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High phytosterol levels in corn cobs point to their sustainable use as a nutritional source

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Abstract

Phytosterols are important structural components of plant cells that affect membrane fluidity, permeability, and membrane-related metabolic regulation. These compounds, which are abundant in vegetable oils and corn kernel oil, are also beneficial for human health. Cultivation of corn (*Zea mays* L.) produces huge amounts of cobs as a by-product, but efforts to utilize cobs are still limited. Here, we investigated phytosterol, crude oil, and fatty acid contents in the kernels and cobs of four major corn cultivars in South Korea and explored the potential use of cobs as a source of phytosterols. Total phytosterol levels were two times higher in cobs (68.0–217.1 mg 100 g^{-1} DW) than in kernels (43.8–89.5 mg 100 g^{-1} DW) and were highest in the kernels and cobs of Sinhwangok at 60 days after pollination. We showed that not only kernels but also cobs can be a rich source of phytosterols. The results also revealed that the amount of phytosterol is depending on a genetic background as well as developmental stages suggesting further investigation would enhance the utilization of corn cobs as a phytosterol source.

Keywords: Maize, Cob, Phytosterol, β-sitosterol, Campesterol, Stigmasterol

Introduction

Phytosterols are not only important structural components of plant cells involved in regulating membrane fluidity, permeability, and membrane-related metabolism but also signaling molecules regulating diverse development processes [1–3]. Phytosterols are derived from squalene 2, 3-oxide and are found in a variety of terrestrial and aquatic plants [4]. Most phytosterols are 4-desmethylsterols; β-sitosterol, campesterol, and stigmasterol are particularly abundant in plants [2]. Different plant species have their own balance of phytosterols, depending on their needs during growth and development [5, 6]. For example, total phytosterol contents are high in oil seeds such as nuts and rapeseed. Vegetable oils from soybean (*Glycine max* (L.) Merr.), wheat germ (*Triticum aestivum* L.), and corn (*Zea mays* L.) also contain high amounts of phytosterols [7]. Cereals are the main sources of phytosterols for the human diet, even though their phytosterol contents are not high, the large amount of cereal consumption in the average human diet provides more than 40% of daily phytosterol requirements [2, 8].

Corn, one of three major cereal crops, has the highest production among cereals worldwide, reaching 1,162 Mt of grain in 2020 (FAO, http://www.fao.org/faostat). However, for every 1 kg of dry corn grain produced, approximately 0.15 kg of cobs is also produced; therefore, about 174.3 Mt of corn cobs can be estimated to be produced in 2020. Corn cobs are mainly composed of cellulose (45%–55%), hemicellulose (25%–35%), and lignin (20%–30%) [9, 10]. Corn cobs are largely considered to be waste products, and efforts to use this agricultural waste have thus far been limited [11]. Studies on the use of corn cobs would enhance agricultural sustainability.

Phytosterols are also beneficial for human health, especially for liver health, lowering cholesterol, and for preventing diabetes and other chronic diseases

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[12]. Promising evidence for the anticancer activities of phytosterols has also been reported [13, 14]. In traditional Korean medicine, the water from boiling corn cobs is used for gargling to treat periodontal disease. Indeed, some studies have shown that phytosterols from corn cobs are beneficial for treating gum disease [15]. Recently, phytosterols from an unsaponified fraction of corn oil are used as periodontal health supplementals [16, 17]. Phytosterol contents in corn cobs depending on developmental stages and genetic backgrounds are very limited compared to those in kernels [18]. In addition, vegetable oil contents are highly affected by growing condition, cultivars, and seed maturation stages [19]. The major sources of commercially available phytosterols are tall oil (derived from wood pulp) and vegetable oils (corn oil, canola oil) [20]. However, these sources cannot satisfy increasing commercial demands [21]. Therefore, in this study, we analyzed phytosterol contents in cobs and kernels from four corn cultivars at three time points to explore the possibility of utilizing corn cobs as a source of phytosterols.

