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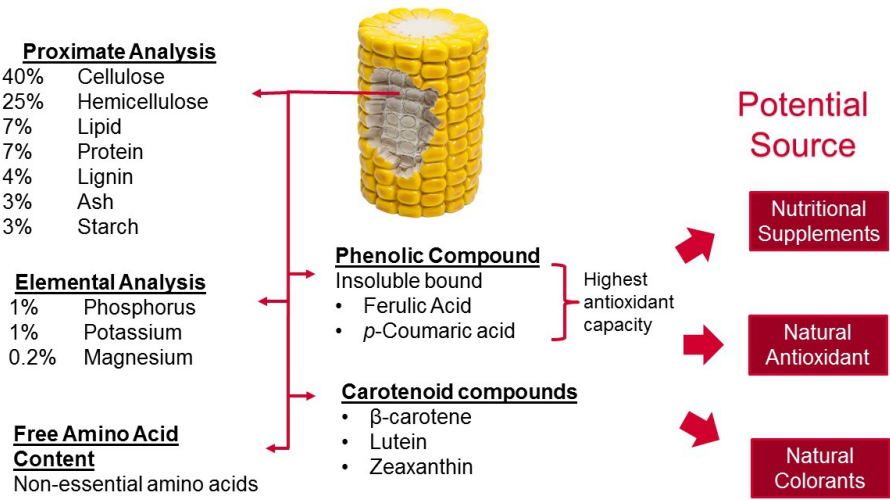
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Graphical Abstract

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Valorization of sweet corn (*Zea mays*) cob by extraction of valuable compounds

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Abstract

The main objective of this study was to investigate the proximate, mineral and phytochemical compositions of sweet corn cob (SCC), often neglected and regarded as agricultural waste. Compositional analysis showed that more than 60% of SCC was composed of insoluble dietary fibre, with cellulose being the major constituent. Results also showed that SCC can be a good source of non-essential protein and minerals (phosphorus, potassium and manganese). SCC had a total phenolic content of 6.74 g GAE kg⁻¹ dry weight (DW), of which bound phenolics were predominant. The bound phenolics fraction showed the highest antioxidant capacity in all three antioxidant capacity assays (TEAC, FRAP and DPPH) and contained the highest amount of ferulic and *p*-coumaric acid. The main carotenoids present in SCC were β -carotene, zeaxanthin and lutein. This investigation shows that SCC can be a potential source of natural colorant (carotenoids), antioxidants (phenolics) and nutritional supplements (proteins and phytochemicals).

Keywords: sweet corn cob; composition analysis; minerals; carotenoid; phenolic; supercritical fluid extraction; antioxidant activity; ferulic acid.

1.0 INTRODUCTION

Corn is one of the most important cereal crops globally ([Shiferaw, Prasanna, Hellin, & Bänziger, 2011](#)). [Kim and Dale \(2004\)](#) reported that the world annual production is about 520 x 10⁹ kg and North America (42%), Asia (26%), Europe (12%) and South America (9%) are the main producers. Most sweet corns are processed into frozen corn kernels, canned corn kernels or corn cobettes, resulting in the production of large amounts of by products, which includes the corn silks, husks and cobs. For every 100kg of corn grain obtained, approximately 18kg of corncobs are produced, most of which is used as animal feed, or remain unused as lignocellulosic waste ([Torre, Aliakbarian, Rivas, Domínguez, & Converti, 2008](#)). Previous studies on corncobs have focused on the production of bioethanol ([Chen, Xia, & Xue, 2007](#)), bio-oil and bio-char ([Mullen et al., 2010](#)), solid biofuel ([Ioannidou et al., 2009](#)) and xylooligosaccharides ([R. Yang, Xu, Wang, & Yang, 2005](#)).

As the world population increases, it is essential that alternative sources of nutrients and protein are explored to overcome the world food shortages. Plant waste contains compounds that have the potential to be used as food ingredients or as nutritional supplements, examples of which are pectin from apple pomace or citrus peel, phenolic compounds from potato peels, as well as lycopene from tomatoes and other red fruits ([Mirabella, Castellani, & Sala, 2014](#)). Proximate and nutritional analysis of fruits and vegetables play an important role in assessing their nutritional significance. Mineral components such as potassium, calcium, sodium, magnesium, phosphorus, iodine and iron are crucial for human nutrition ([Erkan & Özden, 2007](#)). In recent years, there has been continuous research in the functional properties of plant phytochemicals. Amongst them, extensive research has focused on ferulic and *p*-coumaric acid as they are widely distributed in the plant kingdom and have been used as feedstocks for the production of vanillin, or as antioxidants in food preservation ([Kumar & Pruthi, 2014](#)). Various studies have found that

SCC may also be a good source of phenolic compounds and different extraction approaches have been tested including alkaline hydrolysis ([Ares-Peón, Garrote, Domínguez, & Parajó, 2016](#); [Torre et al., 2008](#)), ultrasonic extraction ([Lai, Li, Wu, & Li, 2012](#)), surfactant-based cloud-point extraction ([Dhamole, Demanna, & Desai, 2014](#)) as well as enzymatic hydrolysis ([Pérez-Rodríguez, Torrado Agrasar, & Domínguez, 2017](#)) showing the presence of ferulic and *p*-coumaric acid as well as carotenoids.

The main objective of this study was to investigate the proximate, minerals and phytochemical composition of SCC. In addition, the potential of supercritical fluid extraction to extract carotenoids from SCC was also assessed.

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Ferulic acid (>99%), *p*-coumaric acid (>99%), gallic acid (97.5%) and ascorbic acid were purchased from Sigma Aldrich. β -carotene (>98%), lutein (>95%) and zeaxanthin (>98%) were obtained from Extrasynthese Company, Genay, France. All other reagents and chemicals used in this experiment were of analytical grade.

