

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

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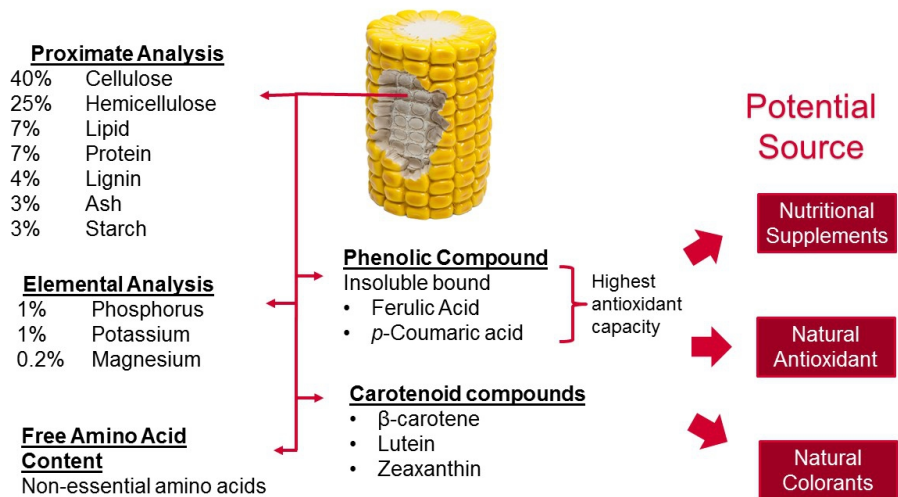


Valorization of sweet corn (Zea mays) cob by extraction of valuable compounds

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Graphical Abstract

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3 **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.

23 1.0 INTRODUCTION

24 Corn is one of the most important cereal crops globally ([Shiferaw, Prasanna, Hellin,](#)
25 [& Bänziger, 2011](#)). [Kim and Dale \(2004\)](#) reported that the world annual production is about
26 520×10^9 kg and North America (42%), Asia (26%), Europe (12%) and South America (9%)
27 are the main producers. Most sweet corns are processed into frozen corn kernels, canned corn
28 kernels or corn cobettes, resulting in the production of large amounts of by products, which
29 includes the corn silks, husks and cobs. For every 100kg of corn grain obtained,
30 approximately 18kg of corncobs are produced, most of which is used as animal feed, or
31 remain unused as lignocellulosic waste ([Torre, Aliakbarian, Rivas, Domínguez, & Converti,](#)
32 [2008](#)). Previous studies on corncobs have focused on the production of bioethanol ([Chen, Xia,](#)
33 [& Xue, 2007](#)), bio-oil and bio-char ([Mullen et al., 2010](#)), solid biofuel ([Ioannidou et al., 2009](#))
34 and xylooligosaccharides ([R. Yang, Xu, Wang, & Yang, 2005](#))

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

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3 48 SCC may also be a good source of phenolic compounds and different extraction approaches
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5 49 have been tested including alkaline hydrolysis ([Ares-Peón, Garrote, Domínguez, & Parajó,](#)
6
7 [2016; Torre et al., 2008](#)), ultrasonic extraction ([Lai, Li, Wu, & Li, 2012](#)), surfactant-based
8 50
9 cloud-point extraction ([Dhamole, Demanna, & Desai, 2014](#)) as well as enzymatic hydrolysis
10 51
11 ([Pérez-Rodríguez, Torrado Agrasar, & Domínguez, 2017](#)) showing the presence of ferulic
12 52
13 and *p*-coumaric acid as well as carotenoids.
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18 54 The main objective of this study was to investigate the proximate, minerals and
19 55
20 phytochemical composition of SCC. In addition, the potential of supercritical fluid extraction
21 56
22 to extract carotenoids from of SCC was also assessed.
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25 57 **2.0 MATERIALS AND METHODS**

26 58 **2.1 Chemicals and reagents**

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30 59 Ferulic acid (>99%), *p*-coumaric acid (>99%), gallic acid (97.5%) and ascorbic acid
31 60
32 were purchased from Sigma Aldrich. β -carotene (>98%), lutein (>95%) and zeaxanthin
33 61
34 (>98%) were obtained from Extrasynthese Company, Genay, France. All other reagents and
35 62
36 chemicals used in this experiment were of analytical grade.
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41 63 **2.2 Sample Preparation**