Materials and methods

Plant materials

Four corn (*Zea mays*) cultivars, Sinhwangok, Kwangpyeongok, Hwangdaok, and Jangdaok, were used in this study. These dent corns were developed by the Rural Development Administration and are major F1 hybrid cultivars for grain production in South Korea [22, 23]. Seeds were sown in the National Institute of Crop Science experimental field (37°16′19.2"N 126°59′34.3"E, Suwon, Republic of Korea) on April 21, 2020 and sampled at 0, 30, and 60 days after pollination (DAP). The planting distance was 60 × 25 cm, and 15–3-6–2000 kg N-P_2O_5-K_2O-compost 10 a^{-1} was applied as fertilizer. The four cultivars were randomly placed in three repetitions. The three sampling time points were as follows: 0 DAP ears (referred to as 0 DAP cobs hereafter), collected when the silks were fully developed, and the silks removed prior to sampling; 30 DAP kernels and cobs, collected during the middle of kernel maturation; and 60 DAP kernels and cobs, collected when the kernels were at physiological maturity (R6 stage) and ready for harvest [24]. The ears of each variety were sampled at 0, 30, and 60 DAP from the three repetitions. The harvested ears were separated into cobs and kernels, freeze-dried, and ground with a coffee grinder. The samples were refrigerated at 4 °C until analysis. The sample names were abbreviated using two letters and a number; for example, SC30 stands for Sinhwangok cobs at 30 DAP.

Determination of crude oil contents

The crude oil content was quantified using a Soxtherm Automatic System (Gerhardt; Bonn, Germany) [25]. Three grams of freeze-dried tissue in 140 mL hexane with boiling stones was placed in an extraction thimble and heated at 180 °C for 30 min to elute the crude oil from the sample. Extraction was repeated 5 times for 80 min, and the solvent was collected. After drying at 105 °C for 1 h, the extraction thimble containing the eluted crude oil was cooled, and the weight was measured to determine the crude oil content.

Analysis of fatty acid composition

The fatty acid composition was analyzed as described by Garcés and Mancha [26]. Each 0.5 g powdered sample was heated at 80 °C in 2 mL of the mixed solvent methanol:heptane:benzene:2,2-dimethoxypropane:H_2SO_4 (37:36:20:5:2, by volume). After the reaction, the sample was cooled to room temperature, and the supernatant, including fatty acid methyl esters (FAMEs), was collected and analyzed by gas chromatography (GC). GC analysis was performed using an Agilent HP-Innowax capillary column (30 m × 0.25 mm × 0.25 μm; Palo Alto, CA, USA) on a Shimadzu GC-2010 plus (Kyoto, Japan). The oven program was set at an initial temperature of 150 °C to a final temperature 240 °C with an increase of 3 °C min^{-1}. The injector and FID temperatures were set at 250 and 260 °C, respectively, and the analysis was performed in split mode with a split ratio of 10:1. The FAME standard mixture (C8-C22) was purchased from Supelco FAME (Bellefonte, PA, USA).

Quantitative analysis of phytosterols

The phytosterol contents of corn cobs and kernels were quantified by GC following saponification of the sample [27]. Each freeze-dried powdered sample (100 mg) was combined with 4 mL of ethanol and 1 mL of 0.1 N ethanolic KOH and saponified at 95 °C for 1 h. After the saponification reaction, the samples were cooled to room temperature, and 5 mL of saturated NaCl and 10 mL of hexane were added to each sample. The supernatant was recovered after vigorous stirring. Extraction was repeated three times, and the recovered supernatants were concentrated with nitrogen. After adding 1 mL of hexane and filtration through a 0.45 μm syringe filter, the samples were analyzed by GC using a Shimadzu GC-2010 plus (Kyoto, Japan) and an Agilent DB-5MS UI (30 m×0.25 mm×0.25 μm; Palo Alto, CA, USA) column. The injector and FID temperatures were set to 270 and 300 °C, respectively, and the oven temperature was increased from 105

to 272 °C at a rate of 20 °C min^{-1}. Campesterol, stigmasterol, and β-sitosterol, which were used as standards, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

All measurements were performed with three biological replicates, and the values were expressed as mean ± standard deviation. ANOVA and correlation analysis were performed with JAMOVI v1.2.27 (jamovi.org). Principal components analysis (PCA) was performed on the autoscaled phytosterol profiles and crude oil contents. Hierarchical cluster analysis (HCA) was based on Euclidean distance. PCA and HCA were performed with MetaboAnalyst (metaboanalyst.ca) [28].

