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Change in Phytochemicals and Antioxidant Activity of Silk, Husk and Cob of Baby Corn (*Zea mays* L.) during Four Phenological Stages

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Baby corn (*Zea mays* L.) is an emerging vegetable crop having a rich profile of functional elements and its widespread use in the food sector for its delicious taste and health benefiting properties. The present investigation analyzed three parts of baby corn, *viz.* silk, cob and husk, at four developmental/maturity stages on the basis of cob size: stage-1 (5.0–7.0 cm; S1), stage-2 (7.1–9.0; S2), stage-3 (9.1–11.0 cm; S3) and stage-4 (11.1–13.0 cm; S4). Xanthophylls and total carotenoid content showed significant ($p<0.05$) difference whereas, increase in β-carotene and chlorophyll was observed from S1 to S3. The phenolic content increased in silk (upto S3) whereas decreasing trend was observed in corn husk extract. Flavonoid content increased by 70.92, 219.8, and 104.2% in silk, 95.4, 241.4 and 290.24% in husk, whereas cob showed reduction of 26.3, 30.0, 40.0% from S2 to S4 over S1, respectively. All extracts showed strong radical scavenging activity, β-carotene linoleate and SOD activity. The study highlights functional attributes of baby corn cob for human consumption *viz a viz* husk portion for industrial use in functional food items.

Keywords: Baby corn, Phenolic content, DPPH activity, β-carotene, Principal component analysis

Introduction

In the last few decades, baby corn (*Zea mays* L.) has gained a significant place in the food industry. It is a high value vegetable, and the cob is mostly used for culinary preparations and soups in different food cultures, while the use of silk and husk is rare. Corn silk has medicinal uses for the treatment of hypertension, tumors, hyperglycemia, hepatitis, cystitis, diabetes, gout, prostatitis and fatigue.[1] The cob (100 g dry weight basis) is rich in crude protein (10.0 g), crude fibre (2.4 g), ash (1.3%), calcium (17.7 mg), phosphorus (197.9 mg) and iron (2.7 mg)[2], while corn silk (100 g dry weight) is also rich in protein (17.6%), ash (3.91%) and dietary fibres (40%).[3] Significant variation ($P<0.05$) among phyto-constituents (phenolics, flavonoids and anthocyanin content) was reported by Sarepoua *et al.*[4] This short growth duration and high yield potential of this high-price crop offer excellent options for economic upliftment and nutritional security.

Regular intake of foods rich in phytoconstituents having antioxidant activity reduces the risk of cancer, cataracts, cardiovascular, neurodegenerative diseases and non-communicable respiratory stresses[5] and micronutrient deficiencies associated complex diseases. Hu and Deng[1] reported that flavonoid extract from corn silk could improve exhaustive exercise activity of mice through enhanced secretion of anti-oxidant enzyme level through inhibition of lipid peroxidation activity by inhibiting lipid peroxidation. Similarly, many of the phyto-constituents showed strong correlation with free radical scavenging capacity during *in vitro* investigations.[6] Baby corn is a part of regular diet as mixed vegetables, boiled foods, soups, sweets and powder[2], but harvesting stage or size of the cob varies with place, market and purpose, which affects the phytochemical constituents. However, husk and corn silks are rarely used parts and constitute the waste products, with very little information reported on husks.

Corn silk can be used as natural color and flavoring food additives in value added products, such as corn

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silk tea, snacks, cosmetics and medicines.[7] So, the information on the compositions of corn silk, cob and husk are important to understand their health benefits. Over the past few years, there has been an increasing interest in the study of the phytochemicals and antioxidant compounds in kernel and silk in corn at immature and mature stages, colored (yellow, purple and pink, etc.) with varietal genotypes (waxy and normal).[5,7–17] Being situated in sub-tropics, plants are subjected to high levels of biotic and abiotic stress, and to mitigate this unfavorable climate, they synthesize high levels of phenols as secondary metabolites to defend in the form of different antioxidant potential. However, till now, no basic information regarding the phenolic content and antioxidant components from commercially available hybrid cultivars in the Island climate of Andaman, India has been reported.

Baby corn represents unfertilized immature cobs of corn preferably harvested within 1–3 days of silk emergence. It has been graded as short (4–7 cm), medium (7–11 cm), and long (11–13 cm); of them, short and medium sizes are common in the international market, while long size cobs are common in local markets.[2] The change in the size of baby corn at advanced stages of maturity affects the phytochemicals and antioxidant activity. However, there were limited reports on progressive changes in phyto-constituents during development stages in three useful parts, *i.e.* cob, silk and husk. Different antioxidant compounds may act *in vivo* through different mechanisms; no single method can fully evaluate the total antioxidant capacity of foods. For this reason, several complementary test systems, namely DPPH, ABTS, FRAP, SOD and β–carotene-linoleic acid activity, have to be used to reveal the antioxidant potential in plant parts. Therefore, the present study was undertaken to determine the phytochemicals and antioxidant activities in all three parts of baby corn for its commercial utilization in the food processing industry.

Material and Methods

Plant Materials and Sample Preparations

Three parts of baby corn (Hybrid HM-4) namely silk, cob, and husks, were collected at four different maturity stages (based on the size of husked baby corn) from disease-free healthy plants grown at 60 × 20 cm^2 distance with the recommended package of practices in the vegetable experimental block at Garacharma Farm, ICAR-CIARI, Port Blair, India. Fresh cobs were harvested from five random plants for morphological observations. Baby corn, in study, represents the cob without silk and husk portion, and corn silk is the stigma and style of the maize plant, while the husk is an outer leafy cover on the cob. Phytochemical analysis was performed in triplicate samples. The marketable size of baby corn is 4 to 13 cm in length and 1.0 to 1.5 cm in diameter.[2] Hence, in the present study, the baby corn graded as stage-1 (S1) represents (dehusked) baby corn of 5.0–7.0 cm; stage-2 (S2) for 7.1–9.0 cm; stage-3 (S3) for 9.1–11.0 cm and stage-4 (S4) for 11.1–13.0 cm size to reveal a comprehensible understanding on the change in phyto-constituents.

Morphological Observations

The fresh weight of cobs and dissected parts (cob, silk and husk) was taken in triplicate using an electronic weighing machine. The cobs were dissected under dim light at room temperature into individual parts, *i.e.* husk (outer and inner), cob and silk and their length and weight were taken using Vernier calliper (Brand: Mitutoyo, Analog type) and digital weighing balance (Make: Labwan, Accuracy: 0.1%, Resolution: 0.01 mg).