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44 64 The preliminary studies on the extraction of free phenolics (Section 2.4) were carried
45 65
46 out using sweet corn of mixed variety purchased from Sainsbury Supermarket (Reading, UK)
47 66
48 in January 2015. The SCC used for the rest of the experiments was harvested in Senegal
49 67
50 (mixed variety) in December 2015 and was provided by Barfoots of Botley Company Ltd
51 68
52 (UK). Corn kernels were removed manually from the cobs using a knife and discarded. The
53 69
54 SCC were then chopped into 5cm pieces in length, placed in the blast freezer (-18°C, 1 hour)
55 70
56 and then freeze dried (Christ Gamma 2-16) until constant weight was achieved. The dried
57 71
58 samples were finely ground in a mill (Apex Comminuting Mill), sieved through a 150 mesh
59
60

1
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3 72 screen (<0.1mm particle size), thoroughly mixed and stored in the freezer (-80°C) until
4
5 73 further analysis.
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7

8 74 **2.3 Proximate composition and minerals analysis of SCC**

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10
11 75 Moisture content was determined by using the Mettler Toledo halogen moisture
12
13 76 analyser (Model: HE73). Ground SCC was analysed for proximate composition by AOAC
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15 77 method for protein (979.09), lipid (963.15) and ash (923.03) ([AOAC, 2005](#)). Structural
16
17 78 carbohydrates and lignin (Klason and acid-soluble) were determined by the NREL procedure
18
19 79 ([Sluiter et al., 2008](#)). Starch content was determined by amyloglucosidase method using
20
21 80 Megazyme total starch assay kit ([J. H. Li, Vasanthan, Rossnagel, & Hoover, 2001](#)). Analysis
22
23 81 of free amino acids content was carried out in accordance to [Elmore, Koutsidis, Dodson,](#)
24
25 82 [Mottram, and Wedzicha \(2005\)](#). Minerals including calcium, magnesium, iron, zinc, copper,
26
27 83 manganese, sodium and potassium were extracted from the samples by dry ashing method
28
29 84 and determined by atomic absorption spectrophotometer (novAA[®] 350) as described in
30
31 85 AOAC 985.35 ([AOAC, 2005](#)). Phosphorus was determined spectrophotometrically as
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33 86 described in AOAC 995.11 ([AOAC, 2005](#)).
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40 87 **2.4 Extraction of free, esterified and insoluble-bound phenolic compounds in SCC**

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42 88 The extraction of free, esterified and insoluble-bound phenolic compounds in SCC
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44 89 was carried out according to the method described by [Sosulski, Krygier, and Hogge \(1982\)](#).
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46 90 **The free phenolic fraction of SCC was extracted based on the preliminary findings indicating**
47
48 91 **that the best extraction condition was using 50% ethanol for an hour (see supplementary**
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50 92 **material, Figure S1).**
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55 93 **2.5 Determination of total phenolic content**

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57
58 94 The phenolic content was determined using the Folin-Ciocalteu method as described
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60 95 by [Singleton and Rossi \(1965\)](#). The absorbance was measured at 760nm with a

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2
3 96 spectrophotometer (CE1021, Cecil), and the phenolic content was expressed as g gallic acid
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5 97 equivalents per kg of dry weight (g GAE kg⁻¹ DW). The calibration curve was established
6
7 98 using gallic acid (50-1000mg/L) as the standard sample (R²= 0.9993).
9

10 99 **2.6 HPLC analysis of phenolic compounds of SCC**

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14 100 The analysis of phenolic compounds in **the free, esterified and insoluble bound**
15
16 101 **fractions** were carried out using HP Agilent 1050 liquid chromatography, equipped with a
17
18 102 DAD detector. The separation of the phenolic compounds was performed using a Zorbax SB-
19
20 103 C18 column (2.1 x 15mm, 1.8 micron). The mobile phase was (A) formic acid/HPLC water
21
22 104 (0.1:100 v/v) and (B) formic acid/acetonitrile (0.1/100 v/v). Solvent B was increased to 25%
23
24 105 (0- 25 min), followed by 90% B for 30 minutes and then a final wash of 100% B for 10
25
26 106 minutes. The injection volume was 5µL with a flow rate of 0.2mL/min. Identification was
27
28 107 carried out by comparing the retention time to the corresponding standards at 280nm and the
29
30 108 amount of individual phenolic compounds (ferulic acid and *p*-coumaric acid) was calculated
31
32 109 using an external calibration curve (R² = 0.9998 for both ferulic and *p*-coumaric acid).
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37 110 **2.7 Determination of antioxidant activities**