Results

Corn kernels have higher crude oil contents than cobs

Corn kernels had 5 to 36 times more crude oil than corn cob samples, depending on the genetic background and developmental stage. The crude oil contents of cobs were 127.8–1331.1 mg 100 g^{-1} DW, and those of kernels were 3735.6–6372.2 mg 100 g^{-1} DW. The crude oil content was lowest in HC30 (127.78 mg 100 g^{-1} DW) and highest in KK60 (6372.22 mg 100 g^{-1} DW). On average, cob samples contained 0.69% crude oil of total dry weight, whereas kernel samples contained 4.67% crude oil, which is 6.7 times more crude oil compared to cobs (Fig. 1A). In three of the four cultivars examined, 60 DAP kernels contained more crude oil than 30 DAP kernels, and Hwangdaok showed a slight decrease in crude oil contents in 60 DAP kernels. Since embryos contain more oil than endosperm, these changes in oil content in kernels might be related to the timing of embryo development [29].

In corn cob samples, the amount of oil was highest at 0 DAP and decreased after pollination as the cobs aged. Two cultivars (Sinhwangok and Kwangpyeongok) showed continuous decreases in oil contents, and the two other cultivars showed the lowest oil levels at 30 DAP. These results might be related to the differences in developmental programs of the cultivars.

Previously reported oil contents in corn kernels range from 3 to 4% [29, 30]. The cultivars used in this study showed a substantial range of genetic variation in crude oil contents. Since oil content in corn cobs during development has not been studied, the information from this study should be helpful for understanding the cob and kernel interaction during seed development.

Corn cobs undergo significant changes in fatty acid composition during maturation

There were clear distinctions in fatty acid composition between corn cobs and kernels. The proportions of palmitic (C16:0) and linolenic (C18:3) acids were markedly higher in cobs than in kernels. The palmitic acid contents ranged from 23.4%–37.7% (average 30.9%) in cobs and 12.7%–20.5% (average 15.4%) in kernels, and the linolenic acid contents were 1.5%–7.6% (average 5.1%) in cobs but only 0.6–2.6% (average 1.1%) in kernels. The changes in fatty acid composition appeared to be more dynamic in cob samples compared to kernels. The cobs underwent significant changes in fatty acid composition, especially between 30 and 60 DAP, with stearic (C18:0) and oleic acids levels increasing by an average of 5.3- and 4.1-fold, respectively. No consistent changes in fatty acid composition in kernels were observed in any of the cultivars examined.

Commercial corn oil extracted from corn germ is rich in linoleic acid (C18:2), with concentrations of up to 57.3% [31]. In this study, the linoleic acid content was 26.7%–60.5% (average 47.7%) in cobs and 49.9%–58.6% (average 54.8%) in kernels (Fig. 1B), indicating that linoleic acid is the most abundant fatty acid in both corn cobs and kernels. Increasing levels of stearic and oleic acids from 30 to 60 DAP in corn cobs coincided with decreases in palmitic acid levels. This observation is reasonable, since palmitic acid is elongated to stearic acid, which is then converted to oleic acid by stearoyl-CoA desaturase [32]. Linolenic acid levels in kernels are a determinant of the quality of corn oils [33]. Therefore, the higher level of linolenic acid in cobs is notable and should be further investigated.

Higher phytosterol levels accumulate in corn cobs vs. kernels

Corn oil contains free phytosterols and esterified (fatty acyl) phytosterols. Among the various phytosterols, we analyzed three phytosterols, campesterol, stigmasterol, and β-sitosterol, representing the three major phytosterols found in corn oil [17, 31, 34]. These three phytosterols were clearly separated by GC under our conditions, using retention times (RTs) of 19.663, 20.119, and 21.084 min (Fig. 2A).

The cobs contained higher levels of phytosterols than kernels in all four cultivars analyzed, which ranged from 86.5 to 217.1 (average 131.4) mg 100 g^{-1} DW in cobs and 43.8 to 89.5 (average 61.2) mg 100 g^{-1} DW in kernels. The phytosterol contents in corn cobs were the lowest in 30 DAP samples, which fluctuated during the 0 to 60 DAP period. The increases in phytosterol contents in 60 DAP cobs were strongly related to the increases in stigmasterol levels; this tendency was not observed in kernels.