Total Phenolics Content (TPC)

The Folin-Ciocalteu colorimetric method was used to measure the total phenolic content[18] with minor modifications. Briefly, 200 µL of the extractions were oxidized with 1 mL of 0.5 N Folin-Ciocalteu reagent, and then the reaction was neutralized with 1 mL of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a UV–2600 spectrophotometer (Shimadzu, Japan) after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalent (mg GAE) per 100 g of flour weight.

Flavonoid Content

Total flavonoid content was determined by a colorimetric method.[19] Approximately 0.5 mL extracts were added to 15 mL polypropylene conical tubes containing 2 mL ddH$_2$O and mixed with 0.15 mL 5% NaNO$_2$. After reacting for 5 min, 0.15 mL of 10% AlCl$_3$.6H$_2$O solution was added to it. After another 5 min, 1 mL of 1 M NaOH was added to the mixture. The reaction solution was well mixed, kept for 15 min, and the absorbance was determined at 415 nm. Qualification was done using Rutin as standard and the results was expressed as milligrams of Rutin Equivalent (mg RE) per 100 g of flour weight.

Determination of Total Carotenoids, β-carotene, Chlorophyll and Xanthophyll

Total carotenoid was determined by the method described by Sadasivam and Manikam[20] with minor modifications. The fresh sample (2 g) was ground well in 10 mL of acetone and centrifuged at 5000 rpm for 8 min in a cooling centrifuge at 4°C. Supernatant was repeatedly ground and filtered till devoid of the color. The solvent was removed by rotary evaporator. The extract was transferred to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The volume was made up by petroleum ether, and the absorbance was read at 450 nm. Total carotenoids were calculated as follows and expressed as milligrams per g of dry weight (DW).

$$\text{Total carotenoids} = \frac{A \times V \times 10^6}{A^{\%} \times W}$$

where, A is the absorbance at 450 nm, V is the total volume of extract (ml), $A^{\%}$ is the extinction coefficient (2592 cm for petroleum ether) and W is sample weight (g).

β-carotene, xanthophyll and chlorophylls a and b (µg/g) were determined spectrophotometrically at 470, 645, and 662 nm, respectively. Their contents estimated using the equations described by Lichtenthaler and Buschmann[21] as follows:

$$\mathrm{Ch_a} = 11.75\mathrm{A}_{662} - 2.35\mathrm{A}_{645}$$

$$\mathrm{Ch_b} = 18.61\mathrm{A}_{645} - 3.960\mathrm{A}_{662}$$

$$\beta\text{-carotene} = 1000\mathrm{A}_{470} - 2.270\,\mathrm{Ch_a} - 81.4\left(\frac{\mathrm{Ch_b}}{227}\right)$$

where, $\mathrm{Ch_a}$ = Chlorophyll a, and $\mathrm{Ch_b}$ = Chlorophyll b. Xanthophyll determined by subtracting β-carotene fraction from total carotenoids.

DPPH Radical Scavenging Activity

Total antioxidant activity was obtained by 2,2-diphenyl-2-picrylhydrazyl (DPPH) method[22] with some modification. The working solution of DPPH was freshly prepared by diluting 3.9 mg of DPPH with 95% ethanol to get an absorbance of 0.856 ± 0.05 at 517 nm. The different concentrations of extract were mixed with 1.5 mL of working DPPH, and the absorbance of the mixture was immediately measured spectrophotometrically after 10 min. The total antioxidant activity of the extract was expressed as mg BHA/g sample equivalent, obtained from the calibration curve.

$$\%\ \text{inhibition of DPPH radical} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where, A_{control} is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the extract/standard.

ABTS Radical Scavenging Activity

The total antioxidant capacity was determined by a colorimetric method[23] with a little modification. The ABTS radical cation was generated by oxidation of 7 mM ABTS with $K_2S_2O_8$ (2.45 mM) in 10 mL of deionized water and kept in darkness at 4°C for 16 h. Once the radicalisation was obtained, the ABTS^+ solution was diluted with 80% ethanol to an absorbance of 0.784 ± 0.01 at 734 nm. Then, 3.9 mL ABTS^+ cation solution was added to 1 mL of extracts and mixed thoroughly. The mixture was incubated for 6 min at room temperature, and the absorbance was tested at 734 nm. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mM Trolox equivalents per 100 g dry weight).

$$\%\ \text{inhibition of ABTS radical} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Ferric Reducing-Antioxidant Power (FRAP) Assay

FRAP assay is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4 diene chloride (TPTZ) to the ferrous form at low pH.[24] Briefly, 0.9 mL of prepared FRAP reagent was mixed with 0.1 mL of diluted sample and the absorbance at 595 nm was recorded after a 15 min incubation at 37°C, and the results were expressed in mM of Fe^{2+} equivalents per g dry weight.

$$\%\ \text{inhibition of ABTS radical} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Super Oxide Dismutage (SOD) Activity

The influence of extracts from baby corn parts on the generation of superoxide anion was measured according to the method described in previous work.[25] Superoxide anion was generated in a non- enzymatic system and determined by a spectrophotometric measurement for the reduction of nitroblue tetrazolium. The reaction mixture, which contained 1 mL of extract in distilled water, 1 mL of PMS (60 µM) in phosphate buffer (0.1 M, pH 7.4), 1 mL of NADH (468 µm) in phosphate buffer, 1 mL of NBT

(150 µm) in phosphate buffer, was incubated at ambient temperature for 5 min and the color was read at 560 nm against blank samples. All analyses were run in triplicate, and mean values were calculated.

$$\%\ \text{inhibition of SOD radical} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

β-carotene-Linoleate Model System

The antioxidant activity of the baby corn extracts was evaluated using β-carotene-linoleate model system as described by Jayaprakasha *et al.*[26] with some modifications. Exactly, 0.1 mg of β-carotene in 0.2 mL of chloroform, 10 mg of linoleic acid and 100 mg of Tween–20 (polyoxyethylene sorbitan monopalitate) were mixed. Chloroform was removed at 40°C under vacuum, and the resulting mixture was diluted with 10 mL of distilled water and mixed well. To this emulsion, 2.0 mL of oxygenated water was added. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 mL of extracts and BHT (50, 100, 150, 200 and 250 µg) in methanol. BHT was used for comparative purposes. A control containing 0.2 mL of methanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50°C in a water bath, and the absorbance at 470 nm was taken at zero time (t = 0). Measurement of absorbance was continued till the color of β-carotene disappeared in the control tubes (t = 180 min) at an interval of 60 min. Antioxidant activity was calculated as the percentage of inhibition (%) relative to the control using the following equation.

$$\%\ \text{inhibition} = \frac{DR_c - DR_S}{DR_c}$$

$$DR_c = \frac{\ln\left(\frac{a}{b}\right)}{t}$$

$$DR_s = \frac{\ln\left(\frac{a}{b}\right)}{t}$$

where, DR_C – degradation rate of β-carotene in the control sample, DR_S – degradation rate of β-carotene in the sample with antioxidant, a = absorbance at time zero, b = absorbance at a defined time t.