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40 111 The trolox equivalent antioxidant capacity (TEAC) assay and 2,2- diphenyl-1-
41
42 112 picrylhydrazyl (DPPH) radical assay were conducted as described by [H. B. Li, Wong, Cheng,](#)
43
44 113 [and Chen \(2008\)](#) and [Zhao, Li, Liu, and Yang \(2014\)](#), respectively. The standard curve for
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46 114 TEAC (R² = 0.987) and DPPH (R² = 0.989) assay were constructed using Trolox at different
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48 115 concentrations (50 – 1000µmol for TEAC and 1 – 170µmol for DPPH). The results were
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50 116 expressed as mmol trolox equivalent (TE)/kg sample.
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55 117 The reducing ability of the extracts was determined using the FRAP assay according
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57 118 to [Benzie and Strain \(1996\)](#) with slight modifications. A total of 10µL of standard, blank
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59 119 (water) or sample were added to each well in a 96-well microtiter plate (Cellstar®). Then,
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3 120 300µL of FRAP reagent was added and the absorbance of the reaction mixture was read at
4
5 121 595nm using GENio Pro™ microplate reader with Magellan software. The standard curve
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7 122 ($R^2= 0.9997$) was constructed using ascorbic acid solution (50-1000µmol). Results were
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9 123 expressed as mmol ascorbic acid (AA)/kg DW.
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13 124 **2.8 Extraction and identification of carotenoid compounds**

15 125 **2.8.1 Conventional extraction**

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19 126 This extraction was based on the method described by [Gorocica-Buenfil, Fluharty,](#)
20
21 127 [Bohn, Schwartz, and Loerch \(2007\)](#). The extracts were re-dissolved in ethanol and were
22
23 128 stored at -18°C until HPLC analysis.
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26 129 **2.8.2 Supercritical fluid extraction (SFE)**

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29 130 Supercritical fluid extraction was carried out in a SFE unit (SciMed, UK), according
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31 131 to [Goto, Kanda, Wahyudiono, and Machmudah \(2015\)](#) with slight modification. SCC powder
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33 132 (5g) was loaded into the extraction vessel. The extraction pressure (350 bars) was controlled
34
35 133 and the temperature was maintained at 60°C throughout the extraction. Carbon dioxide was
36
37 134 fed into the extraction vessel at 15g/min. During the extraction (1 hour), a flow of 15%
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39 135 ethanol was pumped into the system to act as a co-solvent.
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44 136 **2.8.3 HPLC analysis of carotenoid compounds in SCC**

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46
47 137 The analysis of carotenoids present in SCC was performed using a YMC-C30 silica-
48
49 138 based reversed-phase column (250 x 4.6 mm) coupled with a 1260 DAD detector (Agilent
50
51 139 Technologies, UK). The mobile phases were (A) methanol/MTBE/water (82:16:2 v/v/v) and
52
53 140 (B) methanol/MTBE/water (23:75:2 v/v/v). The analysis followed a gradient program for the
54
55 141 mobile phases of 0 min 0% B, 45 min 50% B, 55 min 100% B, 60 min 100% B.
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57 142 Identification was carried out at 450nm by comparing the retention time to the corresponding
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59
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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,](#)

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3 167 [Chen, Gao, & Li, 2001](#)) and stover ([Weiss, Farmer, & Schell, 2010](#)). These results suggest
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5 168 that SCC can be a good source of insoluble dietary fibre.
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8 169 To date, SCC has mainly been used as a carbohydrate source but it may also be an
9
10 170 potential source of protein and minerals. There is an increasing demand for more insight on
11
12 171 the potential of plant-based protein ([Iqbal et al., 2006](#)). A total of 18 free amino acids were
13
14 172 identified in SCC (Table 1) with serine and glutamine as the predominant ones followed by
15
16 173 alanine, proline, aspartic and glutamic acid. Overall, the free amino acid content accounted
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18 174 for 0.7% of the composition in SCC, although free arginine was not measured. **As compared**
19
20 175 **to the essential amino acids present in corn grain** ([Belyea et al., 2004](#)), **SCC contained lower**
21
22 176 **amount of essential amino acids as compared to corn grain. However, the essential amino**
23
24 177 **acids (including lysine, tryptophan, histidine) and non-essentials amino acid (including serine,**
25
26 178 **glutamine and glutamic acid) present in SCC is higher than other cereal products such as**
27
28 179 **whole grain rye, rye bran, wheat bran, barley flour and oat bran** ([Mustafa, Aman, Andersson,](#)
29
30 180 [& Kamal-Eldin, 2007](#)). Therefore, SCC can be used to **complement** other plant protein
31
32 181 sources to increase the overall protein quality of the mixture. [Young and Pellett \(1994\)](#)
33
34 182 reported an improvement on the protein quality of corn and soy flour, when used in
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36 183 combination.
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43 184 The total mineral content measured in this study was 2.18%, which correlated well
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45 185 with the ash content value (3.01%) in SCC. Phosphorus ($10.12 \pm 0.06 \text{ g kg}^{-1} \text{ DW}$) was the most
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47 186 abundant mineral, followed by potassium ($9.62 \pm 0.21 \text{ g kg}^{-1} \text{ DW}$) and magnesium ($1.67 \pm 0.10 \text{ g}$
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49 187 $\text{kg}^{-1} \text{ DW}$). Calcium, lead, zinc, manganese, copper and iron were also present in SCC but in
50
51 188 lower amounts. The quantity of all minerals were higher in the SCC analysed in the present
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53 189 study compared to those reported previously by [Abubakar et al. \(2016\)](#), [Anukam, Goso,](#)
54
55 190 [Okoh, and Mamphweli \(2017\)](#) and [Awosusi et al. \(2017\)](#). The minerals and trace elements
56
57 191 content may vary in plants due to the influence of environmental conditions such as presence
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3 192 of light, water availability elevated CO₂, elevated ozone levels and agricultural technologies
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5 193 ([Nour, Trandafir, & Cosmulescu, 2014](#)).

