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Nutraceutical potential of maize (Zea mays L.) (corn) lab-scale wet milling by- products in terms of β-sitosterol in fiber, gamma-tocopherol in germ, and lutein & zeaxanthin in gluten

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// Abstract

The nutraceutical potential of lab-scale corn wet milling by-products, mainly corn germ, fiber, and gluten, has not been examined keenly in the past. A simplified lab-scale wet milling method was used to fractionate corn into its by-products namely germ, fiber and gluten. They were subject to extraction and characterization of nutraceuticals in comparison to the feed corn kernels. Carotenoids, tocopherols, and tocotrienols were characterized by high-performance liquid chromatography, while the phytosterols were characterized by gas chromatography. γ-tocopherol was high in corn germ compared to fiber, gluten and feed corn kernels. Corn fiber revealed higher β-sitosterol content, than that of feed corn kernels, germ and gluten. Further, corn gluten showed high lutein and zeaxanthin content than that of the feed corn kernels, fiber and germ. Phytosterols, carotenoids, and tocols are hypo-cholesterolemic, eye-protective, and anti-oxidative respectively, in nature. This study demonstrates the significance of corn wet milling by-products and the need for valorization in generating nutraceuticals and functional food ingredients.

Introduction

The industrial corn wet milled starch and by-products find broader applications in food and non-food products, such as modified starches, dextrins, beverages, nutraceuticals, functional foods, textiles, paper, and plastics. Corn wet milling consists of chemical, biochemical, and mechanical processes to disintegrate corn into relatively pure fractions of starch, steep water, germ, fiber, and gluten (Rausch and Eckhoff 2016). In recent years, various wet-milling processes at the laboratory and pilot-plant scale have been developed and compared with the industrial wet milling processes to achieve an industry-like product quality and yield (Dowd 2003; N. Singh and Eckhoff 1996). In general, corn wet milling includes corn steeping at the required temperature, coarse milling to separate germ, fine milling and washing of slurry for fiber separation, and finally isolating starch from gluten. Corn wet milling is challenging to implement in the laboratory because of the tougher separation steps, including manual corn germ flotation and collection and minor yield differences that affect the profitability at the industrial scale. Accordingly, the precision of lab wet milling techniques is of continuing concern. Several separation steps from previous processes have been modified after referring to related studies to improve the similarities between the lab-scale and industrial wet milling (S. K. Singh et al. 1997; S. K. Singh and Eckhoff 1996; Yahl et al. 1971). Changes have been made to improve the lab separation model to provide products, reflecting the similar quality of industrial products. The resulting process can improve the recovery of the by-products and be an excellent industrial process model.

The study of by-products obtained from the lab-scale wet milling process is equally important because of its industrial-scale applications and nutraceutical value. The corn wet milling by-products are recombined in various proportions or supplied individually as cattle and poultry feed in the industry. Each of the by-products obtained by the corn wet-milling process is a source of nutraceuticals such as carotenoids, tocols, and phytosterols(Deepak and Jayadeep 2021). Our appraisal of the existing literature found just a few studies concerning the quantification and characterization of tocols and phytosterols in lab-scale corn wet-milled by-products. However, the health-promoting properties of carotenoids, phytosterols, and

tocopherols are well documented. A study on dry milled corn germ, endosperm, and hull fractions showed tocopherols and tocotrienols in considerable amounts (Ko et al. 2003). An investigation by G.W. Grams et al. on tocol levels in lab-scale corn wet milled by-products showed Corn germ as the major reservoir of tocols (Grams et al. 1971). Tocopherols and tocotrienols have high antioxidant activity and protect the body from oxidative stress. γ- tocopherols show high anti-inflammatory and antioxidant activities (Ahsan et al. 2014; Zielinski et al. 2018). Another study by V. Singh et al. confirmed high phytosterol content in corn fiber (V. Singh et al. 2003). Phytosterols have valuable hypo-cholesterolemic, lipid-lowering, and colon tumor reducing properties(Acosta-Estrada et al. 2018; Jiang and Wang 2005; Moreau et al. 1996; Ramjiganesh et al. 2002). Corn gluten was investigated to be high in carotenoids, particularly xanthophylls and peptides(Heuzé. et al. 2018; Zhu et al. 2019). The carotenoids improve the immune system, attack free radicals, and prevent cancer and retinopathy(Abdel-Aal et al. 2013; Lakshminarayana et al. 2005; Moreno et al. 2007). There is a need to utilize and upcycle the by-products of grain processing to obtain health-beneficial carotenoids, tocols, and phytosterols. Hence these by-products can be precursors for developing functional food ingredients and nutraceuticals. However, there is no exhaustive study on the characterization and analysis of nutraceutical components in the lab-scale corn wet milled by-products.

The objectives of this study were to estimate and characterize the carotenoids, tocopherols, and phytosterols in lab-scale corn wet-milling fractions- corn germ, corn fiber, and corn gluten. We investigated the concentration and distribution of tocols, carotenoids and phytosterols in big flint corn wet milling by-products obtained by a redesigned process. Therefore, the objective of the present study involves an experimental study on the nutraceutical content and quality of Tocopherols and tocotrienols (α, δ, and γ), carotenoids (lutein, zeaxanthin, and β-carotene), Phytosterols (campesterol, stigmasterol, and β-sitosterol) by chemical and HPLC methods in corn germ, fiber, and gluten. This study will recognize and appreciate the potential of these by-products as precursors for nutraceuticals and functional food ingredients.

Materials & Methods

Materials

Commercial flint corn was obtained from the local market in Mysore. The corn moisture was measured in ambient air conditions and was stored at 4 °C. Before milling, the maize grains were hand-picked to remove broken and damaged kernels and foreign materials. The sodium meta bi-sulfite and lactic acid used for steeping were from Sisco Research Laboratories, Mumbai, India. α-tocopherol, γ-tocopherol, δ-tocopherol, and γ-tocotrienol were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Lutein and zeaxanthin were purchased from Cayman Chemicals Ltd., Michigan, USA. Campesterol, stigmasterol, β-sitosterol, and pyridine (anhydrous 99.8 %) were procured from Sigma Chemicals Co. (St. Louis, MO, USA). N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) were procured from Fluka, Madrid, Spain. All other solvents and reagents were of analytical grade (Merck, Madrid, Spain) and were used without further purification. Methanol, acetonitrile, dichloromethane, and

hexane were of HPLC grade, and ethanol and ammonium acetate were purchased from Spectrochem, Mumbai.

Methods

Lab-scale corn wet milling process

Several experiments were conducted during the process development work, which led to a final procedure for recovering steep solubles, Germ, Fiber, Starch, and Gluten. The modified lab corn wet mill process flow was designed after these experiments, considering the previous lab-scale corn wet milling experiments(Dowd 2003; N. Singh and Eckhoff 1996). The process flow chart shows the steps followed to separate these products (Figure 1.).