Statistical Analysis

The variability of phytochemicals in all stages of three parts of baby corn was done by Principal component analysis (PCA) using SAS (Software Version 9.3. SAS Institute Inc., Carry, NC). Means and its comparison (ANOVA) were calculated using SPSS (Version 17.0, SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

Morphological Observations

Morphological changes in baby corn parts during different developmental stages are presented in Table 1. The average weight of individual cob was increased from 17.3 g at 6.2 cm length to 65.5 g at 11.7 cm. It was mainly contributed by the weight of the outer husk (4.9 g at S1 to 25.2 g at S4), while silk weight showed the lowest contribution at all stages (3.5 g at stage1 to 8.7 g at S4) (Table 1). Corn cob had the maximum share in baby corn weight (50.0%), followed by husk portion (27.5%), while corn silk had a 19.4% contribution in total cob weight at S1. The average weight of the husked cob increased from S1 to S4 by 295.8% due to higher dry matter content. The relative contributions of corn cob (50.5 to 46.7%) and corn silk (19.4 to 13.2%) were reduced, while the husk portion (27.5 to 36.9%) increased with the advancement of S1 to S4 (Fig. 1). In comparison to S1, the weight gain during the developmental stage was 493.8% in husk portion, 336.2% in corncob and 248.5% in corn silk. The girth and length of husked baby corn were increased by 62.6% and 85.17%, respectively. Alike variations in morphological

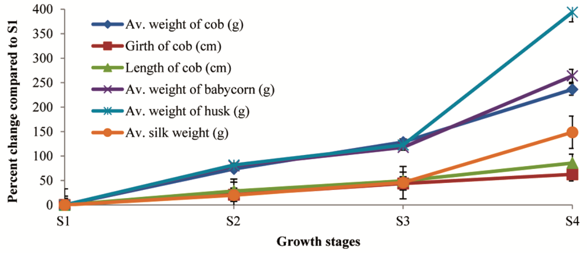

Fig. 1 — Percent change in morphological parameters with respect to S1

Table 1 — Morphological parameters of silk, cob and husk of baby corn at different growth stages

Morphological parameters	Growth stages			
	S1 (5 – 7 cm)	S2 (7.1 – 9 cm)	S3 (9.1 – 11 cm)	S4 (11.1 – 13 cm)
Av. weight of cob (g)	9.1 ± 0.50	15.8 ± 0.60	20.8 ± 0.40	30.6 ± 0.72
Girth of cob (cm)	9.1 ± 0.35	11.0 ± 0.51	13.1 ± 0.70	14.8 ± 0.55
Length of cob (cm)	6.3 ± 0.05	8.1 ± 0.07	9.4 ± 0.20	11.7 ± 0.45
Av. weight of baby corn (g)	18.0 ± 1.14	32.4 ± 1.45	39.2 ± 1.37	65.5 ± 1.42
Av. weight of husk (g)	4.9 ± 0.05	8.9 ± 0.35	10.9 ± 0.70	24.2 ± 0.65
Av. silk weight (g)	3.5 ± 0.25	4.2 ± 0.20	5.1 ± 0.42	8.7 ± 0.30

characteristics in the course of the phenological cycle are related to their physiological and ecological functions that also reflected in their variable phytochemical properties.

Phytochemicals and their Changes

Corn cob had the highest phenolic content (22.55 mg GAE/g) at S4, followed by silk (15.8) at S3 and husk (11.55) at S1 (Table 2), while the lowest content was estimated at S4 of husk (7.8 mg GAE/g). With the successive stages (S1 to S4), cob showed a gradual increase (15.25, 16.1, 17.1 and 22.55 mg GAE/g) and husk followed by a gradual decrease (11.55, 10.2, 8.3 and 7.8 mg GAE/g) in phenolic content. However, silk had a 5.18 and 17.0% increase in phenolic content at S2 and S3, followed by a decrease of 20% at S4. The phenolic content of cob was increased by 5.57, 12.1 and 47.8%, while husk showed 11.6, 28.1 and 32.4% reduction in value compared to S1 (Fig. 2). This means, highest phenols were synthesized when the baby corn are 9–11 cm in length (S3). Significant ($p<0.05$) change in phenolic content was observed except between silk and cob. The highest value at S4 in cob was due to the synthesis of the phenolic compound during developmental stage, which was decreased at S4 due to plant metabolism and losses thorough the defence mechanism. The decreasing trend in husks may be attributed to inherent characteristics of crop and genetic factors, etc. The highest flavonoid corresponded with the highest phenolic content in silk (45.0 mg RE/g) as flavonoid content is the classes of phenolic compounds except cob and husk. It is interesting to note that cob followed a gradual decrease and husk followed a gradual increase in flavonoid content where it was highest (45.0, 11.0, and 4.8 mg RE/g) at S3, S1 and S4 of silk, con and husk respectively.

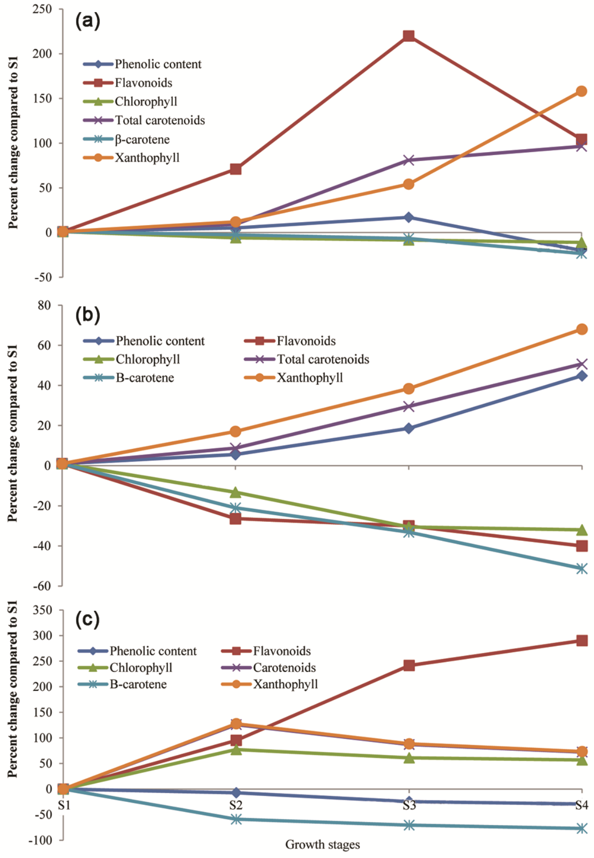

Fig. 2 — Percent change in phytochemicals in different portions of baby corn at four fruit phenological stages: (a) silk, (b) cob, (c) husk