Although the distributions of the three phytosterols varied based on genetic and developmental differences, β-sitosterol was the most abundant phytosterol in both cobs and kernels. The total phytosterol content of SC60

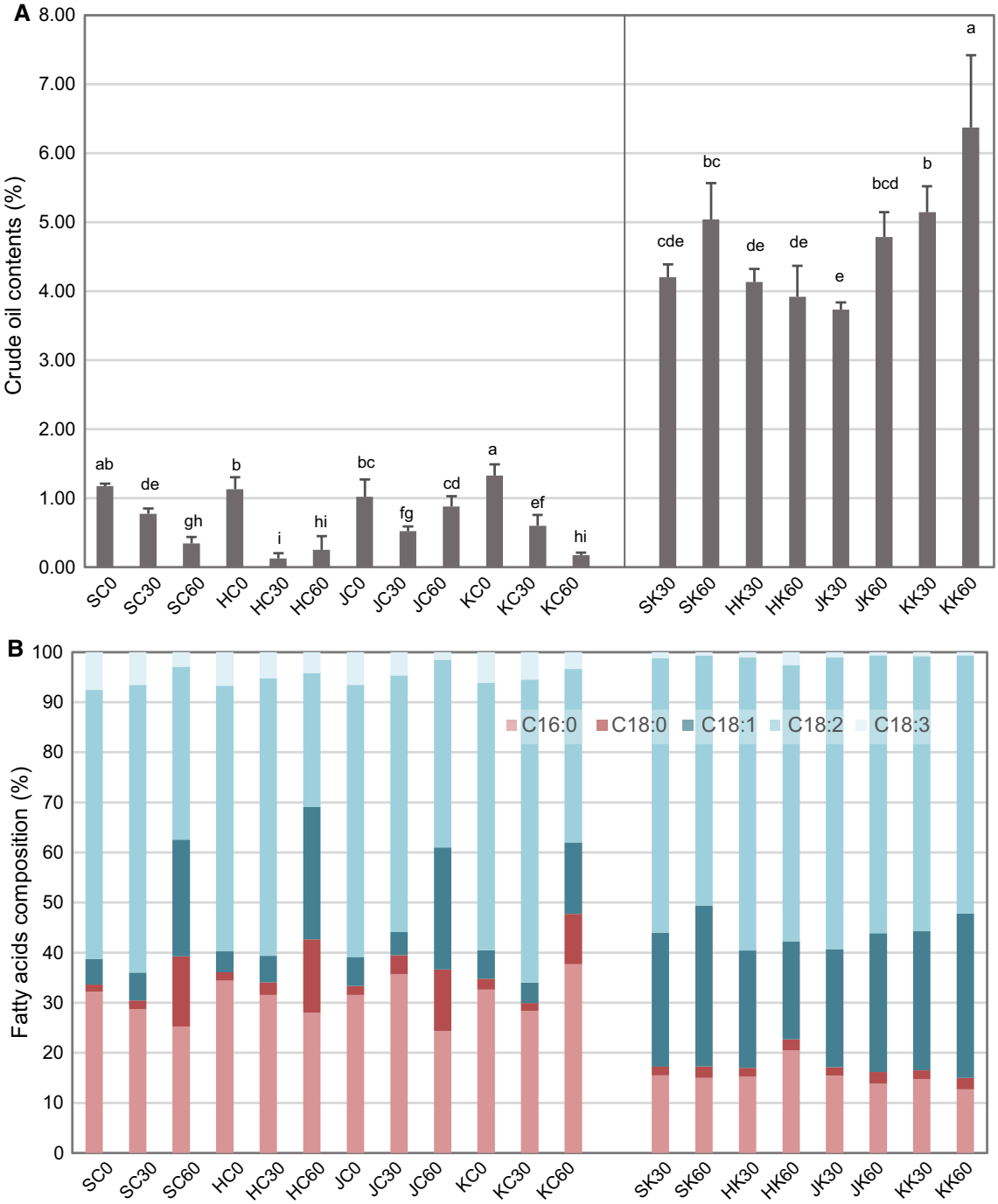

Fig. 1 Comparison of crude oil contents **A** and fatty acid compositions **B** of corn cobs and kernels for the four corn cultivars during the ripening period. Different letters in (A) indicate significant difference at $p < 0.05$ for cob and kernels samples, respectively

was 217.1 mg 100 g^{-1} DW, representing the highest value among the samples analyzed (Fig. 2B).

