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Biochemical and nutritional characterization of coconut (*Cocos nucifera* L.) haustorium

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ABSTRACT

Study was conducted to determine the biochemical constituents in coconut (*Cocos nucifera* L.) haustorium, a spongy tissue formed during coconut germination. Results indicated that 100g of dried coconut haustorium contained 1.05 ± 0.2% ash, 44.2 ± 4.6% soluble sugar, 24.5 ± 3.2% starch, 5.50 ± 0.3% protein, 1.99 ± 0.9% fat, 5.72 ± 0.4% soluble dietary fibre, 20.3 ± 1.9% insoluble dietary fibre, and 146 ± 14.3 mg phenolics. Mineral profiling showed that it contained 145 ± 8.6, 104 ± 9.6, 33.9 ± 8.2, 30.9 ± 1.9, 9.45 ± 2.1, 0.292 ± 0.1, 2.53 ± 0.2 and 1.20 ± 0.1 mg of K, Mg, Ca, P, Mn, Cu, Fe and Zn, respectively. Antioxidant activity assay indicated that 100g haustorium was equivalent to 1918 ± 173, 170 ± 20.4, 72.8 ± 14.7 and 860 ± 116 mg of Trolox as measured by CUPRAC, FRAP, DPPH and ABTS, respectively. Amino acid score indicated that methionine + cysteine (57.6%), phenylalanine + tyrosine (32.6%), leucine (45.7%) and isoleucine (68%) are found less in haustorium. Further studies needed in developing nutritionally balanced formulations using coconut haustorium, which will be useful for lactose intolerant children.

1. Introduction

Coconut palm (*Cocos nucifera* L.) is one of the most important sources of vegetable oil and found throughout the tropics. It contains two distinct endosperms; one in a liquid form called nut water and the other solid form is kernel. The nutrients like carbohydrate, fat, proteins, fibre, minerals present in the matured kernel are utilized during the germination and early development of the embryo. During the process of germination, the embryo grows in two directions – the plumule or the shoot, grows towards the soft eye of the shell and the basal part of embryo develop into an absorbent spongy growth known as haustorium. Haustorium enlarges and fills the entire water cavity in 20–24 weeks after germination (Balachandran & Arumugam, 1995). Haustorium has two distinct portions, the outer oil-rich yellow portion and inner carbohydrate-rich white portion. This organ mobilizes the nutrients from coconut water and later from endosperm and nourishes the developing embryo. Thus, it is assumed that the nutrients present in the haustorium are in readily available form.

Scarcity of labour disrupts harvesting cycles and causes loss of income to the growers. As against the general harvesting cycle of 45–60 days, farmers are harvesting only once in three to four months. Due to the price crash and unavailability of labors, coconut nuts are not been harvested at suitable time and it get matured in the tree. The matured coconut after fallen from the tree starts germination during suitable condition. Considerable amount of harvested matured coconuts that are stored in the godown for oil extraction also starts germinate at certain stages. Some of the early germinating varieties starts germinate during storage time in the coastal conditions. The percentage of such germination varied between 2 to 3% of the total production. Presently, the haustorium formed inside the nut is underutilized, and the information about its nutritional potential is scanty, and limited to few components.

Earlier studies have shown that coconut haustorium composed of sugars, proteins, minerals, alkaloids, polyphenols and growth promoting substances. Balasubramaniam, Atukorala, Wijesundera, and De Silva (1973) found that total starch content of the haustorium increased linearly during maturation, whereas reducing and soluble sugar content increased rapidly and remained at a steady state thereafter. The excess carbohydrates mobilized from the kernel are stored in the haustorium as starch.

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Sugimuma and Murakami (1990) studied the process of haustorium development during germination, cytological and histochemical changes in the haustorium at different developmental stages. The study by Lopez-Villalobos, Doddas, and Hornung (2001) on changes in fatty acid composition during the development of haustorium found that the composition of medium and long chain fatty acids level increased in developing tissues. They further reported that the total fat composition of solid endosperm reduced during development. Though coconut haustorium consists of numerous health-promoting functional compounds and other nutrients, the systematic study on the complete nutritional composition of haustorium is lacking, the present study finds its relevance in the context of this pertinent research gap.

