44±0.30^{a}	0.42±0.22^{b}
C18:1 Oleic	0.58±0.35^{a}	1.18±0.93^{a}	0.47±0.25^{a}
C18:2 Linoleic	9.87±0.85^{b}	22.34±0.94^{a}	4.72±0.46^{c}
C18:3 Linolenic	0.59±0.04^{b}	1.50±0.45^{a}	0.54±0.05^{b}
C20:4 Arachidonic	0.37±0.01^{b}	0.71±0.24^{a}	-
C22:0 Behenic	0.93±0.04^{b}	1.92±0.47^{a}	0.60±0.06^{b}
C24:0 Tetracosanoic	-	0.92±0.16	-
Anti-nutrients			
Tannin (%)	0.02±0.10^{a}	0.04±0.01^{a}	0.01±0.00^{a}
Phytate (mg/100 g)	0.06±0.01^{b}	0.05±0.02^{b}	0.30±0.13^{a}
Trypsin inhibitor (mg/100 g)	1.40±0.00^{a}	1.16±0.01^{b}	1.14±0.00^{a}

Values are means and standard deviations of duplicate analyses; values followed by different superscript letters in a row are significantly ($p < 0.05$) different from each other. J29, J31, and J33 are JFS cultivars.

fatty acid, and a precursor for the synthesis of eicosapentaenoic acid, was found only in J29 and J31 cultivars. Oleic acid (C18:1) was the only monounsaturated fatty acid found in all the three cultivars. The values (0.47 to 1.18%) were low compared to those reported for some edible nut seeds [30]. Palmitic (C16:0), stearic (C18:0), behenic (C22:0), and tetracosanoic acids (C24:0) were the saturated fatty acids found in jackfruit seeds. However, they were all present in trace amount except for palmitic acid (C16:0) which was much higher (6.59%) in J31 cultivar, but in close range with Virginia peanut (6.20%) and almond (7.36%), but higher than hazelnut (5.78%) and pecan (5.90%) seeds [30]. This is the first time fatty acid composition of jackfruit seeds have been reported in literature.

Anti-nutritional factors: Anti-nutrients are chemical substances found in plants which hinder the utilization of some components by the body. The anti-nutritional factors present in JFS are presented in Table 3. Trypsin inhibitor (1.14 to 1.40) showed the highest level contained in all the cultivars. Tannin (0.01 to 0.04%) and phytate (0.05 to 0.30 g/100 g) were also found in all the varieties analysed, although in minute concentrations. The presence of anti-nutritional factors in jackfruit seed has been reported [36, 37]. Tannin values reported for edible seed nuts [30] are comparable with those obtained in this study. However, phytate contents are much lower than the values (1.5 to 3.5 mg/g) reported by the authors. The trypsin inhibitor value (26.61%) reported [38] for jackfruit seed was higher than the value obtained in this study.

Conclusions

Jackfruit seed is a good source of dietary nutrients such as protein, starch and dietary fibre. It is low in simple sugar and fat, which makes it a suitable healthy snack for overweight people. Jackfruit also contains both essential amino acids and fatty acids. However, the presence of anti-nutrients cannot be over looked: they were found in trace amounts. The nutritional health benefits of jackfruit seed can be exploited as an alternative snack product.

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