[Figure 1 near here]

The process begins with steeping of corn kernels for 24 h in a solution of sodium metabisulfite solution (0.2 %) and lactic acid (0.5 %), at 53 °C in a 1 L screw-top glass bottle, which facilitates kernel hydration, lactic acid fermentation, and sulfur dioxide reactions within the endosperm. The bottle was kept in an orbital shaker for efficient mixing and soaking of the kernels. After steeping, the steep water was strained from the bottle containing the steeped grains. The steeped corn was then subjected to coarse grinding in a blender (Sai mill) with blunt-edged blades to render corn de-germination. The inner white germ separated from the corn was manually isolated using forceps by flotation and skimming method. Corn Germs were rinsed with water on a sieve (pore size-354 microns) to remove the bound starch and fiber. The remaining ground corn slurry and the rinse volume were finely ground with sharp-edged blades at high rpm in the blender to make a fine slurry. Corn fiber was isolated from the slurry by washing with water on a sieve (pore size-75 microns) using a mechanical sieve shaker. The flow-through starch-gluten slurry was subject to centrifugation at 8000 rpm to isolate gluten from starch. This step was followed by drying the fractions, i.e., germ, fiber, gluten, and starch, in a hot air oven at 55 °C for 90 minutes. The yield of the fractions was calculated for 100 g of corn kernels utilized.

The corn wet mill by-products were obtained as mentioned in Figure 2.

[Figure 2 near here]

Sample preparation for analysis

The dried corn germ, fiber, gluten fractions, and whole corn were pulverized in a small-sized, high rpm blender mill (KRUPS 75 TYP 203, Solingen, Germany)with sharp-edged blades. Samples (germ, fiber, gluten, and whole corn) were stored at -20°C for further analysis.

Moisture estimation

All the samples were tested for their moisture content estimated by AACC (2000) method by keeping in an air oven at 130 °C for 2 h or until constant weight is achieved(A.A.C.C. 2000).

Vitamin-E (Tocols: Tocopherols & Tocotrienols) extraction

In this process, a redesigned method of direct solvent extraction was employed for extracting tocopherols (TP) and tocotrienols (TT) from the samples, as described by(Jayadeep and Malleshi 2011).Summarily, 10 ml of HPLC grade methanol was used to extract tocols from each gram of pulverized corn germ, fiber, gluten, and whole corn flour separately and mixed in a tube mixer (IKA™ Loopster Digital, fisher scientific) at 27 °C. Centrifugation at 5000 g and 27 °C (room temperature) facilitated the separation of supernatant from the solution. The supernatant was filtered using Whatman 0.45 μm membrane filter (Polyvinylidene difluoride). These tocol extract samples were stored at −20 °C until HPLC analysis.

Characterization of tocols by RP-HPLC (Reverse phase High-Performance Liquid Chromatography)

The HPLC system consisted of a Waters 2690 Alliance Separations Module, a Waters PDA detector, and a Waters 474 Scanning Fluorescence Detector (Millipore, Bedford, MA). The *Millennium 32 Ver. 3.20* Chromatography Software (Waters) did the recording and preparation of Chromatograms. The purified sample was injected through a Kromasil C18(Octadecylsilane) column (5μ, 100 A, 4.6 mm* 250mm). Gradient mode of HPLC was conducted for high throughput detection. The RP-HPLC method used the initial mobile phase conditions of 45% acetonitrile, 45 % methanol (MeOH), 5 % isopropanol (IsOH), and 5 % of aq. Acetic acid (1%), for 6 min. The mobile phase of Acetonitrile: MeOH: IsOH was changed linearly at the ratio of 25:70:5 (v/v/v) over the next 10 min and held there for 12 min before returning to the initial conditions. The flow rate of the mobile phase was 1ml/min with a run time of 30 min. The tocopherols (TPs) and tocotrienols (TTs) in the samples were detected at excitation and emission wavelengths of 298 and 328 nm by a fluorescence detector, respectively(Chen and Bergman 2005). The concentrations of tocols stock and working standards solutions were determined using a spectrophotometer (GENESYS 150, Thermofisher Scientific)using the specific extinction coefficients-75.8 (α-S, Abs_{max}- 292 nm), 91.4 (γ-TP, Abs_{max}- 298 nm), 87.3 (δ-TP, Abs_{max}- 298 nm), and 90.5 (γ-TT, Abs_{max}- 296 nm) (Chen and Bergman, 2005).The TPs and TTs in the samples were identified by comparing retention times with authentic standards.A standard curve was plotted each for α-TP (1.14- 12.1 ng, r^2=0.95), γ-TP (1.13- 5.63 ng, r^2=0.964), δ-TP (0.94– 9.39 ng, r^2=0.999), and γ-TT (1 – 33.15 ng, r^2=0.985) respectively.

The standard tocol concentration corresponding to the peak area was used to determine the respective tocol component concentration in the samples. The content of α-TT and δ-TT in the samples were calculated corresponding to standard α-TP and δ-TP. TheHPLC chromatograms of Tocol (Vitamin-E) standards and tocols extracted from corn germ, fiber, gluten, and whole corn flour are shown in Figure 3.

[Figure 3 near here]

Carotenoid extraction, analysis, and characterization by RP-HPLC

A redesigned method for total carotenoid extraction described by(Alarcão-E-Silva et al. 2001)from the wet milling by-products was used. The samples (1 gm each) were mixed with extraction solvents (acetone: petroleum ether (b.p. 60–80 °C), at 1:1(v/v) ratio) in centrifuge tubes for 1 hr using a tube mixer (IKA™

Loopster Digital, fisher scientific). After mixing, the samples were centrifuged (Remi centrifuge C-30BL, Mumbai, India) at 3000 g at room temperature (27 °C) to separate the supernatant. The supernatant was extracted multiple times till the extraction solvent became colorless. After centrifugation, the supernatant layer was mixed and shaken well with distilled water, four times in volume of acetone, to separate the upper petroleum ether layer from the lower acetone layer using a separatory funnel. The upper carotenoid layer was transferred to a graduated cylinder through a filter paper containing Na_2SO_4 (sodium sulfate) anhydrous to remove residual water and noted the volume for further calculation. A suitable petroleum ether volume was made (b.p. 60–80 °C), and the absorbance of the samples was recorded at a wavelength of 454 nm (λ_{max}) using a UV/Vis spectrophotometer (GENESYS 150, Thermofisher Scientific). The total carotenoid content of the by-product samples was calculated using the specific extinction coefficient() of 2500 at 454 nm.

The carotenoids dissolved in solvents were dried in a vacuum concentrator (Eppendorf concentrator plus). The dried residue was then dissolved in ethanol and stored at -20 °C until RP-HPLC analysis. An aliquot of the sample solution was used for injection into the HPLC system.The experiment was carried out in dim light, and glassware was covered sufficiently with aluminum foil to protect carotenoids from light.The carotenoids were segregated on a C-18 (Octadecylsilane)column, of 250 mm*4.6 mm i.d. (internal diameter), five µm (particle size), 100 A° (pore size) (Kromasil, Supelco, USA).The mobile phase used for the process wasacetonitrile/methanol/dichloromethane (60:20:20 v/v/v) containing 0.1% ammonium acetate. Samples were injected into the system with an isocratic condition maintained at a 1 ml/min flow rate. All the carotenoids were observed at 450 nm (λ_{max}) with a UV-visible detector (Agilent, USA). The individual carotenoid peak identities and λ_{max} values were confirmed by their retention times and characteristic spectra of standard chromatograms, respectively. They were quantified from their peak areas compared to the respective reference standards. The HPLC method was based on(Lakshminarayana et al. 2005). HPLC chromatograms of carotenoid standards and carotenoids extracted from corn germ, fiber, gluten, and whole corn flour are shown in Figure 4.