Most stanol is located in the aleurone cells of corn fibers, and this stanol is esterified and ferulated [35]. The similar phytosterol contents in 30 and 60 DAP kernels can be explained by the notion that phytosterol metabolism has already been saturated in 30 DAP kernels. In cobs, however, phytosterol levels increased from 30 to 60 DAP, pointing to dynamic metabolic changes during late corn cob development.

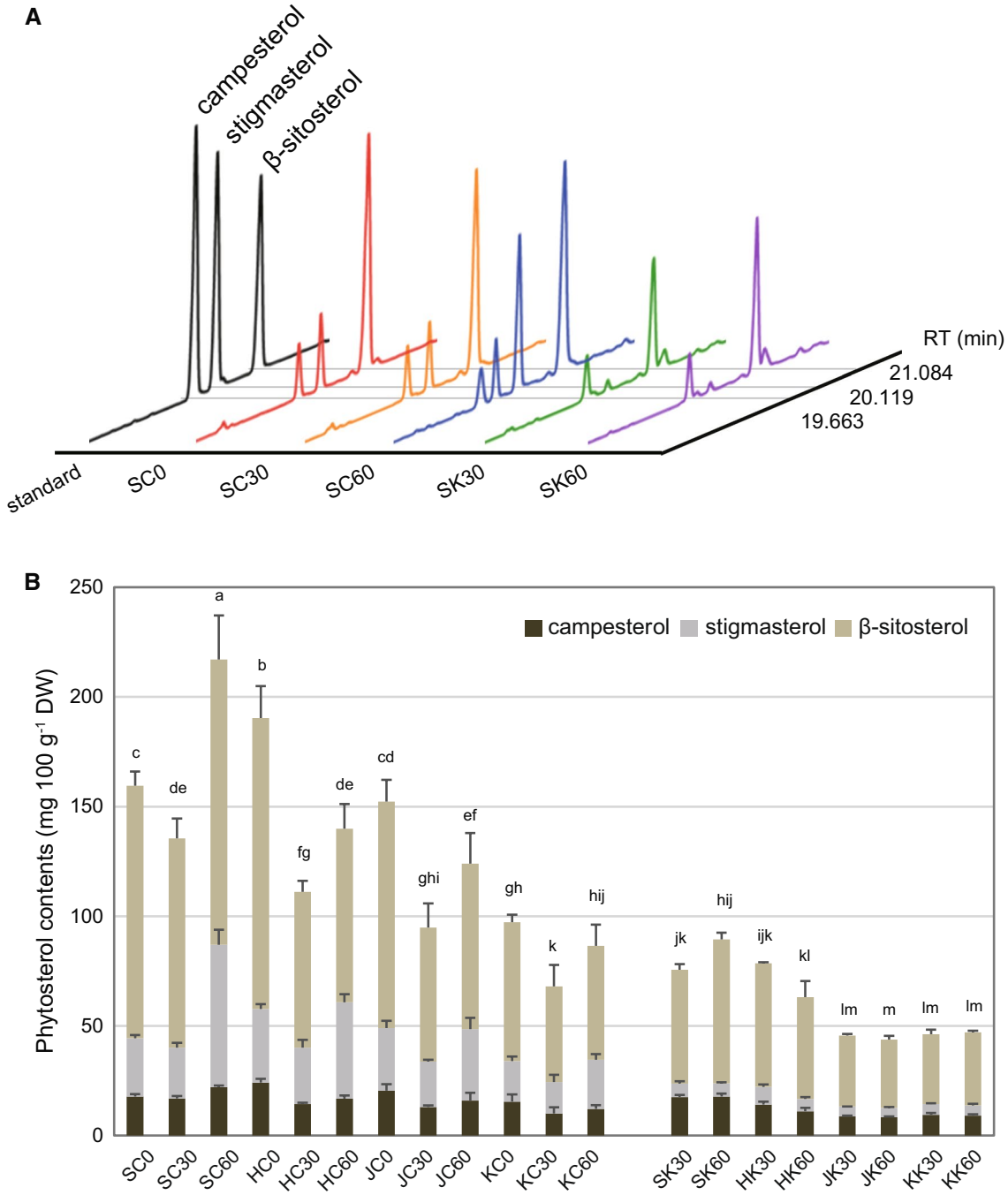

Fig. 2 Quantification of phytosterols in corn samples. **A** chromatograms of phytosterols in cob and kernel samples of corn cultivar Sinhwangok obtained by GC analysis. Cob and kernel samples are colored based on harvest time. **B** comparison of phytosterol contents between corn cobs and kernels during ripening. Different letters indicate significant difference in total phytosterol contents at $p < 0.05$