The study is carried on the evaluation of nutritional composition data for coconut haustorium. Specifically the study aimed to identify the biochemical, nutritional and functional components present in the coconut haustorium. The haustorium contained functional components like phenols, antioxidants, dietary fibres, and nutritional components like sugars, macro and micronutrients. The complete evaluation of nutritional profile of the coconut haustorium is prime important for efficient utilization of haustorium in dietary formulations and as a supplement. Thus, results generated from the study can be used as an integral part in nutrition campaigns to promote it as functional food or for use in food fortifications and formulations.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the study are of analytical grades. Gallic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine), ABTS (2,2-azino-di-(3-ethylbenzothialo zine-6-sulphonic acid) diammonium salt), potassium persulfate, and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma-Aldrich Co St. Louis, MO, United States of America. l(+)-ascorbic acid, 2, 6-dichlorophenol-indophenol, methanol, acetic acid (glacial), sodium acetate, hydrochloric acid (conc.), nitric acid, ferric chloride, ammonium acetate, copper (II) chloride, ethanol, Folin–Ciocalteu's phenol reagent, phenol, sodium hydroxide, sodium bicarbonate, potassium sodium tartrate, sodium sulphate, ammonium molybdate, disodium hydrogen arsenate, D-Glucose, sulphuric acid, AAS standards for sodium, potassium, copper, iron and zinc were purchased from Merck KGaA, Darmstadt, Germany. Dietary fibre kit (K-TDFR-100A/K-TDFR-200A 12/15) was purchased from Megazymes (Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland).

2.2. Sample collection and method of germination

Ten west coast tall variety of coconut (*Cocos nucifera* L.) trees of identical age (30 years) and yield characteristics were selected in the Farm area of ICAR-Central Plantation Crops Research Institute, Kerala, and their inflorescence were tagged. On maturity (12 months from flower opening) bunches were harvested and dried under shade for 30 days. Nuts of identical size and weight (931 ± 91.6 g) were placed in a horizontal plain, two-third of each nut was covered with soil, and moisture was maintained by periodical watering. Three sets (each comprises of three nuts each) of germinated nuts were randomly taken for the experiment at the stages of maturation of 60 days after germination (DAG). It was dehusked, broken carefully and haustorium was collected and weighed. The outer yellow portion was carefully peeled and removed. The spongy inner white portion was sliced and dried at 55 °C. Dried slices were ground using stainless steel grinder and sieved through USA Standard Test Sieve No.18 with American Society for Testing and Materials (ASTM) E11 specifications (Sieve size 1.0 mm). Homogenized samples were stored at −4 °C and used for experiments.

2.3. Measurement of proximate composition

The proximate composition of coconut haustorium was determined using the official methods (AOAC, 2005) viz. Moisture (AOAC 934.01), Ash (AOAC 938.08), Dietary Fibre (AOAC 985.29), Protein (AOAC 2001.11), Fat (AOAC 920.58).

2.4. Sample preparation for sugar, starch, phenolics and antioxidant assay

About 100 mg of dried and powdered coconut haustorium was extracted with 5 ml of 80% (v/v) ethanol by continuous shaking for 30 min in the dark at 60 °C. After centrifugation at 5000*g* for 15 min, the supernatant was collected. The residue was re-extracted twice and the supernatant was pooled and evaporated to dryness. The dried extracts were resuspended in 5 ml of water and stored at −20 °C until analysis. The extract was used to determine the total sugar, reducing sugar, polyphenols and antioxidant capacity using appropriate methods.

2.5. Determination of total soluble sugar, reducing sugar and starch content

Total soluble sugar content in the extract was determined using phenol–sulphuric acid method (DuBois et al., 1956) and reducing sugar content was determined using Nelson-Somogyi's method (Somogyi, 1952). The residue after extraction was used for starch estimation as per AOAC 996.11.

2.6. Fatty acid analysis by gas chromatography

Methyl esters of haustorium oil sample was prepared by following method. To about 0.2 g of haustorium oil taken in a 15 ml screw capped glass vial was mixed with HCl reagent (8.3 ml of acetyl chloride in 100 ml absolute methanol). The mixture was vortexed and incubated at 70 °C in hot air oven for 10 h and cooled to room temperature. Five milliliters of distilled water and one ml of hexane were added to the mixture and vortexed thoroughly. The hexane layer was separated out and used for further analysis. 1 μL of the hexane extract was injected into HP Innowax capillary column of 30 m length (inner diameter: 0.32 m, film thickness: 0.5 μm, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260 °C and 275 °C, respectively. Oven temperature was programmed from 150 °C holding at 1 min to 210 °C at the rate of 15 °C min^{-1}, followed by 210–250 °C at the rate of 5 °C min^{-1} for 12 min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards, run under similar separation conditions, peak integration was performed applying HP3398A software.