[Figure 4 near here]

Phytosterol extraction, analysis, and characterization by GC (Gas chromatography)

Phytosterols (PSs) were extracted from corn germ, fiber, gluten, and whole corn flour using a modified technique as outlined by Hossain and Jayadeep (2020)(Hossain and Jayadeep 2020; Jiménez-Escrig et al. 2006). Initially, the samples were subjected to acid hydrolysis, followed by lipid extraction and saponification to extract the unsaponifiables. 1 g of sample and 4 ml of internal standard (IS) solutions in a leak-proof screw-capped glass tube (0.1 mg di-hydrocholesterol in 1 ml of absolute ethanol) were mixed thoroughly. Each sample in the tube was mixed with hydrochloric acid (6 M) and heated in the water bath at 80 °C for an hour to facilitate acid hydrolysis. Hexane and diethyl ether in the ratio of 1:1 was added to the cooled mixture. The two phases were subject to accelerated separation by centrifugation for 5 min. The lipidic upper phase was transferred to 15 ml centrifuge tubes, and the whole solvent content was evaporated in a speed vac concentrator. The dried residues were saponified using

pyrogallol-ethanol (3% w/v) solution and dissolved. Saturated potassium hydroxide solution was added to the solution and mixed properly. The sample tubes were heated in a water bath at 80 °C for 30 minutes, mixed vigorously at 2-minute intervals, and cooled.Cyclohexane and distilled water were added to each tube and shaken for 10 minutes to mix the contents thoroughly. After this step, the saponified sample and lipid-containing solvent phases were separated by centrifugation at 5000 g for 5 minutes.The upper, unsaponifiable lipid phase was then transferred to centrifuge tubes by passing it through anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture. The sample solvent was vacuum evaporated and dried in a speed-vac concentrator (Eppendorf concentrator plus). The dried residue was then silylated to produce sterol ester derivatives.

Production of trimethylsilyl (TMS) ester derivatives of phytosterols was done by a modified procedure(Jiménez-Escrig et al. 2006). Pyridine (500 µL) was immediately added to the dried PS in the round bottom flask. In addition, BSTFA (N, O- Bis (trimethylsilyl) trifluoroacetamide) containing 1 % TMCS (Trimethylchlorosilane) (500 µL) was added and mixed properly and stored overnight at room temperature for silylation. The excess reagent was then removed with N_2 gas, and the dry PS ester was dissolved in di-chloromethane: hexane (1:1), and the aliquots were stored at -4 °C until analysis.

Gas Chromatography of the standard (stigmasterol, sitosterol, and campesterol) and the sample phytosterols (corn germ, fiber, gluten, and whole corn flour) was performed to obtain standard chromatograms. The GC analysis of PSs was carried out as previously reported(Jiménez-Escrig et al. 2006). The TMS ester derivative sample (1 µl) was injected to flow through the biphenyl polysiloxane column (30 m × 0.25 mm i.d.× 0.25 µm f.t.) (Elite-5, PerkinElmer, Waltham, Massachusetts, USA) built into a gas chromatograph (Shimadzu GC2010) for detection in a flame ionization detector (300 °C). The column temperature program started at 245 °C, was held for 1 minute and increased to 275 °C at the rate of 3 °C/min to be held for 28.5 min. The carrier gas, nitrogen with a flow rate of 1.08 ml/min, flowed with a split ratio of 1:22. Three major PSs—Campesterol, Stigmasterol, and β-Sitosterol were determined by their respective retention times of the standards.. The GC chromatograms of the by-product phytosterol extracts are shown in Fig. 5. All experiments were performed at least twice with triplicate samples each time.

[Figure 5 near here]

Statistical analysis

The values in the tables are represented as Mean± SD (standard deviation) of three independent replicates. The results were analyzed by a two-way ANOVA ($p < 0.001$) test followed by a post-hoc Tukey test (multiple comparison test) using GraphPad prism application (Inc. La Jolla, CA, USA).

Results

Yield and characteristics of lab-scale corn wet milling

The lab-scale corn wet milling process was designed as mentioned in Fig. 1. That resulted in a model of unit steps of the industrial process. Processing as per the model resulted in various end-products, as shown in Fig. 2. The lab-scale corn wet milling end product yields were 58.4 %, 14.4 %, 12.1 %, 4 %, and 4.5 % for starch, gluten, fiber, germ, and steep solubles, respectively. A 6.6 % loss in yield was due to the handling losses from one step to the other. The moisture content was 3.17 %, 6.36 %, and 9.94 % for corn germ, fiber, and gluten, respectively, in the dried products.

Tocopherol and tocotrienol content in corn germ, fiber, and gluten

The estimated vitamin-E content in the by-products and Whole Corn flour has been represented in Table 1. HPLC characterization of tocols in corn germ, fiber, and gluten showed the presence of α-, γ-, and δ-tocopherols and tocotrienols, respectively. Among the by-products, the analyses showed high tocopherol content in corn germ and high tocotrienol content in corn fiber.

[Table 1 near here]

The total TP contents were 58.47 mg/100 g, 1.51 mg/100 g, and 1.11 mg/100 g in corn germ, gluten, and fiber, respectively. The total TT content was 8.47 mg/100 g, 0.50 mg/100 g, and 0.42 mg/100 g in corn fiber, gluten, and germ, respectively. Among the by-products, corn germ showed the highest accumulation of the tocols, followed by corn gluten and fiber. γ- tocopherol and α- tocopherol were high in corn germ. On the other hand, γ-tocotrienol was high in corn fiber. In germ, 91 % of total tocol content was γ-tocopherol, whereas in corn fiber, 49.84 % was γ- tocopherol, and 34 % was γ -tocotrienol of the total tocol content. The Tocol quantities were observed to be of descending order of γ-TP > α-TP/δ-TP > γ-TT > α-TT > δ-TT in the by-products. Whole corn flour was the second-highest in total tocol content after corn germ.

Carotenoid content in corn germ, fiber, and gluten

The total carotenoid, lutein, zeaxanthin, and β-carotene levels were determined in corn germ, fiber, and gluten. The quantities of all the carotenoid components are shown in Table 2.

[Table 2 near here]

The carotenoids characterized by RP-HPLC showed the presence of lutein, zeaxanthin, and β-carotene in all the by-products. Total carotenoids were estimated to be highly concentrated in corn gluten (9.24 mg/100 g), followed by corn fiber and germ. Zeaxanthin (3.55 mg/100 g) was the major component with competitive levels of lutein (2.07 mg/100 g) in corn gluten. β- carotene was not a significant component in the by-products. The total zeaxanthin content was detected at 61.84 %, followed by lutein at 36.03 % of the carotenoids in corn gluten. Compared to corn gluten, corn fiber showed the second-highest total carotenoid content. In the whole corn flour, the carotenoid content was comparatively low due to the matrix effect of starch and other components.