Four cultivars are distinguished by their oil contents and phytosterol profiles

The crude oil content showed a negative correlation with campesterol, stigmasterol, β-sitosterol, and total phytosterol contents (Table 1) in the corn samples. The total phytosterol content had the most significant correlation with β-sitosterol content ($r = 0.980$) among the three phytosterol components, since β-sitosterol comprises the largest proportion of total phytosterols. The crude oil content showed significant negative correlations with

Table 1 Correlation analysis of crude oil and phytosterol contents

	Crude oil	Campesterol	Stigmasterol	β-Sitosterol
Campesterol	− 0.443***	1		
Stigmasterol	− 0.740***	0.679***	1	
β-Sitosterol	− 0.581***	0.881***	0.784***	1
Total phytosterol	− 0.653***	0.880***	0.889***	0.980***

*** $p < 0.001$

total phytosterol contents and the contents of all phytosterol components, suggesting that crude oil could be converted to phytosterols in both corn cobs and kernels.

Hierarchical clustering heatmap analysis divided the samples into groups I and II, which include kernel and cob samples, respectively. Group I members can be distinguished from group II members by their higher crude oil contents (Fig. 3). Group I was subdivided into groups I-1 and I-2, largely based on campesterol contents: All Sinhwangok samples and all Hwangdaok samples except for the 60 DAP samples belong to this group. Group II was also subdivided into two groups, II-1 and II-2. Samples in II-2 tend to contain more phytosterols; many 0 and 60 DAP samples belong to this group. The highest amount of stigmasterol was detected in SC60 samples, and the highest amount of crude oil was detected in KK60 samples. The kernel samples contained high levels of crude oil, whereas cob samples contained low levels of crude oil (Fig. 3). The phytosterol contents showed cultivar-specific variation.

Finally, we performed principal component analysis (PCA) of the phytosterol and crude oil contents of the samples. PC1 accounted for 76.7% of the total variance, in which the three phytosterols loaded in the negative direction and crude oil loaded in the positive direction (Fig. 4). PC1 and PC2 covered 92.7% of the total variance of the samples. Cob and kernel samples are located in separate areas of the score plot (Fig. 4A), mainly due to higher crude oil contents in kernel samples, which are located in the upper right part of the plot. The kernel samples are located in the direction of the negative diagonal slope, which is a similar direction to campesterol and β-sitosterol in the loading plot (Fig. 4B), showing variations in the levels of these phytosterols depending on the stage of maturation.

Discussion

Phytosterol is highlighted for health benefit due to its activity to reduce blood cholesterol level [36]. It has been estimated that 100–500 mg d^{-1} of phytosterol is consumed in different regions of the globe which is supplied from basic diet, functional food, nutrition supplements, and pharmaceutical products [37]. Phytosterols are produced mostly from plant extract such as vegetable oils, grains, and nuts [38]. Economical sources of phytosterol extraction have been under investigation from various sources including red and brown algae [39]. In this study, we quantified the amounts of phytosterols and fatty acids in corn cobs and kernels in three different developmental stages with four cultivars. Corn oil content was ranged from 3.07 to 5.69% of dry weight both in dent and flint corns similar to this study [40]. The phytosterol and fatty acid contents of corn oil differed among varieties, however, their composition ratios are very similar among others including the results of this study [34, 40]. This could be partly explained from the observation that vegetable oil contents are highly affected by growing condition, cultivars, and seed maturation stages [19]. Considerable amounts of phytosterols in corn cobs showed that it could be an alternative source for phytosterol. In addition, comparative analysis of phytosterols in cobs and