2.7. Amino acid profiling of coconut haustorium

Amino acids profiling was done using AccQ.Tag™ method using HPLC with the fluorescent detector (Waters 2475). About 20 mg of lyophilized powdered haustorium sample was hydrolyzed in a reaction vial containing 200 μL of 6N HCl and a crystal of phenol for 20–24hrs at 112–116 °C and derivatized with AccQ.Fluor

derivatizing reagent and AccQ.Fluor Borate Buffer. Derivatized samples were analyzed for amino acid profiling using HPLC with an autosampler (Waters 717 Autosampler) with gradient elution system containing two solvent systems viz. eluent A (10% solution of AccQ.Tagin acetate buffer pH 5.02) and eluent B (60% acetonitrile).

2.8. Mineral analysis

Mineral content was determined in accordance with the official analytical methods (AOAC, 2005) using a GBC Avanta PM atomic absorption spectrometer (AAS) (GBC scientific equipments, Australia) equipped with a D_2 lamp background correction system using an air-acetylene flame. Appropriately diluted acid digested samples were analyzed using AAS calibrated with related minerals in different concentrations for different macro (potassium, calcium and magnesium) and micro-minerals (manganese, copper, iron and zinc). The detection limits for minerals were 3 μg/100 g for K; 1.5 μg/100 g for Ca, 0.3 μg/100 g for Mg; 2 μg/100 g for Mn; 3 μg/100 g for Cu; 6 μg/100 g for Fe and 1 μg/100 g for Zn. Phosphorus present in the haustorium was determined by molybdovanadophosphoric acid method described by Kitson and Mellon (1944) using spectrophotometer. The detection limit for phosphorus estimated using spectrophotometer was 150 μg/100 g sample.

2.9. Determination of total phenolic content (TPC)

Determination of TPC in the extract was done using Folin-Ciocalteu (FC) assay as described by Singleton, Orthofer, and Lamuela-Raventos (1999) with slight modifications. Briefly, 100 μL of extract was added to 900 μL of distilled water and 200 μL of 1 N FC reagent and 2.0 mL of sodium carbonate (7%, w/v). Further, the contents were mixed and allowed to stand for 30 min at room temperature (25 ± 1 °C) at dark. The absorbance was measured at 750 nm using UV–vis spectrophotometer (LAMBDA 25 UV/Vis Systems, PerkinElmer, Inc. MA 02451, USA). Total phenol content was expressed as mg gallic acid equivalent (GAE) per 100 g haustorium.

2.10. Determination of antioxidant potential by FRAP, CUPRAC, ABTS and DPPH methods

Since, multiple reaction characteristics and mechanisms are usually involved; it is difficult to accurately measure the antioxidant potential of the mixed system using single assay. Thus, in this study we used four complementary methods viz. DPPH and ABTS radical scavenging activity; FRAP, and CUPRAC reducing power—based on the single electron transfer mechanism to evaluate the AOA due to their simplicity, stability and accuracy. The FRAP (ferric reducing antioxidant power) assay was done according to Benzie and Strain (1996) with some modifications using 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ solution. Cupric ion reducing antioxidant capacity (CUPRAC) of the extract was determined according to the method of Apak, Guclu, Ozyurek, and Karademir (2004) using $CuCl_2$ solution (10 mM L^{-1}), neocuproine alcoholic solution (7.5 mM L^{-1}), and ammonium acetate (1 M L^{-1}, pH 7.0) buffer solution. In both the methods, Trolox was used as a positive control and results were expressed in mg TE (Trolox equivalent)/100 g dry weight. The $ABTS^{\cdot+}$ radical scavenging activity was done following the procedure or Arnao, Cano, and Acosta (2001) with some modifications. In this method, pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS∗+) is generated by oxidation of ABTS in the presence of potassium per sulfate and is reduced in the presence of such hydrogen-donating antioxidants. About 7.4 mM $ABTS^{\cdot+}$ and 2.6 mM potassium persulfate were used in the assay. Appropriately diluted extracts were allowed to react with $ABTS^{\cdot+}$ solution for 2 h in a dark condition. The absorbance was read at 734 nm using a spectrophotometer. The 2,2-diphenylpicrylhydrazyl (DPPH) assay was done according to the method of Brand-Williams, Cuvelier, and Berset (1995). DPPH radical become stable diamagnetic molecule while accepting electrons, which leads the colour change from purple to yellow. Haustorium extract with different concentrations were allowed to react with DPPH solution for 30 min in dark condition and the absorbance was read at 517 nm.