Phytosterols content in the corn germ, fiber, and gluten

The GC chromatograms showed phytosterol components - Campesterol, Stigmasterol & β- Sitosterol in all by-products in detectable levels. The standard PS response factors of β-sitosterol, campesterol, and stigmasterol were calculated to be 0.99, 0.93, and 1.20, respectively. The phytosterol content in the by-products was estimated corresponding to the standard PS response factors, determined in correlation with the internal standard. The assessed quantity of components- β-sitosterol, campesterol, and stigmasterol in corn germ, fiber, and gluten are furnished in Table 3.

[Table 3 near here]

The PS components were revealed to be rich in corn fiber, followed by corn gluten and corn germ. β-sitosterol was the major component in all the by-products. Among the by-products, the aggregate phytosterol content was shown to be high in corn fiber (362.08± 33 mg/100 g) followed by corn gluten (142.97± 11.51 mg/100 g). On the other hand, whole corn flour contained the third-highest aggregate phytosterol content (112.36 ± 10.28 mg/100 g). Among corn wet milling by-products, corn fiber showed a high density of phytosterol accumulation.

Discussion

The lab-scale setup, made for the recovery of the corn wet milling by-products, is not a exact replica of the industrial unit wherein several mechanization and automation are involved. Compared to the industrial process(Chaudhary et al. 2014), the yield of starch and was low, and that of gluten was high due to unit operations in the laboratory process. This effect may lead to cross-contamination of fractions.

In our lab-scale study of the corn wet milled by-products, the tocol content of corn germ was high, followed by corn fiber and corn gluten. In a previous study by Grams et al., 1971, (Grams et al. 1971), a total tocol content of 8.5 mg/100 g was found in corn germ, of which 6.6 mg/100 g was γ- tocopherol and 1.9 mg/100 g was α- tocopherol. However, the tocotrienol content was not present in measurable amounts. The total tocol content extracted from corn fiber was 5.4 mg/100 g. Our study showed results with different tocopherol and tocotrienol levels compared to this study. This difference is due to using different analytical methods by them (TLC) and by us (HPLC). Corn germ obtained by dry milling by Ko et al., 2003 (Ko et al. 2003) showed a total tocol content of around 46 mg/100 g, similar to our corn germ tocol content (57 mg/100 g). The corn germ is rich in tocopherols, especially γ-TP(Chander et al. 2008; Egesel et al. 2003).

Based on *in-vitro* and animal studies, γ-tocopherol shows powerful anti-inflammatory and antioxidant properties (Dietrich et al. 2006). γ- tocotrienol has shown anti-carcinogenic effects in lung and liver cancer in mice(Yu et al. 1999), and alpha-tocopherol prevented the onset of prostate cancer (Virtamo et al. 2014). There are separate recommendations of Vitamin-E dosage for health benefits for different health conditions. However, the highest RDA (recommended daily allowance) of vitamin E (alpha-tocopherol) recommended by the food and nutrition board for infants is 4 – 5 mg/day, children, 6 – 7 mg/day, adolescents and adults, 15 mg/day, and 15 and 19 mg/day for pregnant and lactating women respectively (Food Nutrition Board, 2000). Corn germ is a potent ingredient for the nutraceutical and

functional food industry since it is an oil reservoir containing ~40-50 % oil. Our study highlighted that corn germ is mainly rich in tocols (58.9 mg/100 g), which shows that 10 g of germ can provide 38.6 % of RDA of Vitamin-E for adults and adolescents. Since corn germ is rich in gamma-tocopherols (53.55 mg/100 g), it has a high nutraceutical potential and can be a functional food ingredient.

Our study showed that corn gluten mainly had high carotenoid content, significantly lutein and zeaxanthin. Lutein and zeaxanthin content in lab-scale wet-milled corn gluten has not been reported in any other study, which is why our study becomes relevant. Consumption of 6 mg/day of L/Z (Lutein/Zeaxanthin) for men and women has been suggested as a dietary recommendation to reduce the risk of age-related macular degeneration (Johnson and Rasmussen 2013). In our study, corn gluten contained ~ 5 mg/100 g of lutein and zeaxanthin, showing a potential food ingredient with health benefits. It was determined in a study that industrial corn gluten meal contained around 19.5 - 49.1 mg/100 g xanthophylls (lutein, zeaxanthin, cryptoxanthin) (Heuzé. et al. 2018). However, there are minimal comparable studies on a laboratory scale.

In our investigation, corn fiber showed high phytosterol content, followed by corn gluten and corn germ. According to Singh et al., 2003, the phytosterol levels of the corn wet milling by-products were higher in corn fiber at 19.3 mg/100 g, followed by corn germ at 16.8 mg/100 g and protein (corn gluten) at 4.8 mg/100 g. However, our study of the characterization of phytosterols in the by-products by gas chromatography found the components at a higher concentration, and there were no similar studies.

Several *in-vitro* and *in-vivo* studies have reported that β- sitosterol has various biological effects such as anxiolytic & sedative effects (López-Rubalcava et al. 2006), analgesic, immunomodulatory (Fraile et al. 2012), anti-microbial (Ododo et al. 2016), anti-cancer(Bouic 2001), anti-inflammatory (Paniagua-Pérez et al. 2017), lipid-lowering effect (Yuan et al. 2019), hepatoprotective(Abdou et al. 2019), protective effect against NAFLD (Non-alcoholic fatty liver diseases)(Feng et al. 2018) and respiratory diseases (Park et al. 2019), wound healing properties (Abbas et al. 2019), antioxidant and anti-diabetic activities (Babu and Jayaraman 2020). Wet-milled corn fiber was observed to be a potential source of phytosterol-based nutraceutical ingredients and health food. According to a review, a daily 2-gram phytosterol supplement reduced LDL levels in people with hypercholesterolemia by 2.7 % and people with genetic hypercholesterolemia by 4.3 to 9.2% (Cabral and Klein 2017). Daily intake of 1.6 g phytosterols was found to lead to an approximately 10% reduction in LDL cholesterol(Hallikainen et al. 2000). The average daily intake of phytosterols is in the range 150–400 mg, but to be effective as a cholesterol-lowering agent, phytosterol intake from enriched foods needs to be between 1.5–2.0 g per day(Azadmard-Damirchi and Dutta 2010; Raju 2013). Corn fiber can complement an available phytosterol supplement and provide a good health value owing to its high phytosterol levels, especially β-sitosterol.

Conclusion

Lab-scale corn wet milling had a good yield of by-products starting with corn gluten followed by fiber and germ. In the present study, the nutraceutical characterization showed the effectiveness of by-products. γ-

TP and α-TP having potent anti-inflammatory and antioxidant properties were high in corn germ. Tocotrienols, especially γ-TT, have powerful antioxidant, anti-cancer, and cholesterol-lowering properties and are high in corn fiber. Corn Gluten was analyzed as having a high carotenoid content, with zeaxanthin being the significant component and competitive Lutein levels. Zeaxanthin & lutein are good against macular degeneration. Among the hypo-cholesterolemic phytosterol components, β-sitosterol was identified as the major component of corn germ, gluten, and fiber. Corn fiber was identified as the significant phytosterol reservoir with a high β- Sitosterol content. Therefore, corn germ, gluten, and fiber can be potential healthy food ingredients for value addition in bakery, beverage, and confectionery products. They can also be used as a source of nutraceuticals.