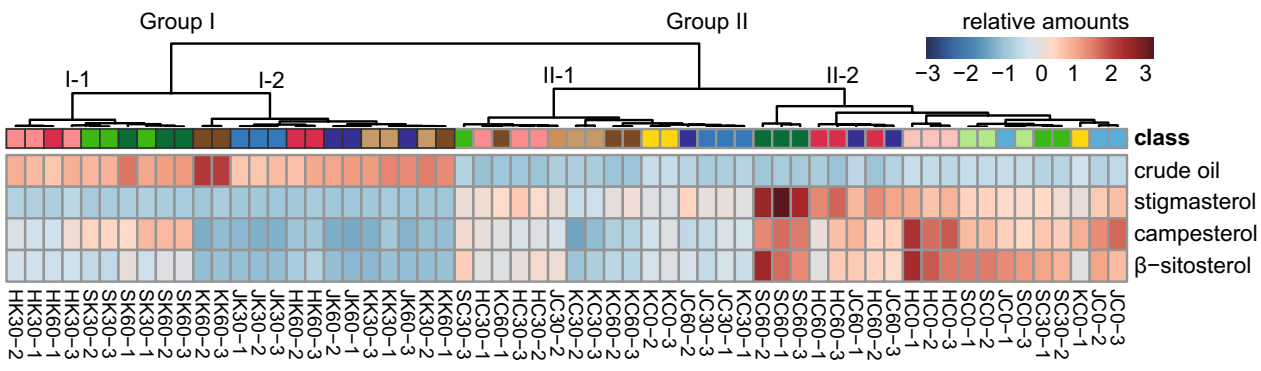

Fig. 3 Clustering heatmap analysis of phytosterol and crude oil contents. Each sample name, with two letters and a number, represents the cultivar name, tissue type (cobs or kernels), and harvesting time (DAP), respectively. Three biological replicates were performed (designated as − 1, − 2, and − 3) per sample