The $ABTS^{\cdot+}$ and DPPH radical scavenging activity (*S%*) was calculated using the following equation:

$$S\% = ((Acontrol - Asample)/Acontrol) \times 100$$

where,

*A*control is the absorbance of the blank control (containing all reagents except the extract) and *A*sample is the absorbance of the test sample. A graph was plotted with the concentration along the *x*-axis and *S%* along the *y*-axis, and IC_{50} value was calculated. IC_{50} value signifies the concentration of tested samples to scavenge 50% of the $ABTS^{\cdot+}$ and DPPH radical. IC_{50} values were expressed in mg TE (Trolox equivalent)/ 100 g coconut haustorium. All experimental results were expressed as mean ± standard deviations.

2.11. Quality control

AS-FRM (Food Reference Material) 14 (rice-2) obtained from Institute of Nutrition, Mahidol University, Thailand was used as reference material to evaluate the analytical methods for moisture, ash, protein, iron and zinc (Courtesy Prapasri Puwastien and Kunchit Judprasong, Institute of Nutrition, Mahidol University, Thailand). Recovery of other minerals was evaluated by comparing the spiked and not spiked haustorium samples with respective mineral standards. The certified value of FRM for moisture, protein, ash, iron and zinc are 11.12 ± 0.53 g/100 g, 8.03 ± 0.22 g/100 g, 1.44 ± 0.03 g/100 g, 1.29 ± 0.17 mg/100 g and 2.22 ± 0.21 mg/100 g, respectively. The values of FRM obtained in the present study for proximates viz. moisture, protein and ash were 10.96 ± 0.81, 7.89 ± 0.42 and 1.39 ± 0.09, respectively. The value of FRM for iron and zinc obtained in the present study was 1.27 ± 0.19 and 2.19 ± 0.20 mg/100 g, respectively. The recovery percent for minerals studied was within the acceptable range (Ca 99.2 ± 1.29%, K 101.0 ± 2.6%, Mg 99.1 ± 1.4%, P 98.2 ± 2.19%, Mn 98.5 ± 2.01, Fe 98.7 ± 1.9%, Cu 99.2 ± 1.1% and Zn 98.9 ± 0.9%).

3. Results and discussions

3.1. Coconut haustorium yield data

Biochemical composition of coconut haustorium was studied in detail to develop a nutritional composition data. Onset of germination, the basal part of the embryo grows into a spongy absorbing tissue called haustorium (Fig. 1.). Since the distance between nut surface and shell surface and the husk thickness varies is highly variable, it was difficult to ascertain the amount of haustorium present inside the nut by measuring the shoot length. The present study revealed that sixty days after the germination (DAG), haustorium occupied the entire space of the coconut. Primary haustorium yield data (Table 1) showed that 60 DAG the shoot length of the germinated nut varied from 27.6–60.5 cm from the surface of the nut, with the mean value of 43.1 ± 12.8 cm. The amount of haustorium harvested in the germinated nut (60 DAG) varied from 80.2 g to 131 g with the mean value of 107 ± 13.5 g nut^{-1}. At this developmental stage, most of the nut water was replaced by

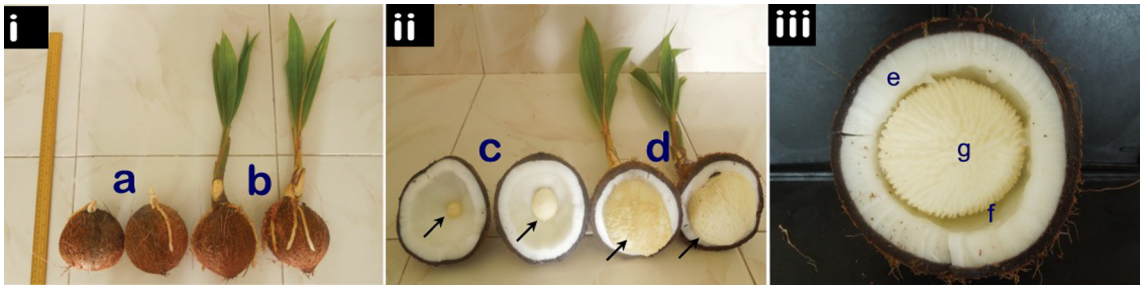

Fig. 1. Different developmental stages of Coconut (*Cocos nucifera* L.) haustorium. i. dehusked germinated coconut (i.a. nuts with shoot emergence i.b. nuts of 60 days after germination); ii. Cut opened germinated nuts (ii.cNuts with shoot emergence have haustorium of small size [indicated by arrow mark], ii.d. Nuts of 60 days after germination having haustorium fully occupied in the entire nut cavity [indicated by arrow mark]); iii. Coconut with matured haustorium [iii.e. coconut kernel iii.f. coconut cavity, iii.g. coconut haustorium, a spongy digestive organ].