Table 2 — Phytochemical content in baby corn parts

	Silk				Cob				Husk			
Parameters	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Phenolic content (mg GAE/100g)	13.5±2.9	14.2±1.9	15.8±2.7	10.8±2.8	15.25±2.5	16.1±2.0	17.1±2.2	22.55±2.5	11.55±2.5	10.2±2.2	8.3±1.9	7.8±1.1
Flavonoids (mg RE/100g)	14.1±1.7	24.2±2.4	45.1±2.2	28.8±2.1	11±2.3	8.1±2.5	7.7±3.0	6.6±2.8	1.23±2.3	2.4±2.8	4.2±2.6	4.8±1.8
Chlorophyll (µg/100g)	69.5±2.4	65.4±3.1	63.7±2.8	61.9±3.0	63.5±2.9	55.1±3.7	44.1±2.8	43.2±3.2	129.4±3.4	229.47±2.6	208.4±3.1	203.1±2.3
Carotenoids (µg/100g)	106.1±3.7	115.4±2.9	187±3.0	202.5±4.2	115.2±4.8	125.3±3.9	154.3±3.6	173.6±4.2	520.4±4.0	1176.4±3.7	974.1±3.3	896.9±2.9
β-carotene (µg/100g)	19.7±3.1	19.2±2.7	18.4±2.7	15.09±2.6	15.7±2.0	12.4±2.8	10.5±2.9	7.65±3.4	4.5±2.6	1.85±2.8	1.33±2.1	1.04±2.7
Xanthophyll (µg/100g)	86.8±4.2	97.2±4.7	133.84±3.8	224.1±4.5	100.2±6.9	117.3±5.2	138.7±5.8	168.4±5.1	516.2±4.9	1175.6±4.2	972.8±3.7	895.1±3.9
DPPH activity (mg BHA/100g)	523.3±2.0	565±2.2	591.66±3.1	515±2.8	626±3.5	640±4.2	650±3.6	665±4.9	576.6±3.8	423.34±3.5	491.66±3.6	361.66±3.4
ABTS activity (Mm Trolox/100g)	402.8±1.4	388.01±1.9	447.2±2.4	394.6±1.8	414.9±1.9	399.67±2.7	354±2.5	324±3.6	255.2±2.9	304.4±3.2	384.1±2.4	401.8±2.8
FRAP activity (mM Fe^{+2}/100g)	58.34±0.7	60.35±1.2	71.1±0.9	68.71±1.8	38.21±1.6	40.69±0.9	46.1±2.1	49.2±1.9	39.53±1.7	40.32±2.0	43.57±1.8	57.35±2.6
SOD activity (mg QE/100g)	325.4±1.9	497.3±2.3	599.1±2.2	501.4±1.4	438.5±2.7	458.7±2.2	507.4±1.3	436.7±1.8	363.2±1.6	453.7±2.5	467.2±2.3	476.88±2.5
B-carotene bleaching activity (%)	70.6±1.1	71.1±1.9	73±1.6	73.6±0.9	53.5±2.4	60.74±1.5	64.09±1.6	80.63±2.4	42.7±1.9	57.1±2.7	64.1±0.9	70.1±2.2

Flavonoid content was increased by 70.92, 219.8, 104.2% in silk and 95.4, 241.4, 290.24% in husk, whereas, cob extract had 26.3, 30.0, 40% reduction in flavonoid content from S1 to S4 (Fig. 2). The increasing pattern of flavonoids in husk may be due to the anthocyanins, which were almost absent in cob and silk. The higher flavonoid content in silk was in agreement with Liu *et al.*[5], who reported that total phenolics and total flavonoids constituted the largest portion of phytochemicals in corn silk. The results of phenolic and flavonoid contents are 2-5 times more than published literature[4,12,15,16] for coloured waxy corn (6.7–19.7 mg/100 g), for cob (44.29–59.82 mg/100 g), silk (58.02–70.63 mg/100 g) and husk (11.79–29.45 mg/100 g)[17], for phenolic and flavonoid content of husk (1.62–14.77 g GAE/100 g and 1.48–6.14 g GAE/100 g)[10], for purple cob (22.46 mg/g) and rice bran (8.24–16.12 mg/g)[13]; Hu and Xu[27] for corn genotypes with colored waxy kernels (0.67–3.88 mg of GAE/g of DW). The reason may be genotype, cultural practices, altitude and seasonal factors, crop ecology and climatic conditions.[16] Here, tropical climate favored synthesis of high levels of phenolic content as a secondary metabolite to defend against biotic and abiotic stresses.[28] The higher values in this study may be due to the type of solvent (methanol) used and the stress level imposed on plants in tropical climates. Flavonoids are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups, as they are also potent antioxidants. The strong antioxidant activity of baby corn fractions could be due to flavonoids, as supported by Ren *et al.*[14], who reported that five flavone glycosides extracted from corn silk showed strong anti-radical activity, indicating a strong antioxidant activity. Corn husk was the richest source of xanthophylls (1175.6 μg/100 g) and carotenoids (1176.4 μg/100 g) at S2, while cob and silk had maximum values of 168.4, 173.6 μg/100 g and 224.1, 202.5 μg/100 g at S4. Both pigments had shown an increasing trend except husk, where carotenoids were the highest at S2, followed by 87.1 and 72.3% decrease at S3 and S4 compared to S1. Similar behavior was also observed for xanthophyll content. Significant difference ($p<0.05$) between three extracts were observed, except between silk and cob phenolic and flavonoid content. The slow increase in total carotenoids could be due to a continuous decrease in lutenin, zeaxanthin, and violaxanthin during ripening.[29]