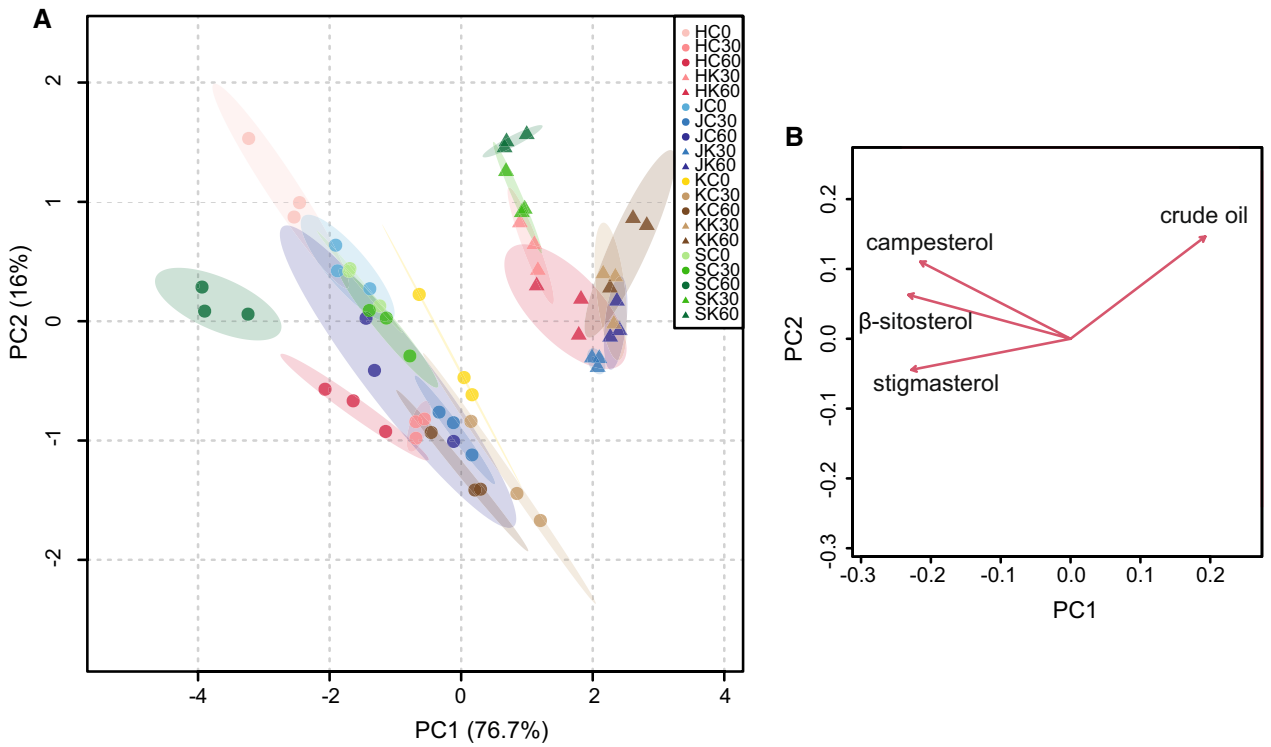

Fig. 4 Score **A** and loading **B** plots of principal component analysis for phytosterol and crude oil contents in the cobs and kernels of the four corn cultivars. Circles represent cob samples, and triangles represent kernel samples, with different colors for cultivars at each harvesting time

kernels suggest that phytosterol biosynthesis occurs both in cobs and kernels from the early developmental stages. Phytosterol is biosynthesized from acetyl-CoA, which is also a precursor for fatty acid biosynthesis [41]. High oil content would be a possible reservoir for abundant phytosterol production (Fig. 1) [42]. Reduced amount of fatty acids during development in cobs and accumulation of phytosterol suggests there should be a cross-talk between these two pathways during seed development. (Figs. 1 and 2).

The amount of phytosterols is directly controlled by activities of the biosynthetic enzymes. Structural genes and its tissue specificity implied where the phytosterols are synthesized. All of the three major phytosterols in corn kernel and cobs are shared 24-methylenelophenol as a substrate which is synthesized from squalene [43]. The biosynthetic pathway is branched by sterol methyl transferase (SMT2) which add methyl group on 24th carbon of 24-methylenelophenol [43]. Whereas, campesterol directly synthesized from 24-methylenelophenol, β-sitosterol and stigmasterol are synthesized from the branched point, 24-ethylidenelophenol [42]. The types of phytosterols in corn can be altered by several enzymes. SMT2 determines the ratio of campesterol to β-sitosterol and stigmasterol [44]. This ratio was significantly different in kernels vs. cob samples ($p < 0.001$), suggesting that SMT2 activity could be different between these two plant organs (Fig. 2B). Stigmasterol further synthesized from β-sitosterol by a cytochrome P450, CYP710A [45]. This enzyme is responsible for the ratio between β-sitosterol and stigmasterol. The average ratio in cobs was much higher than that of kernels (Fig. 2B), indicating higher and continuous activity of CYP710A in cobs rather than kernels.

Twelve structural genes have been identified for phytosterol biosynthesis pathway mostly in Arabidopsis [41, 43, 46]. Its homologs are also found in corn and showed tissue specific expression (Stelpflug et al. 2015; Sun et al; Additional file 1: Fig. S1) [47, 48]. Transcriptome atlas of corn cobs and kernels showed similar expression pattern for the three key genes, which are highly expressed in the early developmental stages and reduced afterward. The expression was commonly high in both cobs and kernels suggesting phytosterols are synthesized in both tissues and are continuously accumulated during development (Additional file 1: Fig.S1).

Various studies have been conducted examining the possible use of corn by-products, such as corn fibers and corn distiller's dried grain [31, 49]. Here we tested the feasibility of using corn cobs as a new source of phytosterols.

The total phytosterol content of cobs ranged from 68.0 to 217.1 mg 100 g^{-1} DW, which is higher than that of kernels (43.8–89.5 mg 100 g^{-1} DW). The total phytosterol content differed depending on the genetic background of the plant and the developmental stage, but the total phytosterol content of cobs was always higher than that of kernels (Fig. 2B).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00736-4.

Additional file 1. Supplementary Fig. S1. Expression levels of the three key genes for phytosterol biosynthesis in cobs and kernels in different developmental stages. Data from qTeller in maizegdb (https://qteller.maizegdb.org/) were redrawn for selected tissues and developmental stages.

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Author contributions
Conceptualization: BHH, HJY, YG; Formal analysis and Data curation: BHH, HJY, YG; Resources: GYS, SJH, SBY, SS, JTW; Validation and Visualization: BHH, HJY, KJH, YG; Writing: BHH, HJY, YG. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed in this study are available from the corresponding author upon reasonable request. The plant materials are commercially available.

Declarations

Competing interests
The authors declare that they have no competing interests.

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