2.2 Sample Preparation

The preliminary studies on the extraction of free phenolics (Section 2.4) were carried out using sweet corn of mixed variety purchased from Sainsbury Supermarket (Reading, UK) in January 2015. The SCC used for the rest of the experiments was harvested in Senegal (mixed variety) in December 2015 and was provided by Barfoots of Botley Company Ltd (UK). Corn kernels were removed manually from the cobs using a knife and discarded. The SCC were then chopped into 5cm pieces in length, placed in the blast freezer (-18°C, 1 hour) and then freeze dried (Christ Gamma 2-16) until constant weight was achieved. The dried samples were finely ground in a mill (Apex Comminuting Mill), sieved through a 150 mesh

screen (<0.1mm particle size), thoroughly mixed and stored in the freezer (-80°C) until further analysis.

2.3 Proximate composition and minerals analysis of SCC

Moisture content was determined by using the Mettler Toledo halogen moisture analyser (Model: HE73). Ground SCC was analysed for proximate composition by AOAC method for protein (979.09), lipid (963.15) and ash (923.03) ([AOAC, 2005](#)). Structural carbohydrates and lignin (Klason and acid-soluble) were determined by the NREL procedure ([Sluiter et al., 2008](#)). Starch content was determined by amyloglucosidase method using Megazyme total starch assay kit ([J. H. Li, Vasanthan, Rossnagel, & Hoover, 2001](#)). Analysis of free amino acids content was carried out in accordance to [Elmore, Koutsidis, Dodson, Mottram, and Wedzicha \(2005\)](#). Minerals including calcium, magnesium, iron, zinc, copper, manganese, sodium and potassium were extracted from the samples by dry ashing method and determined by atomic absorption spectrophotometer (novAA® 350) as described in AOAC 985.35 ([AOAC, 2005](#)). Phosphorus was determined spectrophotometrically as described in AOAC 995.11 ([AOAC, 2005](#)).

2.4 Extraction of free, esterified and insoluble-bound phenolic compounds in SCC

The extraction of free, esterified and insoluble-bound phenolic compounds in SCC was carried out according to the method described by [Sosulski, Krygier, and Hogge \(1982\)](#). The free phenolic fraction of SCC was extracted based on the preliminary findings indicating that the best extraction condition was using 50% ethanol for an hour (see supplementary material, Figure S1).

2.5 Determination of total phenolic content

The phenolic content was determined using the Folin-Ciocalteu method as described by [Singleton and Rossi \(1965\)](#). The absorbance was measured at 760nm with a

spectrophotometer (CE1021, Cecil), and the phenolic content was expressed as g gallic acid equivalents per kg of dry weight (g GAE kg⁻¹ DW). The calibration curve was established using gallic acid (50-1000mg/L) as the standard sample ($R^2 = 0.9993$).

2.6 HPLC analysis of phenolic compounds of SCC

The analysis of phenolic compounds in the free, esterified and insoluble bound fractions were carried out using HP Agilent 1050 liquid chromatography, equipped with a DAD detector. The separation of the phenolic compounds was performed using a Zorbax SB-C18 column (2.1 x 15mm, 1.8 micron). The mobile phase was (A) formic acid/HPLC water (0.1:100 v/v) and (B) formic acid/acetonitrile (0.1/100 v/v). Solvent B was increased to 25% (0- 25 min), followed by 90% B for 30 minutes and then a final wash of 100% B for 10 minutes. The injection volume was 5µL with a flow rate of 0.2mL/min. Identification was carried out by comparing the retention time to the corresponding standards at 280nm and the amount of individual phenolic compounds (ferulic acid and *p*-coumaric acid) was calculated using an external calibration curve ($R^2 = 0.9998$ for both ferulic and *p*-coumaric acid).

2.7 Determination of antioxidant activities

The trolox equivalent antioxidant capacity (TEAC) assay and 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical assay were conducted as described by [H. B. Li, Wong, Cheng, and Chen \(2008\)](#) and [Zhao, Li, Liu, and Yang \(2014\)](#), respectively. The standard curve for TEAC ($R^2 = 0.987$) and DPPH ($R^2 = 0.989$) assay were constructed using Trolox at different concentrations (50 – 1000µmol for TEAC and 1 – 170µmol for DPPH). The results were expressed as mmol trolox equivalent (TE)/kg sample.

The reducing ability of the extracts was determined using the FRAP assay according to [Benzie and Strain \(1996\)](#) with slight modifications. A total of 10µL of standard, blank (water) or sample were added to each well in a 96-well microtiter plate (Cellstar®). Then,

300µL of FRAP reagent was added and the absorbance of the reaction mixture was read at 595nm using GENio Pro™ microplate reader with Magellan software. The standard curve ($R^2= 0.9997$) was constructed using ascorbic acid solution (50-1000µmol). Results were expressed as mmol ascorbic acid (AA)/kg DW.

2.8 Extraction and identification of carotenoid compounds

2.8.1 Conventional extraction

This extraction was based on the method described by [Gorocica-Buenfil, Fluharty, Bohn, Schwartz, and Loerch \(2007\)](#). The extracts were re-dissolved in ethanol and were stored at -18°C until HPLC analysis.

2.8.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction was carried out in a SFE unit (SciMed, UK), according to [Goto, Kanda, Wahyudiono, and Machmudah \(2015\)](#) with slight modification. SCC powder (5g) was loaded into the extraction vessel. The extraction pressure (350 bars) was controlled and the temperature was maintained at 60°C throughout the extraction. Carbon dioxide was fed into the extraction vessel at 15g/min. During the extraction (1 hour), a flow of 15% ethanol was pumped into the system to act as a co-solvent.