194 3.2 Phenolic composition and antioxidant activity of SCC

195 3.2.1 Total Phenolic content and antioxidant activity of SCC

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

204 3.2.2 Quantification of ferulic and *p*-coumaric acid in SCC

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

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

1
2
3 215 the insoluble-bound fraction (0.42g kg^{-1}), followed by soluble conjugated fraction (0.01g kg^{-1})
4
5 216 and free fraction (0.00105g kg^{-1}). Furthermore, previous investigations have shown that the
6
7 217 ferulic acid and *p*-coumaric acid content in yellow corn grain ranged from 0.006 to 1.80g kg^{-1}
8
9 218 and 0.00012 to 0.00050g kg^{-1} , respectively ([Assabgui, Reid, Hamilton, & Arnason, 1993](#); [Hu](#)
10
11 219 [& Xu, 2011](#)). Furthermore, the ferulic and *p*-coumaric acid in agricultural crop residues such
12
13 220 as flax shives (0.25 and 0.61g kg^{-1} , respectively) and wheat bran (3.91 and 0.2g kg^{-1} ,
14
15 221 respectively) ([Buranov & Mazza, 2009](#)) were found to be lower, as compared to SCC. This
16
17 222 research showed that all three phenolic fractions contained higher amount of ferulic acid as
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19 223 compared to sweet corn kernels and other agricultural crop residues suggesting that SCC can
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21 224 be a good source of ferulic acid and *p*-coumaric acid.
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27 225 3.3 Extraction and characterization of carotenoid compounds

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29
30 226 In this study, lutein, zeaxanthin and β -carotene were the three carotenoids identified
31
32 227 and quantified in SCC (Table 4). β -carotene was the main carotenoid in SCC followed by
33
34 228 zeaxanthin and lutein. Supercritical fluid extraction (SFE) was able to extract high amounts
35
36 229 of carotenoids, as compared to conventional extraction. No correlation was found between
37
38 230 carotenoid content and antioxidant activity in all three assays (data not shown) thus the
39
40 231 contribution of carotenoid content in SCC towards antioxidant activity was assumed
41
42 232 negligible. Similarly, [Gil, Tomás-Barberán, Hess-Pierce, and Kader \(2002\)](#) found no
43
44 233 correlation between carotenoid and antioxidant activity of nectarines, peaches and plum.
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49 234 Previous studies carried out by [Kurilich and Juvik \(1999\)](#) reported that the carotenoids
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51 235 present in sweet corn kernels included zeaxanthin (2.16 mg kg^{-1}), lutein (5.95 mg kg^{-1}), and
52
53 236 β -carotene (0.68mg kg^{-1}). Our results showed that there were higher levels of zeaxanthin and
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55 237 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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264 5.0 CONFLICT OF INTEREST

265 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 ($p < 0.05$).

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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 ± 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*