Table 1
Coconut haustorium yield data.

Parameters	Mean ± SD[a]	Range[b]
Initial nut weight (g)	931 ± 91.6	818–1135
Shoot length (cm)	43.1 ± 12.8	27.6–60.5
Total haustorium wt (g/nut)	107 ± 13.5	80.2–131
Haustorium yellow portion wt. (g/nut)	35.1 ± 6.3	26.9–45.8
Haustorium white portion wt. (g/nut)	71.5 ± 8.3	53.2–84.7
Ratio (white/yellow)	2.09 ± 0.3	1.72–2.41

[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.

haustorium, this is an ideal stage to harvest the haustorium for edible purpose (Pictorial depiction of matured coconut halve with matured haustorium is given as Supplementary Material 1.). However, at this stage the outer surface of the haustorium is fully covered with an oily colloidal inner endosperm termed here as 'mucilage'. The amount of oily outer yellow portion ranged from 26.9–45.8 g nut^{-1}, and most of its weight is contributed by oily mucilage, which contained free oil. This oily portion is removed which is prone to rancidity as it contains hydrolyzed oil and free fatty acids. Edible portion i.e. the inner white portion of haustorium is prone to spoilage hence, dried for increasing its shelf life. The amount of edible portion of the haustorium i.e. the inner white portion was ranged from 53.2–84.7 g with the mean average of 71.5 ± 8.3 g nut^{-1}. The ratio between the inner white edible and outer oily yellow portion was about 2.09.

3.2. Proximate composition and amino acid profiling

The haustorium white portion was used for the nutritional composition and proximate analysis; the data is given in Table 2. Except moisture, other parameters are given as on dry weight basis. The haustorium consists of 86.9–89.3% moisture, and about 1.05% ash. The total fat content was very less and ranged from 1.01–3.18% with an average of 1.99%. The total protein content ranged between 4.95 to 6.25% with the mean value of 5.50 ± 0.3%. The available carbohydrate content in the haustorium varied from 59.2–75.6% with the mean value of 67.1 ± 4.8%. Haustorium contained considerable amount of both soluble and insoluble fibre which ranges between 4.96 to 6.20% and 17.1–23.2%, respectively. Matured coconut endosperm (otherwise kernel, meat) contained about 46.99% moisture, and on dry weight basis it contained 6.28% protein, 63.2% total fat, 28.7% carbohydrate, 16.9% dietary fibre and 11.7% sugars (USDA, 2016). Compared to matured coconut endosperm, haustorium contained very less total fat (1.99% against 63.2% in matured coconut kernel), high amount of available

Table 2
Proximate composition of coconut haustorium.*

Parameters	Mean ± SD[a]	Range[b]
Moisture	88.4 ± 0.7	86.9–89.3
Ash	1.05 ± 0.2	0.891–1.52
Protein	5.50 ± 0.3	4.95–6.25
Fat	1.99 ± 0.9	1.01–3.18
Available Carbohydrates**	67.1 ± 4.8	59.2–75.6
Total soluble sugar	44.2 ± 4.6	38.7–53.4
Total reducing sugar	27.5 ± 1.5	24.9–29.7
Starch	24.5 ± 3.2	19.2–28.6
Dietary Fibre		
Soluble	5.72 ± 0.4	4.96–6.20
Insoluble	20.3 ± 1.9	17.1–23.2

[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.
* except moisture other values are in dry weight basis of edible portion.
** monosaccharide equivalent.

carbohydrates (67.1% against 28.7%), total soluble sugar (44.2% against 11.7%) and dietary fibre (26.0% against 16.9%). The fatty acid profile (in%) of coconut haustorium is shown in Table 3. Among the fatty acids, lauric acid was found maximum (43.2 ± 2.5), followed by myristic acid (18.8 ± 0.7) and palmytic acid (12.6 ± 1.0). Among the long chain fatty acids, oleic acid was high (9.42 ± 2.0) followed by stearic acid (5.54 ± 0.4) and linoleic acid (1.68 ± 0.5).