2.8.3 HPLC analysis of carotenoid compounds in SCC

The analysis of carotenoids present in SCC was performed using a YMC-C30 silica-based reversed-phase column (250 x 4.6 mm) coupled with a 1260 DAD detector (Agilent Technologies, UK). The mobile phases were (A) methanol/MTBE/water (82:16:2 v/v/v) and (B) methanol/MTBE/water (23:75:2 v/v/v). The analysis followed a gradient program for the mobile phases of 0 min 0% B, 45 min 50% B, 55 min 100% B, 60 min 100% B. Identification was carried out at 450nm by comparing the retention time to the corresponding

standards and external standard method was used to quantify the amount of individual carotenoids (lutein, zeaxanthin and β -carotene).

2.9 Statistical Analysis

Analyses were carried out in triplicate unless otherwise stated. Values shown in tables and graphs are presented as means \pm standard deviation. The data was analysed by using Minitab statistical software (version 16.1.0). Differences among treatments were determined using a one way ANOVA and Fisher test. Differences were considered as significant, when $p \leq 0.05$. Correlations among data obtained (n=3) were calculated using Pearson's correlation coefficient (r).

3.0 RESULTS AND DISCUSSION

3.1 Proximate composition of SCC

Results of the proximate composition of SCC are presented in Table 1. [Miura et al. \(2004\)](#) reported a lower value of protein (5%), lipid (1%) and ash (2%) content of SCC harvested from Northeastern China. The variation might be due to differences in the source of corn cob as the chemical composition of crops has been seen to vary with climatic conditions, crop cultivar as well as with the soil of the area ([Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006](#)). In addition, the SCC used in this study are of mixed varieties and this might cause variation in the data obtained. Previous investigation showed that corn grains contain higher amount of protein (9.10%) and lower lipid content (4.21%) ([Belyea, Rausch, & Tumbleson, 2004](#)), as compared to SCC. More than 60% of the SCC was composed of insoluble dietary fibre, with cellulose being the major constituent, followed by hemicellulose, as previously reported by [Miura et al. \(2004\)](#), [Awosusi, Ayeni, Adeleke, and Daramola \(2017\)](#) and [Worasuwannarak, Sonobe, and Tanthapanichakoon \(2007\)](#). As compared to other corn industry waste, SCC contain higher amount of cellulose and hemicellulose, as compared to corn straw ([X. Yang,](#)

167 [Chen, Gao, & Li, 2001](#)) and stover ([Weiss, Farmer, & Schell, 2010](#)). These results suggest
168 that SCC can be a good source of insoluble dietary fibre.

169 To date, SCC has mainly been used as a carbohydrate source but it may also be an
170 potential source of protein and minerals. There is an increasing demand for more insight on
171 the potential of plant-based protein ([Iqbal et al., 2006](#)). A total of 18 free amino acids were
172 identified in SCC (Table 1) with serine and glutamine as the predominant ones followed by
173 alanine, proline, aspartic and glutamic acid. Overall, the free amino acid content accounted
174 for 0.7% of the composition in SCC, although free arginine was not measured. As compared
175 to the essential amino acids present in corn grain ([Belyea et al., 2004](#)), SCC contained lower
176 amount of essential amino acids as compared to corn grain. However, the essential amino
177 acids (including lysine, tryptophan, histidine) and non-essentials amino acid (including serine,
178 glutamine and glutamic acid) present in SCC is higher than other cereal products such as
179 whole grain rye, rye bran, wheat bran, barley flour and oat bran ([Mustafa, Åman, Andersson,
180 & Kamal-Eldin, 2007](#)). Therefore, SCC can be used to complement other plant protein
181 sources to increase the overall protein quality of the mixture. [Young and Pellett \(1994\)](#)
182 reported an improvement on the protein quality of corn and soy flour, when used in
183 combination.

184 The total mineral content measured in this study was 2.18%, which correlated well
185 with the ash content value (3.01%) in SCC. Phosphorus ($10.12 \pm 0.06 \text{ g kg}^{-1} \text{ DW}$) was the most
186 abundant mineral, followed by potassium ($9.62 \pm 0.21 \text{ g kg}^{-1} \text{ DW}$) and magnesium ($1.67 \pm 0.10 \text{ g kg}^{-1} \text{ DW}$). Calcium, lead, zinc, manganese, copper and iron were also present in SCC but in
187 lower amounts. The quantity of all minerals were higher in the SCC analysed in the present
188 study compared to those reported previously by [Abubakar et al. \(2016\)](#), [Anukam, Goso,
189 Okoh, and Mamphweli \(2017\)](#) and [Awosusi et al. \(2017\)](#). The minerals and trace elements
190 content may vary in plants due to the influence of environmental conditions such as presence
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of light, water availability elevated CO₂, elevated ozone levels and agricultural technologies (Nour, Trandafir, & Cosmulescu, 2014).

3.2 Phenolic composition and antioxidant activity of SCC

3.2.1 Total Phenolic content and antioxidant activity of SCC

Phenolic compounds in SCC were present in free, esterified and insoluble-bound forms (Figure 1A). The highest level of phenolic compounds were present in the insoluble-bound form (5.41 ± 0.27g GAE kg⁻¹ of sample) followed by the free phenolic (0.9g ± 0.08 GAE kg⁻¹) and esterified phenolic (0.43g ± 0.05g GAE kg⁻¹) fractions. Table 2 showed that in all antioxidant assays, the highest activity was found in the insoluble bound fraction. The overall relationship between antioxidant activity and total phenolic content of SCC (Table 3) was a positive and highly significant correlation ($p \leq 0.01$), suggesting that the phenolic compounds are the most important contributors towards the antioxidant activity of SCC.