The range of carotenoids was in agreement with Khampas *et al.*[8] even for nine genotypes of mature corn (140–290 μg/100 g) except SWY and SWWY variety (330–3110 μg/100 g). Fidrianny *et al.*[10] reported that carotenoid content in ethanolic extract of cob and husk was 0.72, 0.45 g/100 g, respectively, while the n-hexane extract of cob and husk gave higher carotenoid content (1.18 and 3.63 g/100 g, respectively). This is explained by the fact that different polarities of solvents enabled diffusion of different constituents from plant material and consequently affected the yield of the extract, also the genetic background of corn materials and the degree of harvest affected the carotenoid content.[8] Further, degradation or inter-conversion or isomerization of carotenoids during fruit transportation, extraction, analysis and storage, might have also contributed in variation in carotenoid values as these compounds are sensitive to light and heat. Xanthophylls play key roles in protecting plant cells against oxidative stress during photosynthesis. High carotenoid and xanthophylls content in husks may be due to high amount chlorophyll, as these plant pigments responsible for color of different capacities are synthesized in the chloroplast.[30] Unlike silk and cob, a sudden increase in xanthophylls and carotenoid content in S2 and a decrease afterwards may be due to change in the synthesis effect of these two plant pigments by chloroplast. Carotenoids are plant pigments that act as antioxidants and are especially associated with eye health.[31] It suggests the possibility of breeding baby corn varieties rich in carotenoids. These xanthophyll pigments protect the outer retina of eye from oxidative stress and protect the eye from age-related maculopathy. They may do so in partly by stabilizing the microtubule cytoskeleton against insult by reactive oxygen species (ROS).[32] Thus, baby corn husks may be exploited as a source of natural antioxidant for degenerative diseases.

β-carotene was maximum in corn silk at all stages (19.7, 19.2, 18.4, 15.09 μg/100 g), followed by corncob and husk (Table 2). It was decreased by 2.9, 7.0 and 23.8% from S1 in corn silk; 21, 33.1 and 51.2% in cob and 58.8, 70.4 and 76.8% in husk (Fig. 2). Here, significant difference ($p<0.001$) was observed between extracts. Bacchetti *et al.*[9] reported 27.0-39.3 μg/100 g of β-carotene in Italian corn kernels. The highest carotene content was determined in corn silk (19.7 μg/100 g in S1), followed by cob (15.73 μg/100 g), which was lower than that in baby corn (670 μg/100 g) as reported by Hooda and Kawatra[33]. Chlorophyll content also decreased from S1 to S4. and its maximum reduction was observed in cob (31.9%), followed by corn silk (10.93%), while in husk portion showed a maximum increase of 56.95%. The decrease in carotene content may be due to an increase in the level of chlorophyll content.

Free Radical Scavenging Activity

Antioxidants are the compounds responsible for the reduction of oxidative degradation by scavenging free radicals, per-oxide radicals, metal chelators and metal reducing agents. The radical scavenging activities of baby corn parts using five different methods are presented in Table 2.

DPPH Radical Scavenging Activity

The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH value was found to be 523.3, 565.0 and 591.67 mg BHA/100 g in silk, followed by 626.1, 640.0, 650.3 and 665.0 mg BHA/100 g in cob, and 576.67, 423.33, 491.6 and 361.6 mg BHA/100 g in husk. A significant ($p<0.05$) difference between DPPH values for different developmental stages was observed except between silk and husk. The highest value of corn silk at S3 as observed was due to high flavonoid content. The study is in agreement with the report by Ren *et al.*[14] and Harakotr *et al.*[12] (5.6–21.6 TE/g) for colored waxy corn. However, cob and husk had opposite patterns of increasing and decreasing DPPH activity from S1 to S4. The reason may be due to the fact that some of the non-phenolic compounds extracted in the methanolic extraction process, also had antioxidant capacity. Some alkaloids, saponins, tannin and triterpenoids are reported to possess antioxidant activity.[34] This may be why DPPH activity in cob and husk did not corroborate with flavonoid content at the respective stages. There was a steady increase in the inhibition of the radicals with a concomitant increase in the concentration of the extract establishing dose dependence of the extract in scavenging DPPH radicals. DPPH radical scavenging activity increased with increasing concentration (80–800 μg/mL), where silk had scavenging activity of 88.0, 72.81, 92.51, 94.45%, followed by 60.0, 64.5, 59.5, 55.0% in cob, and 54.27, 61.2, 53.8, 47.2% in husk (Fig. 3) at 80 μg/mL. The higher activity in silk may be due to their flavonoid structure supported by Rice-Evans *et al.*[35], who found that orthosubstitution with electron-donating alkyl or methoxy groups of flavonoids increased the stability of the free radical and, hence, its antioxidant potential. In comparison to S1, a 5–17% change in scavenging activity was observed in all three extracts. A slight reduction of scavenging activity in S4 maybe due to the degradation of pigments and subsequently re-absorption into the plants.[36] The radical scavenging activity was found to be in conformity with the reports of Sarepoua *et al.*[16] and Simla *et al.*[17], ranging between 9.12 to 52.35% for purple waxy corn. The study indicates the potentiality of baby corn at the young stage as a more effective radical scavenger than matured color kernel, even in husk too (>50% at all stage). The reason may be due to the fact that in this study, small kernels and cobs were included as single sample because the whole structure is edible.

The DPPH scavenging activity decreased by 17.5% at S2, followed by an increase of 7% at S4 for silk portion. In the case of cob and husk, similar patterns of increase were observed with 6–12% followed by a decrease of 12% at S4 in comparison to S1 (Fig. 4). This showed that the scavenging activity of husk is at par with that of silk and cob.

ABTS$^+$ Scavenging Activity

The ABTS assay is based on the inhibition of the absorbance of the ABTS radical cation ($ABTS^{\bullet+}$) by antioxidants. Cob and husk had the highest ABTS value at S1 (414.9 mM Trolox/100 g) and S4 (395.2 mM Trolox/100 g), while silk had its maximum value (447.2) at S3 corresponding to their flavonoid contents. This is in agreement with published literature.[15–17] With the developmental stage, ABTS value increased by 11% at S3 followed by a 2.2% decrease in activity indicating non-significant changes. Nevertheless, cob showed a 21.9% reduction and husk showed a 54.8% increasing in value at S4 compared to S1. The reported value of ABTS activity was as high as 1.8–2.2 mM trolox/100 g, for Italian corn[9] and as low as 200–690 μM/100 g, for sweet, waxy and field corn.[8] The higher values observed in this study may be due to the geographic location, growing season, soil type, mineral status and the level of maturation.[37] The radical scavenging activity was found to be 72.8, 81.9, 95.21, and 85.21% in silk, 40.57, 38.5, 37.5, 31.9% in cob, and 25.65, 31.4, 40.2, 45.8% in husk at 80 μg/mL concentration. In comparison to S1, husk showed maximum increase of