Declarations

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Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding Declaration

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Conflict of Interest Statement

No potential conflict of interest was reported by the author(s).

Author’s Contribution

Deepak T S- wrote the main manuscript text including figures and tables, processing and interpretation of data, revising and preparation of manuscript

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References

1. A.A.C.C. (2000). *Approved methods of the American Association of Cereal Chemists* (10th editi.). St 456 Paul, Minnesota.
2. Abbas, M., Al-Rawi, N., Abbas, M., & Al-Khateeb, I. (2019). Naringenin potentiated β-sitosterol healing effect on the scratch wound assay. *Research in Pharmaceutical Sciences*, *14*(6), 566–573. https://doi.org/10.4103/1735-5362.272565
3. Abdel-Aal, E.-S., Akhtar, H., Zaheer, K., & Ali, R. (2013). Dietary Sources of Lutein and Zeaxanthin Carotenoids and Their Role in Eye Health. *Nutrients*, *5*(4), 1169–1185. https://doi.org/10.3390/nu5041169
4. Abdou, E. M., Fayed, M. A. A., Helal, D., & Ahmed, K. A. (2019). Assessment of the hepatoprotective effect of developed lipid-polymer hybrid nanoparticles (LPHNPs) encapsulating naturally extracted β-Sitosterol against CCl4 induced hepatotoxicity in rats. *Scientific Reports*, *9*(1), 19779. https://doi.org/10.1038/s41598-019-56320-2
5. Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldivar, S. O. (2018). *Minor constituents and phytochemicals of the Kernel*. *Corn: Chemistry and Technology, 3rd Edition*. https://doi.org/10.1016/B978-0-12-811971-6.00014-0
6. Ahsan, H., Ahad, A., Iqbal, J., & Siddiqui, W. A. (2014). Pharmacological potential of tocotrienols: A review. *Nutrition and Metabolism*, *11*(1), 52. https://doi.org/10.1186/1743-7075-11-52
7. Alarcão-E-Silva, M. L. C. M. M., Leitão, A. E. B., Azinheira, H. G., & Leitão, M. C. A. (2001). The Arbutus Berry: Studies on its Color and Chemical Characteristics at Two Mature Stages. *Journal of Food Composition and Analysis*, *14*(1), 27–35. https://doi.org/10.1006/jfca.2000.0962
8. Azadmard-Damirchi, S., & Dutta, P. C. (2010). *Phytosterol Classes in Olive Oils and their Analysis by Common Chromatographic Methods*. *Olives and Olive Oil in Health and Disease Prevention*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-374420-3.00027-9
9. Babu, S., & Jayaraman, S. (2020). An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. *Biomedicine and Pharmacotherapy*, *131*, 110702. https://doi.org/10.1016/j.biopha.2020.110702
10. Bouic, P. J. D. (2001). The role of phytosterols and phytosterolins in immune modulation: A review of the past 10 years. *Current Opinion in Clinical Nutrition and Metabolic Care*, *4*(6), 471–475. https://doi.org/10.1097/00075197-200111000-00001
11. Cabral, C. E., & Klein, M. R. S. T. (2017). Phytosterols in the treatment of hypercholesterolemia and prevention of cardiovascular diseases. *Arquivos Brasileiros de Cardiologia*, *109*(5), 475–482. https://doi.org/10.5935/abc.20170158
12. Chander, S., Meng, Y., Zhang, Y., Yan, J., & Li, J. (2008). Comparison of nutritional traits variability in selected eighty-seven inbreds from Chinese maize (Zea mays L.) germplasm. *Journal of Agricultural*

and Food Chemistry, *56*(15), 6506–6511. https://doi.org/10.1021/jf7037967

13. Chaudhary, D. P., Kumar, S., & Langyan, S. (Eds.). (2014). *Maize: Nutrition Dynamics and Novel Uses*. New Delhi: Springer India. https://doi.org/10.1007/978-81-322-1623-0
14. Chen, M.-H., & Bergman, C. J. (2005). A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. *Journal of Food Composition and Analysis*, *18*(2–3), 139–151. https://doi.org/10.1016/j.jfca.2003.09.004
15. Deepak, T. S., & Jayadeep, P. A. (2021). Prospects of Maize (Corn) Wet Milling By-Products as a Source of Functional Food Ingredients and Nutraceuticals. *Food Technology and Biotechnology*, *60*(1), 1–27. https://doi.org/10.17113/ftb.60.01.22.7340
16. Dietrich, M., Jacques, P. F., Traber, M. G., Cross, C. E., Hu, Y., & Block, G. (2006). Does γ-Tocopherol Play a Role in the Primary Prevention of Heart Disease and Cancer? A Review. *Journal of the American College of Nutrition*, *25*(4), 292–299. https://doi.org/10.1080/07315724.2006.10719538
17. Dowd, M. K. (2003). Improvements to laboratory-scale maize wet-milling procedures. *Industrial Crops and Products*, *18*(1), 67–76. https://doi.org/10.1016/S0926-6690(03)00034-7
18. Egesel, C. O., Wong, J. C., Lambert, R. J., & Rocheford, T. R. (2003). Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Science*, *43*(3), 818–823. https://doi.org/10.2135/cropsci2003.8180
19. Feng, S., Gan, L., Yang, C. S., Liu, A. B., Lu, W., Shao, P., et al. (2018). Effects of Stigmasterol and β-Sitosterol on Nonalcoholic Fatty Liver Disease in a Mouse Model: A Lipidomic Analysis. *Journal of Agricultural and Food Chemistry*, *66*(13), 3417–3425. https://doi.org/10.1021/acs.jafc.7b06146
20. Fraile, L., Crisci, E., Córdoba, L., Navarro, M. A., Osada, J., & Montoya, M. (2012). Immunomodulatory properties of beta-sitosterol in pig immune responses. *International Immunopharmacology*, *13*(3), 316–321. https://doi.org/10.1016/j.intimp.2012.04.017
21. Grams, G. W., Blessin, C. W., & Inglett, G. E. (1971). Distribution of Tocopherols in Wet- and Dry-Milled Corn Products. *Cereal Chemistry*, *48*(October), 356–359.
22. Hallikainen, M. A., Sarkkinen, E. S., & Uusitupa, M. I. J. (2000). Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner. *Journal of Nutrition*, *130*(4), 767–776. https://doi.org/10.1093/jn/130.4.767
23. Heuzé., V., Tran., G., Sauvant., D., Renaudeau., D., Lessire., M., & Lebas., F. (2018). Corn Gluten Meal, Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/715. Accessed 26 April 2019
24. Hossain, A., & Jayadeep, A. (2020). Analysis of bioaccessibility of campesterol, stigmasterol, and β-sitosterol in maize by in vitro digestion method. *Journal of Cereal Science*, *93*(December 2019), 102957. https://doi.org/10.1016/j.jcs.2020.102957
25. Jayadeep, A., & Malleshi, N. G. (2011). Nutrients, composition of tocotrienols, tocopherols, and c-oryzanol, and antioxidant activity in brown rice before and after biotransformation. *CYTA - Journal of Food*, *9*(1), 82–87. https://doi.org/10.1080/19476331003686866