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)*

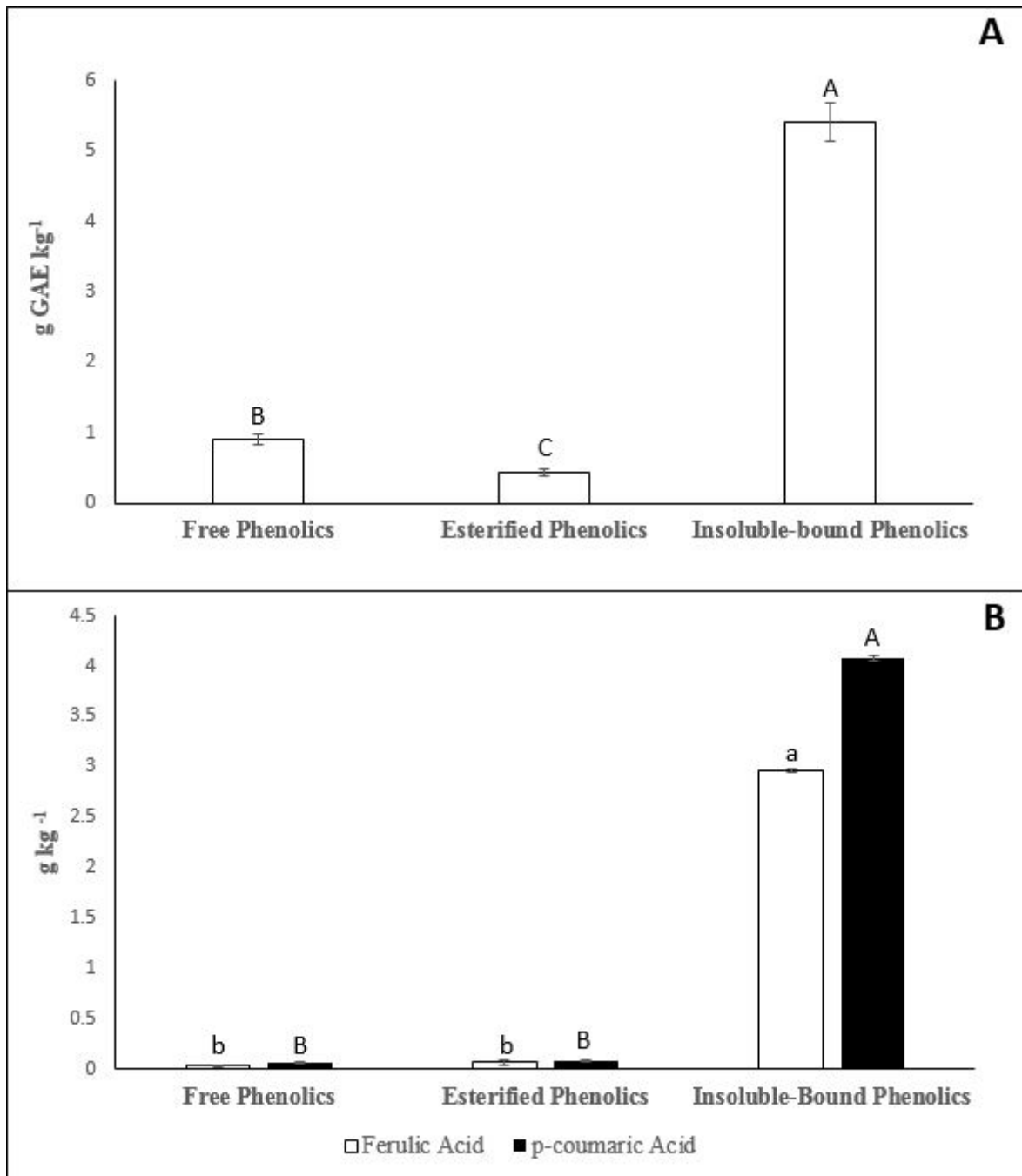


Figure 1

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 \pm 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.