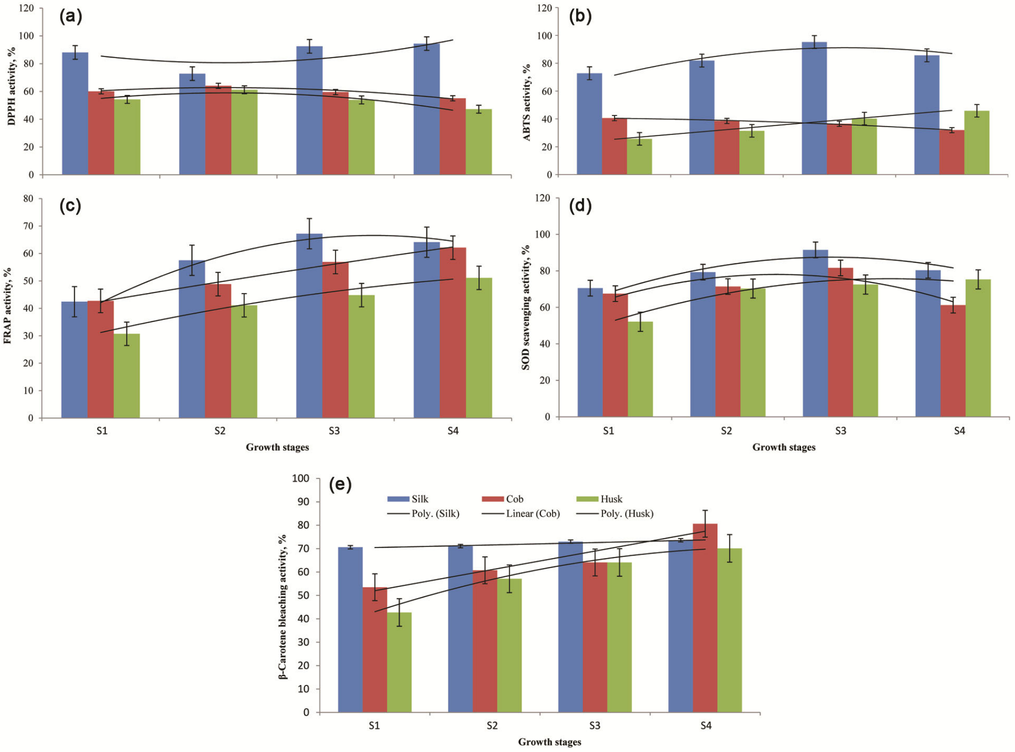

Fig. 3 — Antioxidant capacity of silk, cob and husk at different maturity stages: (a) DPPH activity, (b) ABTS activity, (c) FRAP activity, (d) SOD scavenging activity, (e) β-Carotene bleaching activity

123.4%, followed by silk (30.81% at S3), while cob had 21.3% reduction in scavenging activity (Fig. 4). A significant ($p<0.05$) difference in scavenging activity was observed except for cob and husk. The highest activity of silk corroborated with the high flavonoid content of silk compared to the other two extracts. A concentration-dependent activity was observed in DPPH activity. The relatively low scavenging potential for husk extract may be due to its low extraction potential in solvent to remove the phytochemicals from its complex tissue to the solvent medium, and also evident from its low flavonoid content. The results of the present investigation explains that the methanolic extract of baby corn parts may contain an enormous amount of hydrogen donor molecules, which may reduce the production of radicals and the decolorization in the DPPH and ABTS assays. It was also seen that the ABTS radical is more sensitive to phenolic-containing compounds than the DPPH radical.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}–TPTZ] complex and producing a colored ferrous tripyridyltriazine [Fe^{2+}–TPTZ]. In the current study, FRAP value was measured to be the highest (71.1 mM Fe^{2+}/g at S3), followed by husk (57.36 mM Fe^{2+}/g) and cob (49.2 mM Fe^{2+}/g) at S4. Khampas *et al.*[8] reported only 4–14 µM/100 g of FRAP value for 13 genotypes of corn. It is interesting to note that with the developmental stage, FRAP value was increased by 3.4, 21.8, 17.7 % in silk, 6.4, 20.6, 28.7% in cob, and 1.9, 10.2, 45.1% in husk from the S1. The highest content in silk at S3 was due to its flavonoid content, such as ABTS and DPPH activity. However, the anomaly behavior of cob may be due to presence of C2 – C3 double bond and 4-oxo (keto double bond at position 4 of the C ring) in flavonoid structure (mainly flavones) that increased the delocalizing electrons from B ring, leading to high scavenger activity at S4 though having the least flavonoid content (6.6 RE/g). This is in agreement with Tawaha *et al.*[38], who reported that various phenolic compounds respond differently in this assay, depending on the number of phenolic groups they have, and total phenolic content does not necessarily incorporate all the antioxidants that may be present in

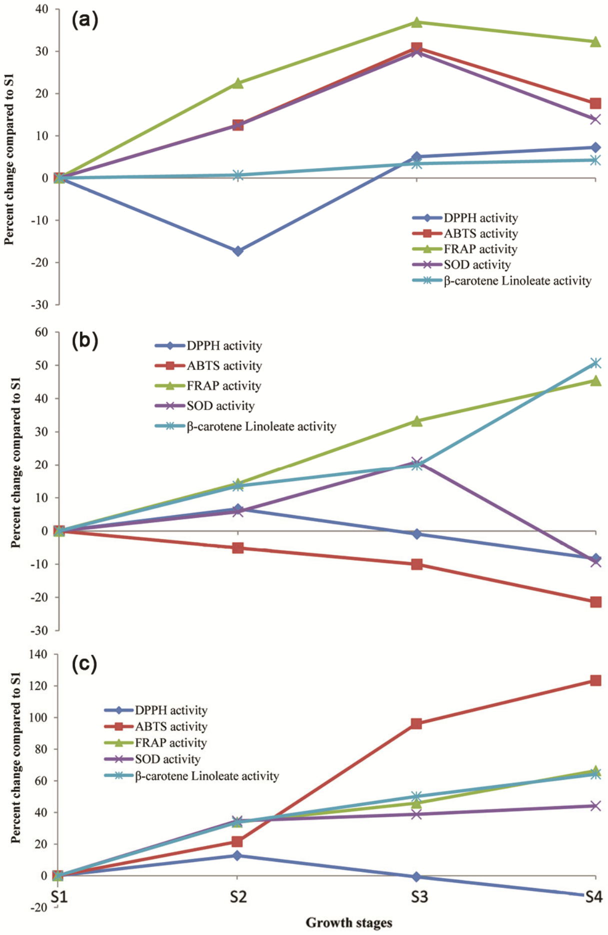

Fig. 4 — Change in radical scavenging activity of (a) silk, (b) cob and (c) husk at different maturity stage with respect to S1