3.2.2 Quantification of ferulic and *p*-coumaric acid in SCC

The total amount of ferulic and *p*-coumaric acid present in all three fractions of SCC (Figure 1B) was 3.06 ± 0.19 and 4.23 ± 0.25g kg⁻¹ DW, respectively. Ferulic and *p*-coumaric acid in the insoluble-bound phenolic fraction of SCC was highest, with 2.96 and 4.08 g kg⁻¹, respectively. Free and esterified phenolic fractions had significantly lower ($p < 0.05$) ferulic and

p-coumaric acid content as compared to the insoluble-bound fraction. Strong correlation was found between ferulic and *p*-coumaric acid content of SCC for all three antioxidant assays (Table 3) carried out in this study. This showed that both ferulic and *p*-coumaric acid content contributed towards the antioxidant activity of SCC. Dewanto, Wu, and Liu (2002) reported that the presence of ferulic acid in sweet corn kernels was highest in

the insoluble-bound fraction (0.42 g kg^{-1}), followed by soluble conjugated fraction (0.01 g kg^{-1}) and free fraction (0.00105 g kg^{-1}). Furthermore, previous investigations have shown that the ferulic acid and *p*-coumaric acid content in yellow corn grain ranged from 0.006 to 1.80 g kg^{-1} and 0.00012 to 0.00050 g kg^{-1} , respectively ([Assabgui, Reid, Hamilton, & Arnason, 1993](#); [Hu & Xu, 2011](#)). Furthermore, the ferulic and *p*-coumaric acid in agricultural crop residues such as flax shives (0.25 and 0.61 g kg^{-1} , respectively) and wheat bran (3.91 and 0.2 g kg^{-1} , respectively) ([Buranov & Mazza, 2009](#)) were found to be lower, as compared to SCC. This research showed that all three phenolic fractions contained higher amount of ferulic acid as compared to sweet corn kernels and other agricultural crop residues suggesting that SCC can be a good source of ferulic acid and *p*-coumaric acid.

3.3 Extraction and characterization of carotenoid compounds

In this study, lutein, zeaxanthin and β -carotene were the three carotenoids identified and quantified in SCC (Table 4). β -carotene was the main carotenoid in SCC followed by zeaxanthin and lutein. Supercritical fluid extraction (SFE) was able to extract high amounts of carotenoids, as compared to conventional extraction. No correlation was found between carotenoid content and antioxidant activity in all three assays (data not shown) thus the contribution of carotenoid content in SCC towards antioxidant activity was assumed negligible. Similarly, [Gil, Tomás-Barberán, Hess-Pierce, and Kader \(2002\)](#) found no correlation between carotenoid and antioxidant activity of nectarines, peaches and plum.

Previous studies carried out by [Kurilich and Juvik \(1999\)](#) reported that the carotenoids present in sweet corn kernels included zeaxanthin (2.16 mg kg^{-1}), lutein (5.95 mg kg^{-1}), and β -carotene (0.68 mg kg^{-1}). Our results showed that there were higher levels of zeaxanthin and β -carotene content in SCC, compared to those reported for sweet corn kernels. Therefore,

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238 SCC could potentially be a relatively good source of carotenoid compounds as compared to
239 sweet corn.

240 In this study, two different extraction methods were compared (Table 4), the SFE and
241 conventional method. SFE resulted in significantly higher levels of carotenoids than
242 conventional extraction. This confirms the effectiveness of the SFE techniques in the
243 extraction of carotenoid compounds in SCC. It is well-known that carotenoids are highly
244 sensitive to light, air, heat and pH ([Panfili, Fratianni, & Irano, 2004](#)). SFE allowed the
245 extraction of carotenoid compounds without exposure to lights and air, as the extraction is
246 carried out in an air-tight and closed chamber. The content of lutein and zeaxanthin in SCC
247 was twice as high in SFE extraction, as compared to conventional extraction. Furthermore,
248 the content of β -carotene was three times higher in SFE extraction. This could be due to the
249 higher sensitivity of β -carotene towards oxidation ([Yuan, Gao, Zhao, & Mao, 2008](#)), as
250 compared to lutein and zeaxanthin. In addition, the combination of low viscosity and high
251 diffusivity of supercritical fluid can enhance the penetration into porous solid material and
252 consequently, result in faster and more effective extraction ([Lang & Wai, 2001](#)). The
253 supercritical fluid extraction of carotenoid content in SCC was higher as compared to other
254 vegetable by-products such as persimmon peel ([Takahashi et al., 2006](#)), tomato seed and
255 tomato peel ([Knoblich, Anderson, & Latshaw, 2005](#)). Results from this study indicate that
256 SCC can be a potential source of carotenoid compounds.

257 In conclusion, this research has shown that SCC appears to be a promising source of
258 natural colorant (carotenoids) and antioxidants (phenolics). The knowledge generated from
259 this study may be useful to explore the use of agricultural waste as a source of functional
260 food or value added products.

261 **4.0 ACKNOWLEDGEMENT**

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5.0 CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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Figure Captions

Figure 1. Total phenolic content (A) and amount of ferulic and *p*-coumaric acid (B) in free, esterified and insoluble-bound fractions of sweet corn cob. Values are presented in means \pm standard deviation of triplicate samples; fractions with different letters within the same phenolic compound are significantly different ($p < 0.05$).

For Peer Review

Table 1. Composition, minerals and amino acid content analysis of sweet corn cob.