The amino acid profile and essential amino acid score for coconut haustorium is presented in Table 4. The potential food value of the haustorium protein content (as a source of amino acids) is justified by comparing with the FAO reference pattern (FAO/WHO, 1990). Amino acid profile of haustorium showed that aspartic acid

Table 3
Fatty acid composition of coconut haustorium (in%).

Fatty acid	Mean ± SD[a]	Range[b]
Caproic acid	0.39 ± 0.06	0.28–0.46
Caprylic acid (8:0)	3.71 ± 0.3	3.35–4.01
Capric acid (10:0)	4.55 ± 0.1	4.44–4.62
Lauric acid (12:0)	43.2 ± 2.5	40.3–44.7
Myristic acid (14:0)	18.8 ± 0.7	18.1–19.3
Palmitic acid (16:0)	12.6 ± 1.0	11.7–13.7
Stearic acid (18:0)	5.54 ± 0.4	5.18–5.96
Oleic acid (18:1)	9.42 ± 2.0	8.21–11.8
Linoleic acid (18:2)	1.68 ± 0.5	1.40–2.21

[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.

Table 4
Amino acid composition and essential amino acid score of coconut haustorium compared to the essential amino acid reference value suggested by FAO/WHO (mg/g protein).

Amino acid	Mean ± SD[a]	Range[b]	Per cent	FAO/WHO Reference	Essential Amino acid score
Essential					
Cystine	9.00 ± 1.6	6.6–10.2	0.88	25	57.6
Methionine	5.40 ± 0.8	4.4–6.4	0.53		
Valine	56.1 ± 5.2	50.7–63.3	5.50	35	160
Iso leucine	19 ± 3.9	16.1–24.8	1.86	28	68.0
Leucine	30.2 ± 6.7	26.4–40.3	2.96	66	45.7
Tyrosine	12.2 ± 6.9	1.9–16.6	1.20	63	32.5
Phenylalanine	13.5 ± 4.8	10.1–20.6	1.32		
Histidine	32.4 ± 2.5	29.2–35.2	3.18	19	170
Lysine	78.2 ± 14.2	66.1–96.6	7.67	58	135
Threonine	33.4 ± 3.6	29.4–37.6	3.28	34	98.4
Non essential					
Aspartic acid	305 ± 10.4	278–319	29.87		
Proline	95.0 ± 12.1	76.1–115	9.32		
Serine	33.9 ± 5.1	26.5–37.8	3.32		
Glutamic acid	64.7 ± 12.3	45.2–78.7	6.35		
Glycine	25.9 ± 5.1	21.2 = 30.9	2.54		
Alanine	117 ± 11.5	98–118	11.43		
Arginine	45.7 ± 2.3	40.2–47.8	4.48		

[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.

was the major amino acid (29.9%), followed by alanine (11.4%) proline (9.32%) and glutamic acid (6.35%). Essential amino acid scores indicated that histidine, valine and lysine were present in high level as compared to FAO/WHO reference values and threonine was on par with the reference value. Haustorium is highly deficient in aromatic amino acid with chemical score of 32.5%, followed by leucine (45.7%) followed by the sulphur containing amino acids (57.6%) and isoleucine (68%).This data suggests that efforts on developing suitable formulation from dried coconut haustorium using other crop groups such as pseudo-cereals and millets are needed to develop nutritionally balanced food. However the high amount of aspartate and glutamate (36%) may be attributed to its flavor enhancing properties.

The carbohydrate content in the haustorium varied from 59.2 g to 75.6 g with the mean value of 67.1 ± 4.8 g 100 g^{-1}. The study showed that about 66% of the available carbohydrates are simple soluble sugars, out of which 64% of the sugars are glucose and fructose. The starch content varied between 19.2 g to 28.6 g100 g^{-1}. Balasubramaniam et al. (1973) stated that the concentration of total soluble sugars and reducing sugars of the haustorium increased steadily till haustorium attain 10 g fresh weight, and these constituents remain constant thereafter suggested that steady state of sugar composition is maintained. These sugars are used as energy source to the growing embryo and to synthesize various structural components. Studies showed that coconut haustorium contained only sucrose, glucose, fructose and starch as carbohydrate entities (Sugimuma & Murakami, 1990). It is estimated that 65%–75% of people worldwide have low lactase levels (Vesa, Marteau, & Korpela, 2000), which may lead to lactose intolerance and difficulty digesting dairy products. Thus, it is essential to identify the food with less or nil lactose content for lactose intolerant child or adults. The coconut haustorium contained available carbohydrates of about 67.1 g/100 g dw of which about 66% is simple soluble sugar. The haustorium could be used as a source of hydrolyzed sugars along with other nutrients for developing food supplements for lactose intolerance children.