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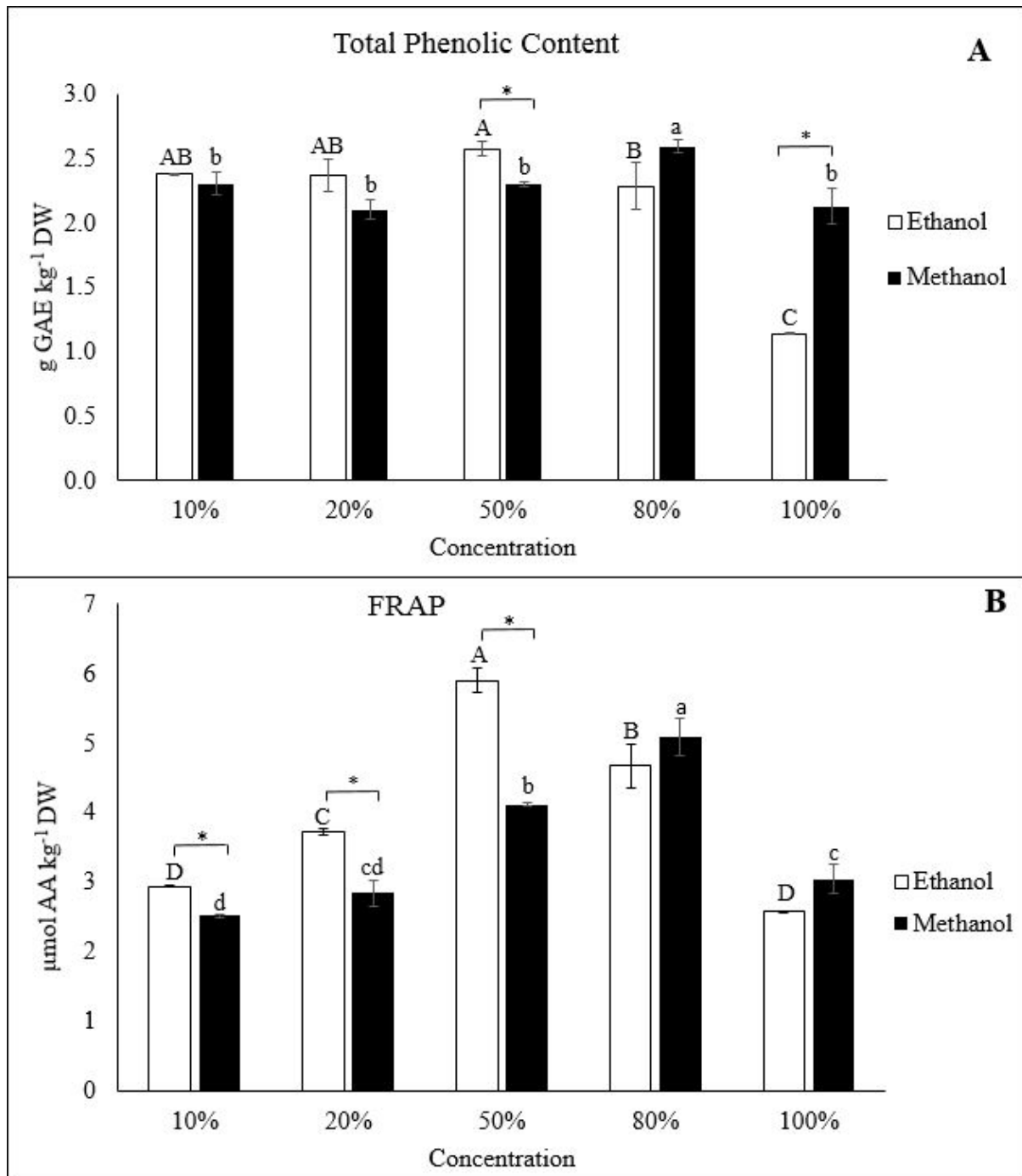


Figure 1

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3 Referee(s)' Comments to Author:
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9 Comments to the Author
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11 Abstract
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15 L7: Units g GAE kg⁻¹ ...please use the same notation in all the manuscript as
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17 required by the author's guide.
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20 All units are now based as per kg throughout the manuscript as required by the author's guide.
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23 L6 and L11-13: In my opinion, the amounts of protein and minerals found in SCC are low to
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25 considered as a "good" or "promising" source of these components. On the other hand, the
26
27 comparison of ferulic content between the SCC and sweet corn kernels in the results and
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29 discussion section don't contribute to this asseveration.
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33 I suggest revise your results and consider to use other words.
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36 The amount of ferulic acid in both sweet corn cob and sweet corn kernels has been revised
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38 concluding that the content of ferulic acid in corn kernels (0.42g kg⁻¹ of insoluble bound,
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40 0.01g kg⁻¹ of soluble conjugated and 0.001g kg⁻¹ of free ferulic acid) is lower as compared to
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42 sweet corn cob. Hence, SCC can be a potential source of phenolic compounds (Line 209 to
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44 212).
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51 Introduction
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54 L48: Please revise the citation of Lai et al 2012. The correct first name is Fu-Rao
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57 This has now been corrected (Line 332)
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22 L164-165: I'm not sure about the SCC could be a "good" source of protein and minerals. The
23
24 amounts of protein and minerals reported are low (6.7 and 2.18 %) compared with other
25
26 sources. I suggest delete the term "good" or use a more appropriate word.
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30 Suggestion of SCC could be a “good source of protein and minerals” has been removed from
31
32 the manuscript.
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35
36 L187-191: Extraction time... It is not clear which solvent (ethanol, methanol or both) was
37
38 used in this part. Please specify. If both were proved, the same effect on the extraction time
39
40 was observed? Any explanation for these results?
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43 This section has been removed to fulfil the requirement for word count. The graphs have been
44
45 added to supplementary data for reference (Line 87-90).
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49 L192-193: This sentence is incorrect. Please discuss the results carefully. First, there are no
50
51 significant differences in phenolics between ethanol and methanol using 10%, and a means
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53 comparison between treatments is necessary to be sure about the differences between
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55 treatments using 20 and 80 % concentrations. This is the same case of FRAP at 80%.
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5 added to supplementary data for reference. (Line 87-90)
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9 L193-195. Incorrect. This trend is true only for FRAP. In phenolics, there are no significant
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11 differences between 10, 20 and 50%, and between 20 and 80%. The only clear trend observed
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13 in both variables is a decrease from 80 to 100 %.
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16 This section has been removed to fulfil the requirement for word count. The graphs have been
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18 added to supplementary data for reference. (Line 87-90)
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21 L195-196: Please re-write your discussion according to the statistical analysis, not based on
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23 the visual trends.
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26 This section has been removed to fulfil the requirement for word count. The graphs have been
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28 added to supplementary data for reference. (Line 87-90)
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31 L192-201: There is a lack of discussion and explanation about these results. For example,
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33 what could be the reason for the decreasing of phenolics extraction using a concentration
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35 higher than 50% for ethanol and higher than 80% for methanol? Any hypothesis?
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39 This section has been removed to fulfil the requirement for word count. The graphs have been
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41 added to supplementary data for reference. (Line 87-90)
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44 L225-227. According to previous lines 221-222. sweet corn kernels have a significantly
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46 higher amount of ferulic than those your results.
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49 The amount of ferulic acid has been corrected (Line 209 to 2121).
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52 L252: please, if possible use a more recent reference
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55 A more recent reference has been used (Line 245) by Yuan et al., (2008).
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3 YUAN, Y., GAO, Y., ZHAO, J. & MAO, L. 2008. Characterization and stability evaluation
4 of β -carotene nanoemulsions prepared by high pressure homogenization under various
5 emulsifying conditions. *Food Research International*, 41, 61-68.
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10 L256-259: please, consider a careful revision of this conclusion based on the previous
11 comments and the results.
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16 Conclusion has been altered slightly to show that SCC can be a promising source of
17 carotenoid and phenolic compounds (Line 252 – 255).
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20 21 22 23 24 References