Component (%w/w based on dry matter)*	
Protein	6.70 ± 0.06
Lipid	7.18 ± 0.08
Ash	3.04 ± 0.05
Lignin	
Klason Lignin	1.03 ± 0.00
Acid Soluble lignin	3.08 ± 0.00
Cellulose	40.40 ± 1.73
Hemicellulose	
Galactose + Xylose + Mannose**	19.12 ± 0.80
Arabinose**	4.45 ± 0.06
Starch	3.21 ± 0.08
Minerals content (g kg ⁻¹ DW)*	
Iron	0.01±0.00 ^d
Copper	0.01±0.00 ^d
Zinc	0.04±0.00 ^d
Lead	0.07±0.00 ^d
Calcium	0.21±0.06 ^d
Magnesium	1.67±0.10 ^c
Potassium	9.62±0.21 ^b
Phosphorus	10.12±0.06 ^a
Manganase	0.08±0.12 ^d
Amino acid composition (g kg ⁻¹ DW)*	

<i>Non-essential amino acids :</i>	
Serine	1.36 ± 0.13^a
Glutamine	1.16 ± 0.24^a
Glutamic	1.09 ± 0.08^b
Alanine	0.90 ± 0.06^c
Aspartic	0.86 ± 0.09^c
Proline	0.69 ± 0.06^d
Asparagine	0.35 ± 0.07^e
Tyrosine	0.08 ± 0.04^{fg}
Glycine	0.04 ± 0.01^{fg}
Ornithine	0.04 ± 0.00^{fg}
<i>Essential amino acids :</i>	
Lysine	0.15 ± 0.02^f
Tryptophan	0.13 ± 0.01^{fg}
Histidine	0.12 ± 0.00^{fg}
Threonine	0.09 ± 0.02^{fg}
Leucine	0.07 ± 0.01^{fg}
Phenylalanine	0.05 ± 0.01^{fg}
Valine	0.05 ± 0.01^{fg}
Isoleucine	0.02 ± 0.01^g

**All content based on the freeze-dried sweet corn cob. Values presented as mean \pm standard deviation; Superscripts indicate significantly different at $p \leq 0.05$.*

***Presented as polymers, contributing to hemicellulose content*

Table 2. Trolox equivalent antioxidant capacity (TEAC), DPPH radical scavenging capacity, ferric reducing antioxidant potential (FRAP) of free, esterified and insoluble bound fractions of sweet corn cob

Fractions	TEAC (mmol TE kg ⁻¹)	DPPH (mmol TE kg ⁻¹)	FRAP (mmol AA kg ⁻¹)
Free phenolics	9.54 ± 1.03 ^b	1.74 ± 0.13 ^b	1.83 ± 0.21 ^b
Esterified phenolics	4.69 ± 0.59 ^b	0.43 ± 0.18 ^c	1.07 ± 0.11 ^b
Insoluble-bound phenolics	131.23 ± 23.87 ^a	3.68 ± 0.30 ^a	10.86 ± 0.56 ^a

**Values are presented in means ± standard deviation of triplicate samples; mean values within the same column with different letters are significantly different (p<0.05).*

Table 3. Pearson's correlation coefficient between total phenolic content (TPC), trolox equivalent antioxidant capacity (TEAC), DPPH radical scavenging capacity, ferric reducing antioxidant potential (FRAP), ferulic acid and *p*-coumaric acid content of sweet corn cob^a.

	TPC	TEAC	DPPH	FRAP
ABTS	0.981			
DPPH	0.936	0.885		
FRAP	0.993	0.981	0.932	
Ferulic acid content	0.995	0.984	0.900	0.990
<i>p</i>-coumaric acid content	0.996	0.984	0.902	0.990

^a 95% confidence level

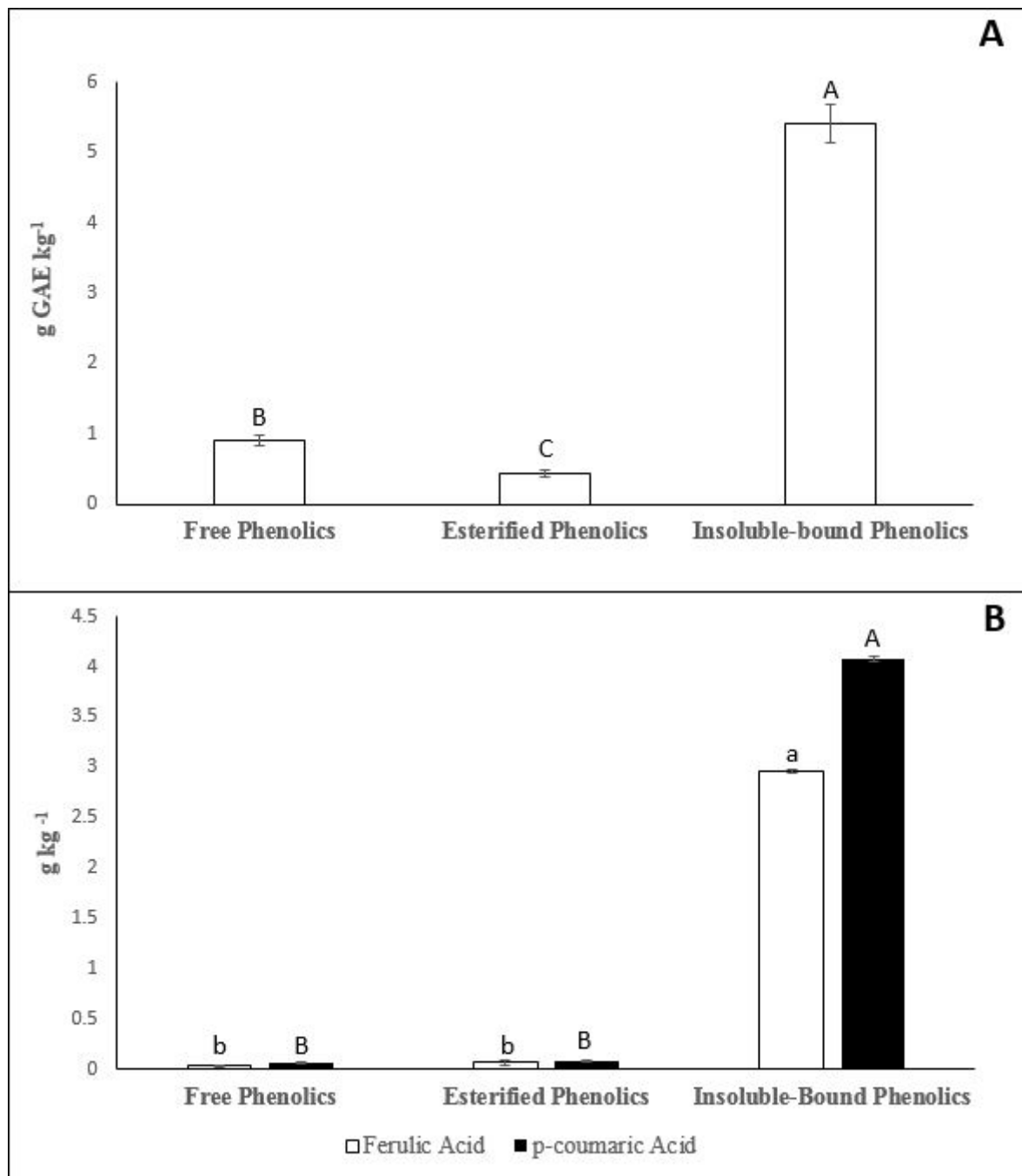
**All correlations are significant at $p \leq 0.01$ level*