3.3. Mineral composition in coconut haustorium

The amount of macro and micro-minerals present in the 100 g coconut haustorium (dry weight basis) and their contribution in Recommended Dietary Allowances (RDA) for children (NIN, 2009) are given in the Table 5. Among the macro-minerals studied, potassium content varied between 131 to 160 mg with the mean value of 145 ± 8.6 mg. The magnesium content ranged from 89.0–122 mg with the mean value of 104 ± 9.6 mg. Calcium and phosphorus contents ranged between 23.6–46.2 and 27.1–33.4, with the mean value of 33.9 ± 8.2 and 30.9 ± 1.9 mg, respectively. Among the micro-minerals studied, manganese found high with the mean value of 9.45 ± 2.1 mg followed by iron (2.53 ± 0.2 mg), zinc (1.21 ± 0.1 mg) and copper (0.292 ± 0.1 mg). The body requires small amount for a various minerals for normal functions. These include the formation of bones and teeth; as essential constituents of body fluids and tissues; as components of enzyme cofactors and for normal nerve function. The present study revealed that coconut haustorium is rich in various minerals, which are essential for normal function of the cell. The intake of 100 g of haustorium could contribute about 20.89 to 25.48% of RDA of the potassium, 197.78–272.78% of RDA of the magnesium, 4.71 to 9.24% calcium, 4.52 to 5.56% of phosphorus, 127–264% of manganese, 43.4–56.6% iron, 20.73 to 25.45% of zinc and 9.63 to 34.07% copper. Thus, coconut haustorium is rich source of magnesium, manganese and especially iron, and it can certainly be a high potential diet in many developing countries, where the average diet is deficient in iron. Since calcium and phosphorus content is low, fortification of haustorium with calcium and phosphorous is essential for to transform it into a nutritionally balanced food.

Table 5
Mineral constituents in coconut haustorium.*

Minerals*	Mean ± SD[a]	Range[b]	% Contribution in RDA**
Calcium	33.9 ± 8.2	23.6–46.2	4.71–9.24
Potassium	145 ± 8.6	131–160	20.9–25.5
Magnesium	104 ± 9.6	89.0–122	198–273
Phosphorus	30.9 ± 1.9	27.1–33.4	4.52–5.57
Manganese	9.45 ± 2.1	6.35–13.2	127–264
Iron	2.53 ± 0.2	2.17–2.83	43.4–56.6
Copper	0.292 ± 0.1	0.132–0.464	9.63–34.1
Zinc	1.20 ± 0.1	1.14–1.40	20.7–25.5

[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.
* Values are in mg/100 g haustorium on dry weight basis of edible portion.
** For children (NIN, 2009).

3.4. Phenolic content and antioxidant potential of coconut haustorium

Total phenolic content (TPC) and antioxidant potential of coconut haustorium are given in Table 6. The TPC in the coconut haustorium was estimated using Folin–Ciocalteu's phenol reagent employing the reduction of a phosphowolframate–phosphomolyb date complex to blue products by phenolic compounds. The TPC ranged from 123 mg to 166 mg with a mean value of 146 ± 14.3 mg 100 g^{-1} of haustorium. Free radicals generated during the normal metabolic process of aerobic cells are highly reactive and unstable molecules that likely to react with food lipids, nucleic acids, sugars and sterols (Lee, Koo, & Min, 2004) that lead to numerous physiological alterations. Though the living system contains the array of compounds to balance the negative effect of free radicals, it is essential to provide some of them through diet for keeping the free radical concentration at a lower side. Consumption of natural food materials that are rich sources of antioxidants is an ideal way to enhance the level of antioxidant system in the body. Present study revealed that the coconut haustorium contained about 146 mg TPC, which will provide good supplement of antioxidants.