26. Jiang, Y., & Wang, T. (2005). Phytosterols in cereal by-products. *Journal of the American Oil Chemists' Society*, *82*(6), 439–444. https://doi.org/10.1007/s11746-005-1090-5
27. Jiménez-Escrig, A., Santos-Hidalgo, A. B., & Saura-Calixto, F. (2006). Common Sources and Estimated Intake of Plant Sterols in the Spanish Diet. *Journal of Agricultural and Food Chemistry*, *54*(9), 3462–3471. https://doi.org/10.1021/jf053188k
28. Johnson, & Rasmussen, H. (2013). Nutrients for the aging eye. *Clinical Interventions in Aging*, *8*, 741. https://doi.org/10.2147/CIA.S45399
29. Ko, S.-N., Kim, C.-J., Kim, H., Kim, C.-T., Chung, S.-H., Tae, B.-S., & Kim, I.-H. (2003). Tocol levels in milling fractions of some cereal grains and soybean. *Journal of the American Oil Chemists' Society*, *80*(6), 585–589. https://doi.org/10.1007/s11746-003-0742-9
30. Lakshminarayana, R., Raju, M., Krishnakantha, T. P., & Baskaran, V. (2005). Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *53*(8), 2838–2842. https://doi.org/10.1021/jf0481711
31. López-Rubalcava, C., Piña-Medina, B., Estrada-Reyes, R., Heinze, G., & Martínez-Vázquez, M. (2006). Anxiolytic-like actions of the hexane extract from leaves of Annona cherimolia in two anxiety paradigms: Possible involvement of the GABA/benzodiazepine receptor complex. *Life Sciences*, *78*(7), 730–737. https://doi.org/10.1016/j.lfs.2005.05.078
32. Moreau, R. A., Powell, M. J., & Hicks, K. B. (1996). Extraction and Quantitative Analysis of Oil from Commercial Corn Fiber. *Journal of Agricultural and Food Chemistry*, *44*(8), 2149–2154. https://doi.org/10.1021/jf950743h
33. Moreno, F. S., Toledo, L. P., de Conti, A., Heidor, R., Jordão, A., Vannucchi, H., et al. (2007). Lutein presents suppressing but not blocking chemopreventive activity during diethylnitrosamine-induced hepatocarcinogenesis and this involves inhibition of DNA damage. *Chemico-Biological Interactions*, *168*(3), 221–228. https://doi.org/10.1016/j.cbi.2007.04.011
34. Ododo, M. M., Choudhury, M. K., & Dekebo, A. H. (2016). Structure elucidation of β-sitosterol with antibacterial activity from the root bark of Malva parviflora. *SpringerPlus*, *5*(1), 1210. https://doi.org/10.1186/s40064-016-2894-x
35. Paniagua-Pérez, R., Flores-Mondragón, G., Reyes-Legorreta, C., Herrera-López, B., Cervantes-Hernández, I., Madrigal-Santillán, O., et al. (2017). Evaluation of the Anti-Inflammatory Capacity of Beta-Sitosterol in Rodent Assays. *African journal of traditional, complementary, and alternative medicines : AJTCAM*, *14*(1), 123–130. https://doi.org/10.21010/ajtcam.v14i1.13
36. Park, Y. J., Bang, I. J., Jeong, M. H., Kim, H. R., Lee, D. E., Kwak, J. H., & Chung, K. H. (2019). Effects of β-Sitosterol from Corn Silk on TGF-β1-Induced Epithelial-Mesenchymal Transition in Lung Alveolar Epithelial Cells. *Journal of Agricultural and Food Chemistry*, *67*(35), 9789–9795. research-article. https://doi.org/10.1021/acs.jafc.9b02730
37. Raju, M. P. (2013). The Role of Phytosterols Enriched Foods-A Review. *IOSR Journal Of Environmental Science, Toxicology And Food Technology*, *7*(6), 40–47. https://doi.org/10.9790/2402-0764047

38. Ramjiganesh, T., Roy, S., Freake, H. C., McIntyre, J. C., & Fernandez, M. L. (2002). Corn Fiber Oil Lowers Plasma Cholesterol by Altering Hepatic Cholesterol Metabolism and Up-Regulating LDL Receptors in Guinea Pigs. *The Journal of Nutrition*, *132*(3), 335–340. https://doi.org/10.1093/jn/132.3.335
39. Rausch, K. D., & Eckhoff, S. R. (2016). Maize: Wet Milling. In *Encyclopedia of Food Grains* (2nd ed., Vol. 3–4, pp. 467–481). Elsevier. https://doi.org/10.1016/B978-0-12-394437-5.00239-4
40. Singh, N., & Eckhoff, S. R. (1996). Wet milling of corn - A review of laboratory-scale and pilot plant-scale procedures. *Cereal Chemistry*, *73*(6), 659–667. https://www.cerealsgrains.org/publications/cc/backissues/1996/Documents/73_659.pdf
41. Singh, S. K., & Eckhoff, S. R. (1996). Effect of pump rate and table slope on starch recovery for a 100-g laboratory wet-milling procedure. *Cereal Chemistry*, *73*(1), 51–53. https://www.cerealsgrains.org/publications/cc/backissues/1996/Documents/73_51.pdf
42. Singh, S. K., Johnson, L. A., Pollak, L. M., Fox, S. R., & Bailey, T. B. (1997). Comparison of Laboratory and Pilot-Plant Corn Wet-Milling Procedures. *Cereal Chemistry Journal*, *74*(1), 40–48. https://doi.org/10.1094/CCHEM.1997.74.1.40
43. Singh, V., Moreau, R. A., & Hicks, K. B. (2003). Yield and Phytosterol Composition of Oil Extracted from Grain Sorghum and Its Wet-Milled Fractions. *Cereal Chemistry Journal*, *80*(2), 126–129. https://doi.org/10.1094/CCHEM.2003.80.2.126
44. Virtamo, J., Taylor, P. R., Kontto, J., Männistö, S., Utriainen, M., Weinstein, S. J., et al. (2014). Effects of α-tocopherol and β-carotene supplementation on cancer incidence and mortality: 18-Year postintervention follow-up of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *International Journal of Cancer*, *135*(1), 178–185. https://doi.org/10.1002/ijc.28641
45. Yahl, K., Watson, S., Smith, R., & Barabolok, R. (1971). Laboratory wet-milling of corn containing high levels of aflatoxin and a survey of commercial wet-milling products. *Cereal chemistry*. http://agris.fao.org/agris-search/search.do?recordID=US201301131995
46. Yu, W., Simmons-Menchaca, M., Gapor, A., Sanders, B. G., & Kline, K. (1999). Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutrition and Cancer*, *33*(1), 26–32. https://doi.org/10.1080/01635589909514744
47. Yuan, C., Zhang, X., Long, X., Jin, J., & Jin, R. (2019). Effect of β-sitosterol self-microemulsion and β-sitosterol ester with linoleic acid on lipid-lowering in hyperlipidemic mice. *Lipids in Health and Disease*, *18*(1), 157. https://doi.org/10.1186/s12944-019-1096-2
48. Zhu, B., He, H., & Hou, T. (2019). A Comprehensive Review of Corn Protein-derived Bioactive Peptides: Production, Characterization, Bioactivities, and Transport Pathways. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 329–345. https://doi.org/10.1111/1541-4337.12411
49. Zielinski, H., Ciska, E., & Kozlowska, H. (2018). The cereal grains: focus on vitamin E. *Czech Journal of Food Sciences*, *19*(No. 5), 182–188. https://doi.org/10.17221/6605-cjfs