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Table 4. Concentration of individual carotenoid compound present in sweet corn cob extracted by conventional extraction and supercritical fluid extraction (mg kg⁻¹ DW).

Extraction Method	Lutein	Zeaxanthin	β-carotene
Conventional	1.67 ± 0.11 ^b	3.98 ± 0.43 ^b	49.35 ± 3.60 ^b
Supercritical fluid	3.81 ± 0.02 ^a	8.47 ± 0.09 ^a	177.29 ± 4.35 ^a

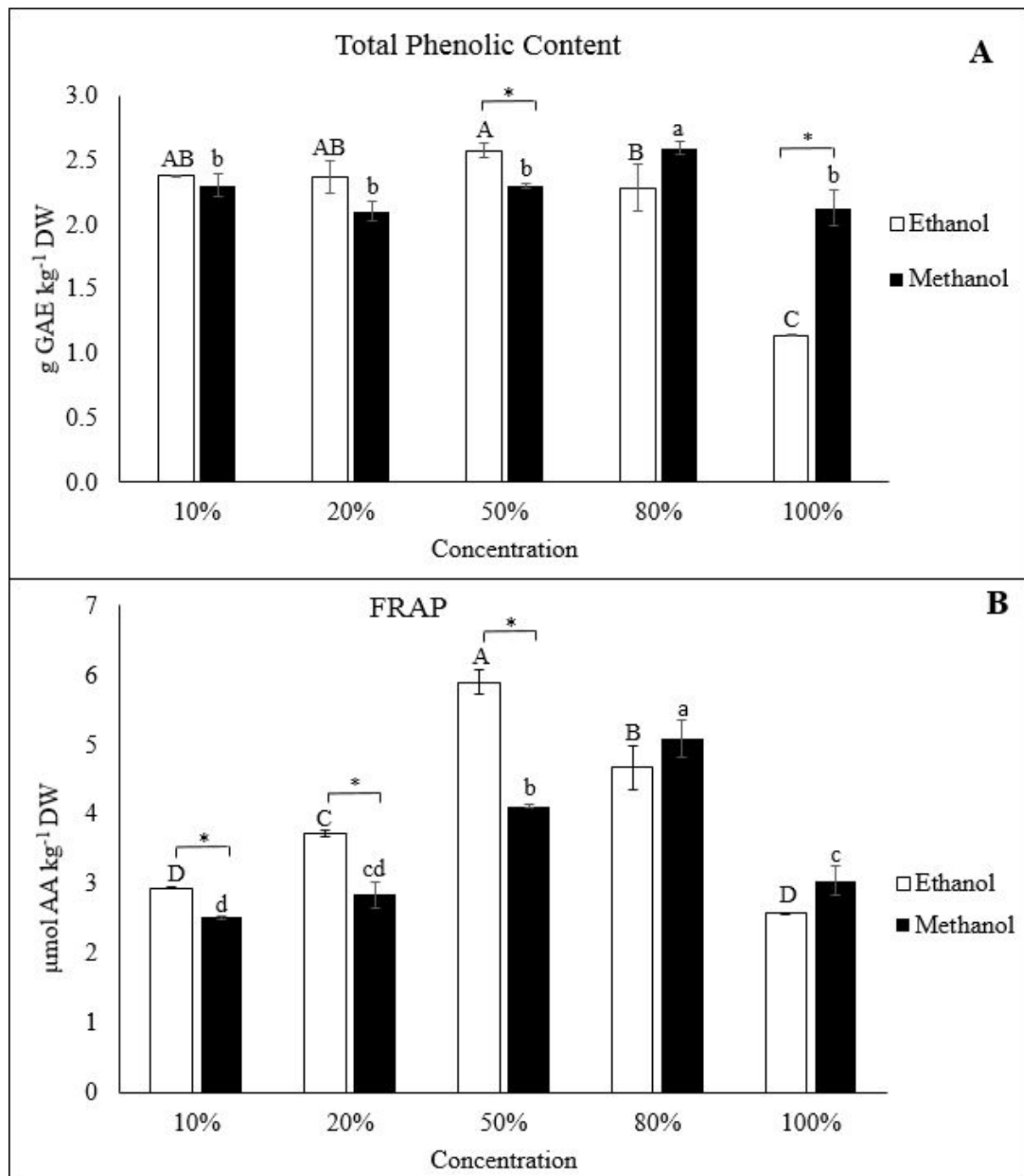
**Values are presented in means ± standard deviation of duplicate samples; mean values within the same carotenoid compound with different letters are significantly different (p<0.05)*

**Figure 1**

Supplementary Material

Figure Captions

Figure 1. Effect of different concentration of ethanol and methanol on (A) total phenolic content and (B) ferric reducing antioxidant power (FRAP) of sweet corn cob. Values are presented in means ± standard deviation of duplicate samples; uppercase and lowercase showed significant different ($p<0.05$) between ethanolic and methanolic extract of sweet corn cob, respectively. Asterisks (*) indicate significant difference ($p< 0.05$) between solvents at the same concentration.