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27 L277: Names are incorrectly written
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30 These have now been corrected as suggested (Line 269).
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33 L281: The correct citation is: *Phytopathology*, 83, 949-953.
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36 This has now been corrected as suggested (Line 274).
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39 L329: use "Food" instead of "food"
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42 This has now been corrected as suggested (Line 330).
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45 L330: Please revise carefully the reference. Names are incorrectly written as well as the
46 volume number and the final page number.
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50 These have now been corrected as suggested (Line 331)
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53 L344. use the capital letter in "l"-lactic
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56 This has now been corrected as suggested (Line 345)
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59 L361: pages number incomplete
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3 These have now been corrected as suggested (Line 363)
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6 L365-367: please complete the reference
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9 This has now been corrected as suggested (Line 367-369)
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12 L370: Use "Food" instead of "food"
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15 These have now been corrected as suggested (Line 372)
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18 L374: separate VAN HASSELT; use "leaf" instead "lead"
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21 This reference has been replaced by a more recent reference by Yuan et al. (2008) (Line 392).
22
23

24 YUAN, Y., GAO, Y., ZHAO, J. & MAO, L. 2008. Characterization and stability evaluation
25 of β -carotene nanoemulsions prepared by high pressure homogenization under various
26 emulsifying conditions. *Food Research International*, 41, 61-68.
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30 L383: the last page should be 1212S
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33 This has now been corrected as suggested (Line 391)
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36 Table 1: What do the letters aside the SD mean?
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39 Superscripts in Table 1 indicate significantly different at $p \leq 0.05$. This has now been added to
40 the footnote of Table 1.
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44 Figure 1: Please, describe what does the capital and lowercase letter above the bars means
45 specifically.
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49 Figure moved under Supplementary date to fulfil the requirement for word count.
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9 Materials and Methods
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11
12 Page 5, lines 65-66: The manually removing of the kernels from the cob was doing with a
13 knife?. If yes, please clarify this. To have clear that the cob that was used in the study
14 simulated what is discharged in the industry.
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20 Sample preparation now reads “Corn kernels were removed manually from the cobs using a
21 knife and discarded” (Line 67).
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25 Page 5, lines 84-91: authors talk about optimization of extraction. However, they do not
26 mention the statistical model used to test that the extraction conditions chosen was the best. It
27 could be better to eliminate the term “optimization” in this section
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32 Section 2.4 has been removed to fulfil the requirement for word count.
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35 Page 6, lines 103-112. Authors need to precise the phenolic compounds analyzed with the
36 HPLC. Phenolic acids. Did you run the analysis at 280 nm only?. Ferulic and *p*-coumaric
37 acids are derivatives from cinnamic acid and they are commonly quantified at 320 nm. Which
38 phenolic compounds fraction did you use for HPLC analysis?, the free?, the esterified? The
39 insoluble-bound?
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47 The analysis were run at 280, 320 and 365nm and 280nm was chosen as both ferulic and *p*-
48 coumaric acid showed their highest absorbance (max λ) at this wavelength. The phenolic
49 fractions subjected to HPLC analysis are now listed in Section 2.7, line 98.
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54 Page 7, lines 124-126: why do you use ascorbic acid and not Trolox or ferrous sulfate?
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3 Several standards including ascorbic acid, trolox and ferrous sulphate are normally used in
4 FRAP assay. In our study, a well-known antioxidant, ascorbic acid, was chosen following our
5 standard laboratory SOP.
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13 Results and Discussion