Tables

Table 1. Tocopherol and Tocotrienol levels (μg/100 g) in lab-scale corn wet milling by-products and whole corn flour

Tocol (Vitamin-E components)	Corn Germ	Corn Fiber	Corn Gluten	Whole Corn flour
Tocotrienols				
δ- Tocotrienol	23.8 ± 0.5^{a}	54.6 ± 5.01^{a}	50 ± 0.75^{a}	28 ± 2.4^{a}
γ-Tocotrienol	367 ± 18.2^{c}	661 ± 8.5^{b}	452 ± 3.9 ^{bc}	1098± 4.2^{a}
α-Tocotrienol	32.74 ± 0.14^{b}	132 ± 10.2^{b}	ND*	677 ± 3.2^{a}
Tocopherols				
δ Tocopherol	1738.1 ± 5.04^{a}	108.6± 9.02^{bc}	97.4 ± 8.7^{c}	262.94± 3.6^{bc}
γ-Tocopherol	53559 ± 472.6^{a}	978 ± 2.1^{d}	1396.5 ± 136.4^{c}	10682 ± 97.9^{b}
α-Tocopherol	3174.3 ± 27.06^{a}	28 ± 1.93^{c}	18 ± 0.85^{c}	1277 ± 22.7^{b}
Total Tocols	58894.9 ± 523.5^{a}	1962.2 ± 36.7^{c}	2013.9 ± 150.6^{c}	14024.94 ± 134^{b}

ND*-Not Detected. All values (μg/100 g) are expressed as mean ± standard deviation (*SD*); ($n = 3$). Different letters in the same row indicate significant differences for individual components among the samples ($p < 0.001$)

Table 2. Carotenoid levels in lab-scale corn wet milling by-products and whole corn flour (μg/100 g)

Carotenoids	Corn Germ	Corn Fiber	Corn Gluten	Whole Corn Flour
Lutein	64.6 ± 4.4^{c}	524 ± 38^{b}	2074 ± 142^{a}	398 ± 32.3^{b}
Zeaxanthin	79.5 ± 2.6^{c}	661 ± 17^{b}	3559 ± 346^{a}	235.3 ± 4^{c}
β- carotene	16.4 ± 2.6^{a}	29.97 ± 1.1^{a}	122 ± 11^{a}	35.41 ± 3.5^{a}
Total carotenoids	408.9 ± 11.6^{c}	1091.5 ± 21.8^{b}	9248.9 ± 15.3^{a}	1080 ± 1.6^{b}

All values (μg/100 g) are expressed as mean ± standard deviation (SD); (n = 3). Different letters in the same row indicate significant differences for individual components among the samples ($p < 0.001$)

Table 3. Phytosterol components in lab-scale corn wet mill by-products and whole corn flour (mg/ 100 g)

Phytosterol components	Corn Germ	Corn Fiber	Corn Gluten	Whole Corn Flour
Campesterol	3.72 ± 0.33^{a}	20.11 ± 1.80^{a}	8.72 ± 0.78^{a}	7.67 ± 0.69^{a}
Stigmasterol	34.38 ± 2.75^{a}	47.61 ± 3.80^{a}	ND*	10.56 ± 0.84^{b}
β-Sitosterol	31.11 ± 2.9^{d}	294.36 ± 27.4^{a}	134.25 ± 10.73^{b}	94.13 ± 8.75^{c}

ND*-Not detected. All values (mg/100 g) are expressed as mean ± standard deviation (SD); (n = 3). Different letters in the same row indicate significant differences for individual components among the samples ($p < 0.001$).

Figures

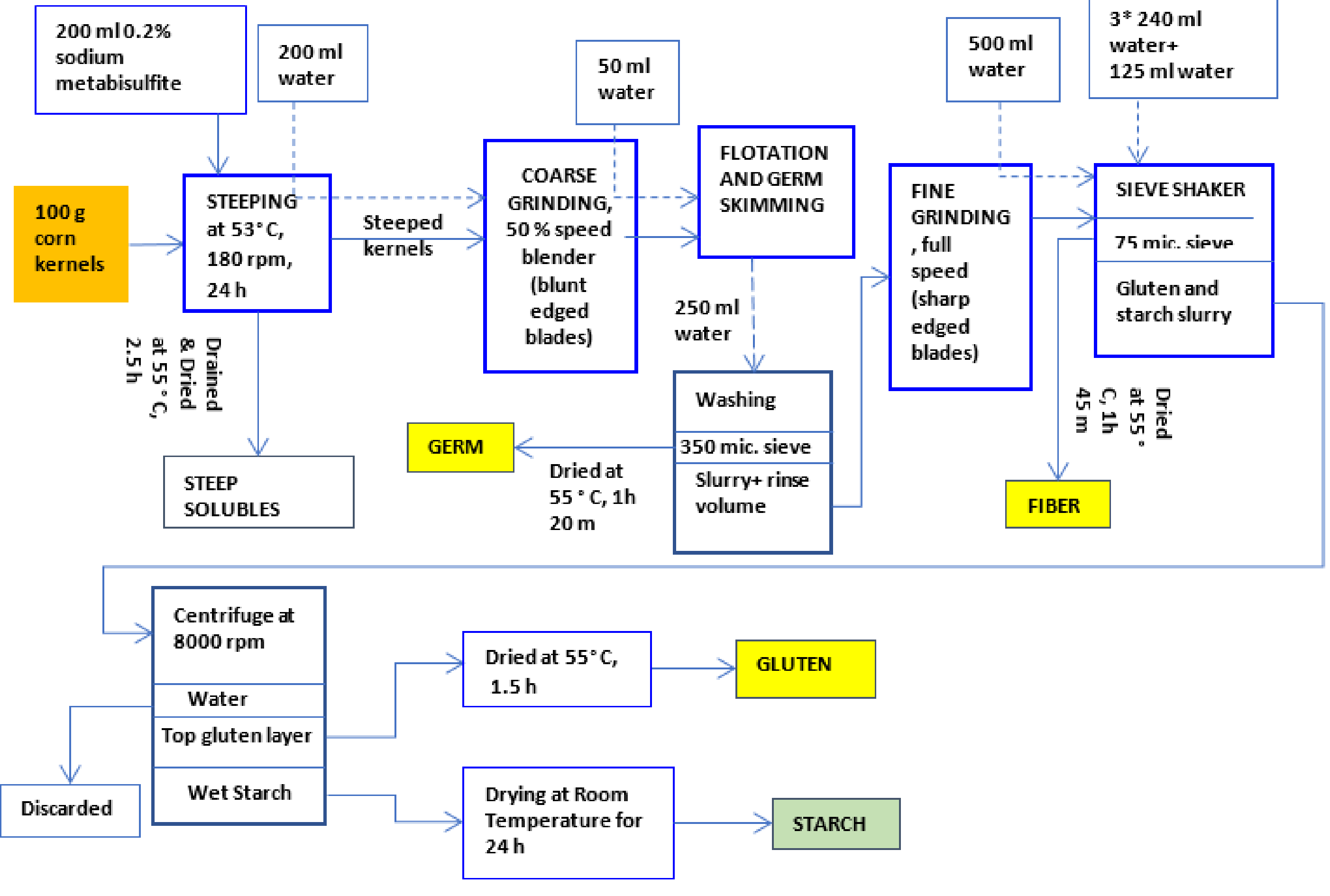
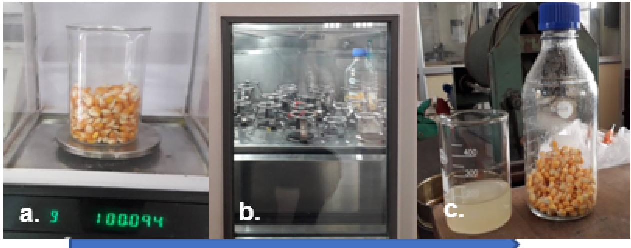