**Figure 1**

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Referee(s)' Comments to Author:

Referee: 1

Comments to the Author

Abstract

L7: Units g GAE kg⁻¹ ...please use the same notation in all the manuscript as required by the author's guide.

All units are now based as per kg throughout the manuscript as required by the author's guide.

L6 and L11-13: In my opinion, the amounts of protein and minerals found in SCC are low to considered as a "good" or "promising" source of these components. On the other hand, the comparison of ferulic content between the SCC and sweet corn kernels in the results and discussion section don't contribute to this asseveration.

I suggest revise your results and consider to use other words.

The amount of ferulic acid in both sweet corn cob and sweet corn kernels has been revised concluding that the content of ferulic acid in corn kernels (0.42g kg⁻¹ of insoluble bound, 0.01g kg⁻¹ of soluble conjugated and 0.001g kg⁻¹ of free ferulic acid) is lower as compared to sweet corn cob. Hence, SCC can be a potential source of phenolic compounds (Line 209 to 212).

Introduction

L48: Please revise the citation of Lai et al 2012. The correct first name is Fu-Rao

This has now been corrected (Line 332)

Materials and methods

L100: L7: Units g GAE/kg DW ...please use the same notation in all the manuscript as required by the author's guide

All units are now based as on per kg now throughout the manuscript as required by the author's guide.

Results and Discussion

L164-165: I'm not sure about the SCC could be a "good" source of protein and minerals. The amounts of protein and minerals reported are low (6.7 and 2.18 %) compared with other sources. I suggest delete the term "good" or use a more appropriate word.

Suggestion of SCC could be a “good source of protein and minerals” has been removed from the manuscript.

L187-191: Extraction time... It is not clear which solvent (ethanol, methanol or both) was used in this part. Please specify. If both were proved, the same effect on the extraction time was observed? Any explanation for these results?

This section has been removed to fulfil the requirement for word count. The graphs have been added to supplementary data for reference (Line 87-90).

L192-193: This sentence is incorrect. Please discuss the results carefully. First, there are no significant differences in phenolics between ethanol and methanol using 10%, and a means comparison between treatments is necessary to be sure about the differences between treatments using 20 and 80 % concentrations. This is the same case of FRAP at 80%.

This section has been removed to fulfil the requirement for word count. The graphs have been added to supplementary data for reference. (Line 87-90)

L193-195. Incorrect. This trend is true only for FRAP. In phenolics, there are no significant differences between 10, 20 and 50%, and between 20 and 80%. The only clear trend observed in both variables is a decrease from 80 to 100 %.

This section has been removed to fulfil the requirement for word count. The graphs have been added to supplementary data for reference. (Line 87-90)

L195-196: Please re-write your discussion according to the statistical analysis, not based on the visual trends.

This section has been removed to fulfil the requirement for word count. The graphs have been added to supplementary data for reference. (Line 87-90)

L192-201: There is a lack of discussion and explanation about these results. For example, what could be the reason for the decreasing of phenolics extraction using a concentration higher than 50% for ethanol and higher than 80% for methanol? Any hypothesis?

This section has been removed to fulfil the requirement for word count. The graphs have been added to supplementary data for reference. (Line 87-90)

L225-227. According to previous lines 221-222. sweet corn kernels have a significantly higher amount of ferulic than those your results.

The amount of ferulic acid has been corrected (Line 209 to 2121).

L252: please, if possible use a more recent reference

A more recent reference has been used (Line 245) by Yuan et al., (2008).

YUAN, Y., GAO, Y., ZHAO, J. & MAO, L. 2008. Characterization and stability evaluation of β -carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Research International*, 41, 61-68.

L256-259: please, consider a careful revision of this conclusion based on the previous comments and the results.

Conclusion has been altered slightly to show that SCC can be a promising source of carotenoid and phenolic compounds (Line 252 – 255).

References

L277: Names are incorrectly written

These have now been corrected as suggested (Line 269).

L281: The correct citation is: *Phytopathology*, 83, 949-953.

This has now been corrected as suggested (Line 274).

L329: use "Food" instead of "food"

This has now been corrected as suggested (Line 330).

L330: Please revise carefully the reference. Names are incorrectly written as well as the volume number and the final page number.

These have now been corrected as suggested (Line 331)

L344. use the capital letter in "l"-lactic

This has now been corrected as suggested (Line 345)

L361: pages number incomplete

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These have now been corrected as suggested (Line 363)

L365-367: please complete the reference

This has now been corrected as suggested (Line 367-369)

L370: Use "Food" instead of "food"

These have now been corrected as suggested (Line 372)

L374: separate VAN HASSELT; use "leaf" instead "lead"

This reference has been replaced by a more recent reference by Yuan et al. (2008) (Line 392).

YUAN, Y., GAO, Y., ZHAO, J. & MAO, L. 2008. Characterization and stability evaluation of β -carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Research International*, 41, 61-68.

L383: the last page should be 1212S

This has now been corrected as suggested (Line 391)

Table 1: What do the letters aside the SD mean?

Superscripts in Table 1 indicate significantly different at $p \leq 0.05$. This has now been added to the footnote of Table 1.

Figure 1: Please, describe what does the capital and lowercase letter above the bars means specifically.

Figure moved under Supplementary date to fulfil the requirement for word count.