an extract or fraction. At 80 µg/mL of each extract, the scavenging activity increased, being maximum (67.2%) for silk at S3, and 62.1 and 52.1% at S4 for cob and husk. In comparison to S1, silk showed an increase of 22.49, 36.9 and 32.3%, while cob showed an increase of 14.2, 33.25, 45.4% and husk showed 33.87, 45.29 and 66.46% increase in scavenging activity. The result was consistent with corn silk using different solvent [methanol (56.41%), ethanol (51.16%) and water extract (35.01%)] at 400 µg/mL.[15] Higher scavenging activity of silk indicated its higher reduction potential than husk and cob but lower as compared to DPPH and ABTS. This is in agreement with Oktay *et al.*[39], who reported the presence of phytochemicals in several plants significantly correlated with high FRAP values. These authors suggest that moderate ferrous-ions chelating abilities showed by baby corn parts could be beneficial to health. Iron can stimulate lipid peroxidation by the Fenton reaction, and accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. However, this study did not conform with the findings by Harakotr *et al.*[12], who stated that the greatest DPPH scavenging activity was associated with the greatest ferric ion-reducing activity.

Super Oxide Scavenging Activity

The superoxide radical has been the subject of much research in recent years due to its role as an agent of oxygen toxicity to cells.[40] Superoxide is also one of the most harmful radicals, and its scavenging is necessary because it is a precursor for other major ROS, like hydrogen peroxide, hydroxyl and singlet oxygen. Superoxide dismutase (SOD) is an enzyme that removes the superoxide radical, repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. SODS, a class of metal-containing proteins, catalyse the dismutation of superoxide radical anions into H_2O_2 and molecular oxygen. There are three different types of SOD categorized by their metal cofactor: Cu/Zn (Cu/Zn–SOD), Mn (Mn–SOD), and Fe (Fe–SOD).[41] However, in this study, the total SOD activity was determined in three parts of baby corn at different stages. The antioxidant dismutates superoxide radicals and breaks down hydrogen peroxides and hydroperoxides into harmless molecules (H_2O_2/alcohol and O_2). All extracts of baby corns scavenged superoxide radicals and thus inhibited formazan formation. From Table 2, superoxide value was observed to be increased from S1 to S4, being highest at S3 (599.1 and 507.4 mg quercetin/100 g) for silk and cob and S4 in husk extract (476.8 mg quercetin/100 g at S4). SOD scavenging activity increased in a dose-dependent manner and a non-significant difference was observed based on quercetin content, where it was found to be 70.5, 79.3, 91.5, 80.3% in silk, 67.5, 71.4, 81.6, 61.2% in cob and 52.1, 70.3, 72.5, 75.3% in husk. This is in agreement with Nurhanan *et al.*[15] for corn silk (72.8). The highest activity at S3 in cob may be attributed to some kind of flavonoids. Fidrianny *et al.*[11] reported that the glycoside of flavonoid had lower antioxidant activity than its aglycone. The glycosidic linkage at positions 3 or 7 (in ring C and A) on flavonoid structure and the carbohydrate might have led to lower scavenging activity in corn cob due to steric hinderance.[42] In comparison to S1, husk had the maximum increase in scavenging activity (44.19%) at S4, followed by 29.7 and 20.8% at S3 in silk and cob. This implied silk, cob and husk were strong enough to scavenge SOD radical at S2 followed by cob at S4.

β-Carotene Bleaching Activity

The free radical linoleic acid attacks the highly unsaturated *β*-carotene, and the presence of different antioxidants can hinder the extent of *β*-carotene-bleaching by neutralising the linoleate free radical and other free radicals formed in the system. The absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant the color was retained for a long time. BHT, the positive control used in this test, had 92% of antioxidant activity at 0.2 mg/mL. The oxidation caused by the free radicals formed during the peroxidation of linoleic acid, attacked the chromophore of the β-carotene emulsion, resulting in a whitening reaction.[43] The present study showed a continuous increase in β-carotene inhibition with the successive stages in all extracts with significant differences ($p<0.05$) in cob and husk. The highest bleaching activity was found in cob (80.63%), followed by silk (73.6%), and husk (70.1) at S4. However, in comparison to S1, husk had the highest increase (64.1%), followed by cob (50.7%) and silk (4.24%), as shown in Fig. 4. This indicated that the selection of silk at any stage has similar effect in inhibiting the formation of peroxide ion. Nurhanan *et al.*[15], reported that the methanol extract of corn silk exhibited the highest activity (66.04%), followed by the ethanol (52.92%), water (38.65%) and ethyl acetate extracts (26.33%). The increasing pattern did not correspond to the level of flavonoids in silk and husk. The reason may be due to changes in the pH and mineral content of the extract, where with the developmental stages, some minerals were synthesized along with the increasing in sugar content, which affected the pH of the extract, thus affecting the bleaching activity as conformed by Dawidowicz *et al.*[44] Plant extracts can differ quantitatively and qualitatively not only in the composition of antioxidants but also in natural acids, which makes their pH different.

Principal Component Analysis (PCA)

The datasets containing phytochemicals and antioxidant activity were subjected to PCA in order to decrease the number of descriptors associated with the data set, while still explaining the maximum amount of variability present in the data. The PCA can compress data based on similarities and differences, by reducing the number of dimensions without much loss of information and defining the number of principal components. PCA (Table 3) reduced the original 11 variables into two principal components (PC1 and PC2) in all three extracts. PC1 and PC2 accounted for 60.58 and 33.97%, 85.85 and 12.21% and 77.86 and 17.91% in silk, cob and husk extracts, respectively. So, the first two PCs explained more than 90% of variability in the data sets of these three extracts. Based on the guidelines[45], an attribute was

Table 3 — Loading of the variables for the first two principal components

Variables	PC1	PC2
Total phenolic content (TPC)	−0.445*	0.895
	0.945**	−0.320
	−0.925***	0.332
Flavonoids	0.619	0.785
	−0.911	−0.222
	0.940	0.300
Chlorophyll	0.936	−0.158
	−0.092	−0.392
	0.332	0.943
Carotenoids	0.970	0.185
	0.982	0.110
	0.156	0.988
β-carotene	−0.933	0.360
	−0.989	−0.100
	−0.721	−0.689
Xanthophyll	0.954	−0.297
	0.999	—
	0.157	0.987
DPPH activity	—	0.964
	0.996	—
	−0.663	−0.577
ABTS activity	0.193	0.844
	−0.981	−0.110
	0.928	0.309
Ferric Reducing Antioxidant Power (FRAP) activity	0.874	0.459
	0.980	0.161
	0.937	—
Super Oxide Dismutage (SOD) activity	0.648	0.681
	—	0.997
	0.700	0.721
β-carotene bleaching activity	0.983	0.160
	0.982	−0.185
	0.873	0.487
Eigen value	6.670	3.730
	9.440	1.340
	8.860	1.970
Variance explained (%)	60.650	33.970
	85.850	12.210
	77.860	17.910

Extraction method: Principal component analysis.