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16 Page 10-11, lines 207-210: The correlations coefficients are very high, however, these are
17 going to be affected by the number of samples that are involved in the analysis. Commonly,
18 as the number of samples increase the values of Pearson coefficient decrease. How many
19 samples do you use for the Pearson correlation analysis?
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26 In Section 2.9, details have been added in for Pearson correlation analysis (Line 148 to 149).

27 Three samples were used for Pearson correlation analysis.
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32 Page 11, lines 212-213; Please check the redaction of this paragraph, you mentioned three
33 fractions of SCC, and presented only two values.
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37 Line 202 to 203 stated the total ferulic and *p*-coumaric acid of SCC including all three
38 fractions (free, esterified and insoluble-bound)
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42 Page 11, lines 212-227: In corn grain the insoluble bound fraction contains higher values of
43 the proportion ferulic acid/*p*-coumaric acid. In this study the value of this proportion is lower
44 than 1.0. How do the authors explain this result? Why do not compare the values obtained for
45 phenolic compounds and carotenoids in corn cob with other sources currently in use for the
46 commercial extraction of these compounds. By doing so, one could clearly see if the corn cob
47 has the potential to compete in these uses.
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56 The comparison of phenolic and carotenoid content with other crop by products were
57 compared to highlight the potential of SCC to be used as a source of phenolic and carotenoid
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3 compounds. For phenolic content, comparison has been made with flax shives and wheat
4 bran (Line 215 to Line 217). In addition, carotenoid content of SCC was compared with other
5 vegetable by-products including tomato seed, as well as tomato and persimmon peel (Line
6 248-250).
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For Peer Review

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11 Comments to the Author
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14 Please see the following suggestions:
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20 Lines 157 – 163 Knowing the variety of corn used as source of SCC could further explain
21 deviations in composition. If samples included mixed varieties, this might also be a source of
22 variation. If the latter is the case, please state it in M&M. It would not constitute a problem,
23 because generally, this could be a normal case in the future when this process might be
24 applied in a larger scale. In spite of possible variations coming from different sources of cobs,
25 the method of extraction proposed is valid.
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34 *At Section 2.2, the statement “of mixed variety” was added in Line 64 – 66.*
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37 Line 161. Hemicellulose content is reported for the sample analyzed: state the method used in
38 M&M section.
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42 *The method for the determination of hemicellulose content was included in Section 2.3 (Line*
43 *75 – 77) and followed the NREL procedure as described by Sluiter et al. (2008).*
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47 *SLUITER, A., HAMES, B., RUIZ, R., SCARLATA, C., SLUITER, J., TEMPLETON, D. &*
48 *CROCKER, D. 2008. Determination of Structural Carbohydrates and Lignin in Biomass. In:*
49 *GOLDEN, N. R. E. L. (ed.). Colorado.*
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54 Line 170 Complement might be more appropriate than compliment
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58 *This has now been corrected as suggested (Line 178).*
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3 Lines 171-172. The comment regarding “Young and Pellett (1994) reported an improvement
4 on the protein quality of corn and soy flour, when used in combination” is made for corn and
5 soybean flours. A more detailed discussion comparing the composition of SCC with corn
6 flour (from grains) might be useful and very interesting for the readers, as well as the
7 expression of amino acid content per g or 100g of protein might also help comparisons with
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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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3 This manuscript is very interesting. Probably a stronger statement regarding the usefulness of
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5 SCC as a byproduct, that is usually burnt or used as animal feed, might help to raise
6
7 awareness and the possibility to use it more as a source of these fine chemicals.
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13 Regarding the protein quality discussion, perhaps adding a comparison between the amino
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15 acid composition of the cob and the grains of corn might help, in order to have a richer
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17 discussion.
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