Referee: 2

Materials and Methods

Page 5, lines 65-66: The manually removing of the kernels from the cob was doing with a knife?. If yes, please clarify this. To have clear that the cob that was used in the study simulated what is discharged in the industry.

Sample preparation now reads “Corn kernels were removed manually from the cobs using a knife and discarded” (Line 67).

Page 5, lines 84-91: authors talk about optimization of extraction. However, they do not mention the statistical model used to test that the extraction conditions chosen was the best. It could be better to eliminate the term “optimization” in this section

Section 2.4 has been removed to fulfil the requirement for word count.

Page 6, lines 103-112. Authors need to precise the phenolic compounds analyzed with the HPLC. Phenolic acids. Did you run the analysis at 280 nm only?. Ferulic and *p*-coumaric acids are derivatives from cinnamic acid and they are commonly quantified at 320 nm. Which phenolic compounds fraction did you use for HPLC analysis?, the free?, the esterified? The insoluble-bound?

The analysis were run at 280, 320 and 365nm and 280nm was chosen as both ferulic and *p*-coumaric acid showed their highest absorbance (max λ) at this wavelength. The phenolic fractions subjected to HPLC analysis are now listed in Section 2.7, line 98.

Page 7, lines 124-126: why do you use ascorbic acid and not Trolox or ferrous sulfate?

Several standards including ascorbic acid, trolox and ferrous sulphate are normally used in FRAP assay. In our study, a well-known antioxidant, ascorbic acid, was chosen following our standard laboratory SOP.

Results and Discussion

Page 10-11, lines 207-210: The correlations coefficients are very high, however, these are going to be affected by the number of samples that are involved in the analysis. Commonly, as the number of samples increase the values of Pearson coefficient decrease. How many samples do you use for the Pearson correlation analysis?

In Section 2.9, details have been added in for Pearson correlation analysis (Line 148 to 149). Three samples were used for Pearson correlation analysis.

Page 11, lines 212-213; Please check the redaction of this paragraph, you mentioned three fractions of SCC, and presented only two values.

Line 202 to 203 stated the total ferulic and *p*-coumaric acid of SCC including all three fractions (free, esterified and insoluble-bound)

Page 11, lines 212-227: In corn grain the insoluble bound fraction contains higher values of the proportion ferulic acid/*p*-coumaric acid. In this study the value of this proportion is lower than 1.0. How do the authors explain this result? Why do not compare the values obtained for phenolic compounds and carotenoids in corn cob with other sources currently in use for the commercial extraction of these compounds. By doing so, one could clearly see if the corn cob has the potential to compete in these uses.

The comparison of phenolic and carotenoid content with other crop by products were compared to highlight the potential of SCC to be used as a source of phenolic and carotenoid

compounds. For phenolic content, comparison has been made with flax shives and wheat bran (Line 215 to Line 217). In addition, carotenoid content of SCC was compared with other vegetable by-products including tomato seed, as well as tomato and persimmon peel (Line 248-250).

For Peer Review

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Referee: 3

Comments to the Author

Please see the following suggestions:

Lines 157 – 163 Knowing the variety of corn used as source of SCC could further explain deviations in composition. If samples included mixed varieties, this might also be a source of variation. If the latter is the case, please state it in M&M. It would not constitute a problem, because generally, this could be a normal case in the future when this process might be applied in a larger scale. In spite of possible variations coming from different sources of cobs, the method of extraction proposed is valid.

At Section 2.2, the statement “of mixed variety” was added in Line 64 – 66.

Line 161. Hemicellulose content is reported for the sample analyzed: state the method used in M&M section.

The method for the determination of hemicellulose content was included in Section 2.3 (Line 75 – 77) and followed the NREL procedure as described by Sluiter et al. (2008).

SLUITER, A., HAMES, B., RUIZ, R., SCARLATA, C., SLUITER, J., TEMPLETON, D. & CROCKER, D. 2008. Determination of Structural Carbohydrates and Lignin in Biomass. *In*: GOLDEN, N. R. E. L. (ed.). Colorado.

Line 170 Complement might be more appropriate than compliment

This has now been corrected as suggested (Line 178).

Lines 171-172. The comment regarding “Young and Pellett (1994) reported an improvement on the protein quality of corn and soy flour, when used in combination” is made for corn and soybean flours. A more detailed discussion comparing the composition of SCC with corn flour (from grains) might be useful and very interesting for the readers, as well as the expression of amino acid content per g or 100g of protein might also help comparisons with FAO standards. Knowing how protein from SCC compares with flour from the grains is interesting.

Previous study by Belyea et al. (2004) showed that corn grains contained higher amount of essential amino acids as compared to SCC (Line 172 – 174). However, as compared to other cereal products such as barley flour, oat flour, whole grain rye and wheat bran, SCC contained higher amount of certain essential and non-essential amino acids (Line 174 – 177). Therefore, SCC can be used to complement other plant protein flour to improve the overall quality of the mixture. The expression of amino acid was kept at g kg⁻¹ for the ease of comparison with amino acids in corn grain and other cereal products.

Lines 179-182 Variations might depend also on the variety of corn used.

The corn cobs used in this study are of mixed variety and at Section 2.2, the statement “of mixed variety” was added in Line 64 – 66. In addition, an extra statement was added in Line 157 – 158 to explain the variation due to mixed variety.

Line 182 Eliminate extra period sign at the end of this line.

This has now been corrected as suggested (Line 190)

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This manuscript is very interesting. Probably a stronger statement regarding the usefulness of SCC as a byproduct, that is usually burnt or used as animal feed, might help to raise awareness and the possibility to use it more as a source of these fine chemicals.

Regarding the protein quality discussion, perhaps adding a comparison between the amino acid composition of the cob and the grains of corn might help, in order to have a richer discussion.

For Peer Review