A wide array of compounds present in the plant edibles, fruits and vegetables possess antioxidant activity (AOA). The study revealed that the antioxidant reducing potency measured by CUPRAC method gave more AOA value in terms of trolox equivalent (TE) for 100 g haustorium, as compared to other methods studied. The DPPH radical scavenging activity (IC_{50}) ranged between 52.5–93.3 mg with the mean value of 72.8 ± 14.7 mg TE 100 g^{-1}, and ABTS radical scavenging activity ranged from 713 mg to 1072 mg TE 100 g^{-1} of coconut haustorium. From the present study, demonstrated that haustorium have more ABTS radical scavenging activity as compared to DPPH. ABTS method offers a number of advantages over the DPPH assay. The ABTS method can be used in a wide pH range whereas DPPH method is limited to higher pH applications. In addition, since ABTS is soluble in aqueous and organic solvents, it can detect both hydrophilic and lipophilic antioxidants in the system (Cano, Hernandez-Ruiz, Garcia-Canovas, Acosta, & Arnao, 1998).

The CUPRAC and FRAP reducing power values ranged between 1701 to 2303 mg TE, and 143–205 mg TE 100 $g^{-1}$of haustorium, respectively. In CUPRAC method, the redox reaction was carried out at a pH 7 as opposed to the acidic conditions (pH 3.6) of FRAP or basic conditions (pH 10). At more acidic conditions than the physiological pH, the reducing capacity may be suppressed due to protonation on antioxidant compounds, whereas in more basic conditions, proton dissociation of phenolics would enhance a sample's reducing capacity. In the earlier studies, it was found that the CUPRAC reagent is fast enough to oxidize thiol-type antioxidants, whereas according to the protocol developed by Benzie and Strain (1996), the FRAP method does not measure certain thiol-type antioxidants like glutathione. The reason for this might be the half-filled d-orbitals of highspin Fe (III) attributing it a chemical inertness, while the electronic structure of Cu (II) enables fast kinetics (Apak et al., 2004). Since, the FARP assay was performed under acidic condition, most of the antioxidant activity is suppressed due to protonation, which leads to lesser reducing power when compared to CUPRAC assay. At the same time, FRAP able to detect antioxidant potential of the compounds that are reactive at acidic pH. These compounds also play a crucial role in free radical scavenging activity in the human gastrointestinal tract, which is commonly exposed to substances capable of inducing oxidative stress, such as foods (mainly meats) that contain large amounts of lipids, hydroperoxides, free metals, and myoglobin (Kanner, Gorelik, Roman, & Kohen, 2012). Under gastric condition, phenolic compounds have been shown to inhibit lipid peroxidation (Lapidot, Granit, & Kanner, 2005), an effect associated with their reaction with peroxyl radicals. Nonetheless, the low stomach pH could influence the ability of phenolic compounds to neutralize peroxyl radicals via hydrogen donation. Chikku and Rajamohan (2012) reported the cardioprotective potency of coconut haustorium on isoproterenol induced myocardial infarction in rats is mainly due to increased antioxidant status which in turn reduced oxidative stress. In present study, it is demonstrated that that haustorium contained considerable amount of antioxidant compounds, which are active even under acidic conditions.

Table 6
Phenolic content and antioxidant potential of coconut haustorium.*

Parameters	Mean ± SD[a]	Range[b]
Total phenolics content (mg GAE)	146 ± 14.3	123–166
Radical scavenging activity (mg TE IC_{50})		
DPPH	72.8 ± 14.7	52.5–93.3
ABTS	860 ± 116	713–1072
Reducing power (mg TE)		
CUPRAC	1918 ± 173	1701–2303
FRAP	170 ± 20.4	143–205

GAE – Gallic acid equivalent
TE – Trolox equivalent
[a] Results are represented as mean ± standard deviation (n = 9) of three independent experiments.
[b] Range represents the lowest and highest observed average values in across the sample.
* Values are in mg/ 100 g haustorium on dry weight basis of edible portion.

4. Conclusions

The present study revealed that the nutritional composition of coconut haustorium, which is rich in nutrients. It comprises of about 66% carbohydrates, with approximately 64% is soluble sugars. It contained a considerable amount of dietary fibre, polyphenols, minerals and have high antioxidant potential. Since it has high carbohydrate content as a glucose, fructose and sucrose, it could be used in baby food formulations for lactose intolerance. Since calcium and essential amino acids are found low, fortification of haustorium with calcium and essential amino acids is essential for to transform it into a nutritionally balanced food. Developing nutritionally balanced food formulations, of late, assumes paramount importance, especially in the context of developing countries. The present study highlights the potential of haustorium as an invaluable and comparatively cheaper food component especially in the developing counties where the livelihood/food security has become an issue invoking grave concerns.

Conflict of interest

Authors do not have any conflict of interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.10.127.

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