Rotation method: Varimax with Kaiser normalisation (Eigen value >1).

*The most significant loadings are highlighted in boldface.

*, ** and, *** value in respective row is loading for silk, cob and husk, respectively

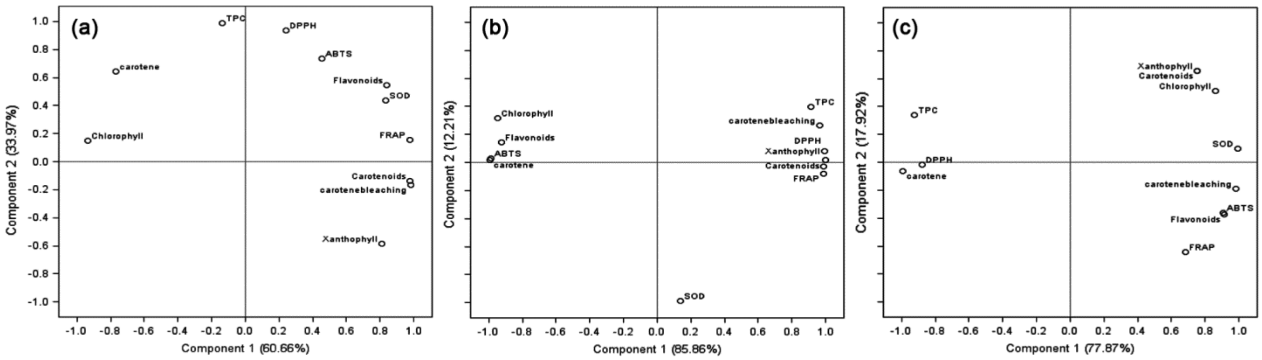

Fig. 5 — Loading plot of phytochemicals on PC1–PC2 for extracts of different portion of baby corn: (a) silk, (b) cob, (c) husk

correlated to load heavily on a given component if the factor of loading was greater than 0.72. Here, four phytochemicals, *i.e.* β–carotene (loading −0.933, −0.989, −0.721), FRAP activity (loading 0.874, 0.980, 0.937) and β-carotene bleaching (loading 0.983, 0.982, 0.873) were loaded on PC1, indicating a strong correlation and high contribution among these attributes in three extracts. However, phenolic content, flavonoid content, DPPH activity, ABTS activity (loading 0.895, 0.785,0.964, 0.844) of silk, and chlorophyll, carotenoids, xanthophylls and SOD (loading 0.943, 0.988, 0.987, 0.721) of cob were loaded at PC2. On the contrary, only SOD activity (loading 0.997) of cob was attributed by PC3 indicating high contributions from SOD scavenging activity. From the scree plot (using SPSS software), the curve flattened after two components for silk and husk, and three components for cob having Eigen values less than 1. Hence, up to two principal components for silk and husk, and three principal components for cob were chosen to explain the variability of 14 independent parameters having Eigen values less than 1 under this study. As seen from the biplot graph (Fig. 5), PC1 was related to the analysis of phytochemicals in silk and husk except for four phytochemicals in two extracts (phenolic content, flavonoid content, DPPH, ABTS in silk and chlorophyll, carotenoids, xanthophylls, SOD in husk). However, PC1 was related to all phytochemicals except for SOD, which was related to PC2. This result clearly indicated that in silk extract, the antioxidant activity (DPPH, ABTS, FRAP and SOD) were mainly contributed by the flavonoid content as they all have situated at the second quadrant (positive side) of PC1, whereas, non-phenolic compounds such as carotenoids and xanthophyll were mainly contributing to β–carotene bleaching activity. Similarly, total phenolic content was mainly associated with DPPH, β–carotene bleaching activity and FRAP, however, flavonoid content was contributing to only ABTS activity in cob portion. So, there was a high degree of homogeneity that separated the scavenging potentials based on phenolic and flavonoid content. This could be due to their high corresponding values in PC1 and PC2 axes. The ABTS activity on the upper left quadrant had a high negative score in PC1 and near zero in PC2. When comparing with loading, it could be seen that the extract had low carotenoids and DPPH (related to PC2) and the highest value of SOD (related to PC2). In this way, two PCs were separated based on their loading and score values. In the case of husk, flavonoid content was directly contributed to β-carotene bleaching activity, ATBS and FRAP activity, only DPPH activity was provided by total phenolic content. It was interesting to see that SOD scavenging activity was contributed by non-phenolic compounds such as carotenoids and xanthophyll as also evidenced by their respective content (Table 2). Thus, the antioxidant activity of silk, cob and husk is the result of synergetic or antagonistic interaction between phenolic and flavonoid compounds. This reveals the difference in the constitution of phytochemicals in three plant parts of the same species at different growth stages.

Conclusions

This study is a holistic analysis of functional constituents and antiradical activity of baby corn at different maturity stages. The collected information are very relevant in determining the comparative advantage of the harvesting stages of baby corn for proper morphological features vis-a-vis its phytochemical constituents. Significant increase in xanthophyll and total carotenoid, while a decrease in carotenes and chlorophyll in all three parts highlights role of corn cob stages in functional value of produce.

The trend in other phytochemicals suggest for harvest at S3 (*i.e.* 9.1–11.0 cm length) to harness the health benefits of the baby corn. Further, the corn silk and husks are considered as agricultural waste, however, the present study found rich profile of natural antioxidants in both fractions. Thus, they may be of special interest to various industries, *e.g.,* dietary supplements, food additives, and cosmetics. The various biological activities could be of interest for the food industry for preparation of baby foods and nutraceuticals or functional food items.

Conflict of Interest

The authors declared no conflict of interest.

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