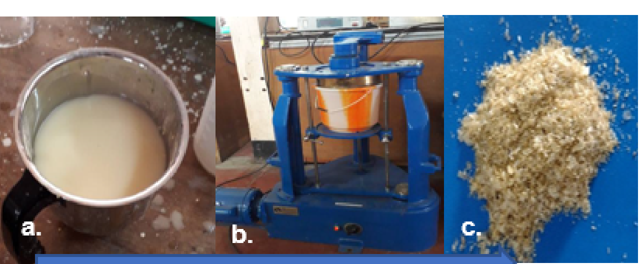
Figure 1

Process flowchart of lab-scale corn wet milling (Yellow represents the by-products we have worked upon)

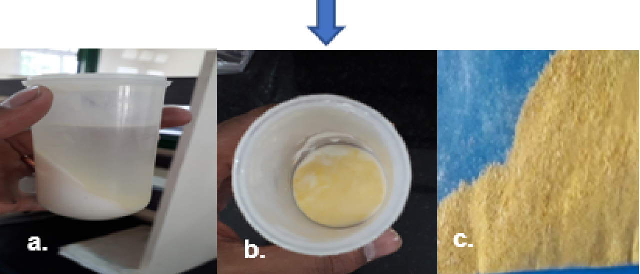


a. Big flint corn, b. Steeping unit at 53°C, c. Steep solubles & steeped corn

a. Coarse grinding and flotation of slurry, b. Wet Germ isolation, c. Dried Germs



a. Fine grinding of germ-free slurry, b. Washing & sieving of slurry for fiber isolation, c. dried fiber



a. & b. Centrifugal separation of starch and gluten slurry
c. Dried Gluten

Figure 2

Pictorial representation of lab-scale corn wet milling process

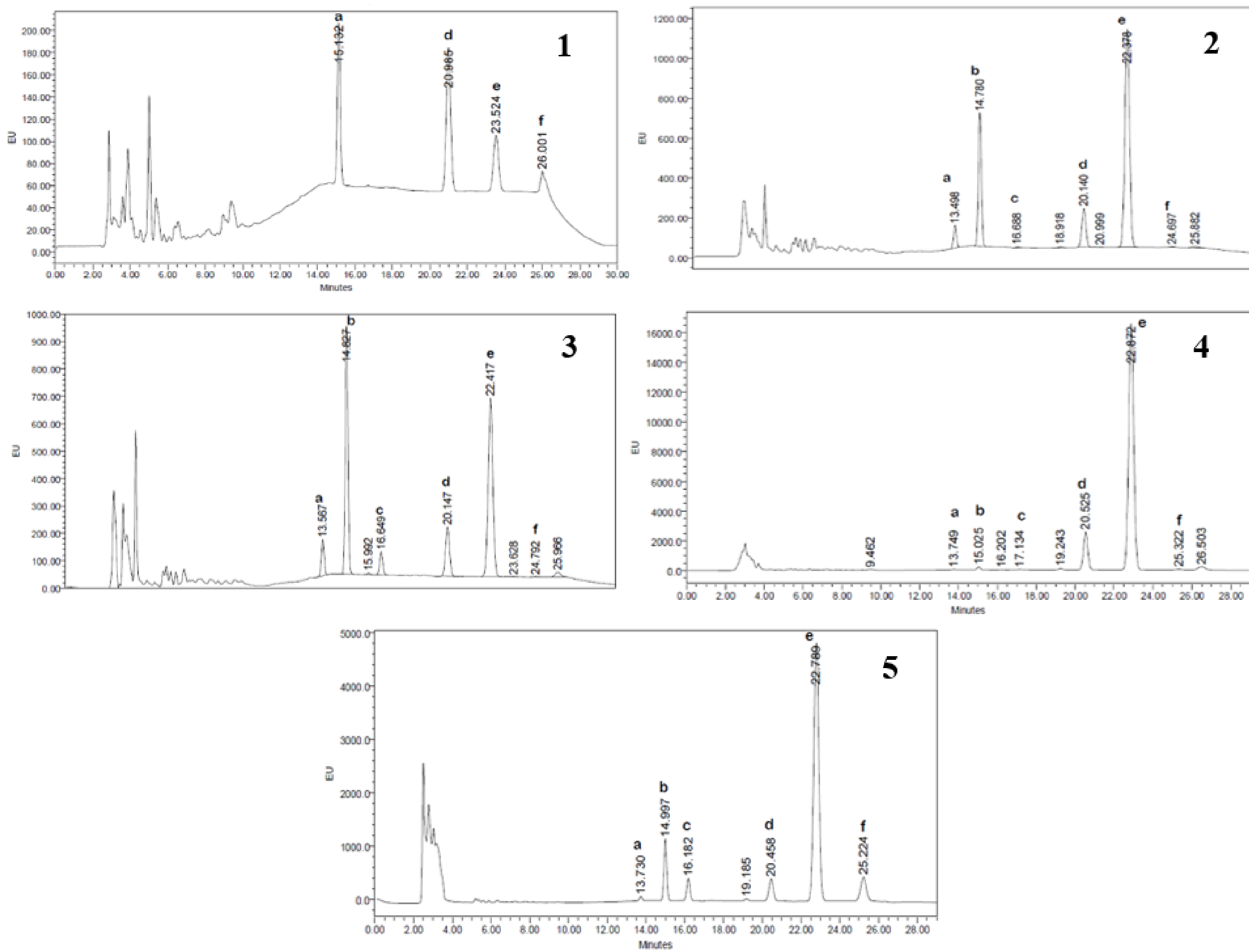


Figure 3

HPLC Chromatograms of 1. Std. Mix. 2. Corn Gluten 3. Corn Fiber 4. Corn Germ and 5. Whole Corn flour extracts, showing peaks of a. δ- tocotrienol, b. γ- tocotrienol, c. α-Toco-trienol d. δ- tocopherol, e. γ-tocopherol, f. α- tocopherol

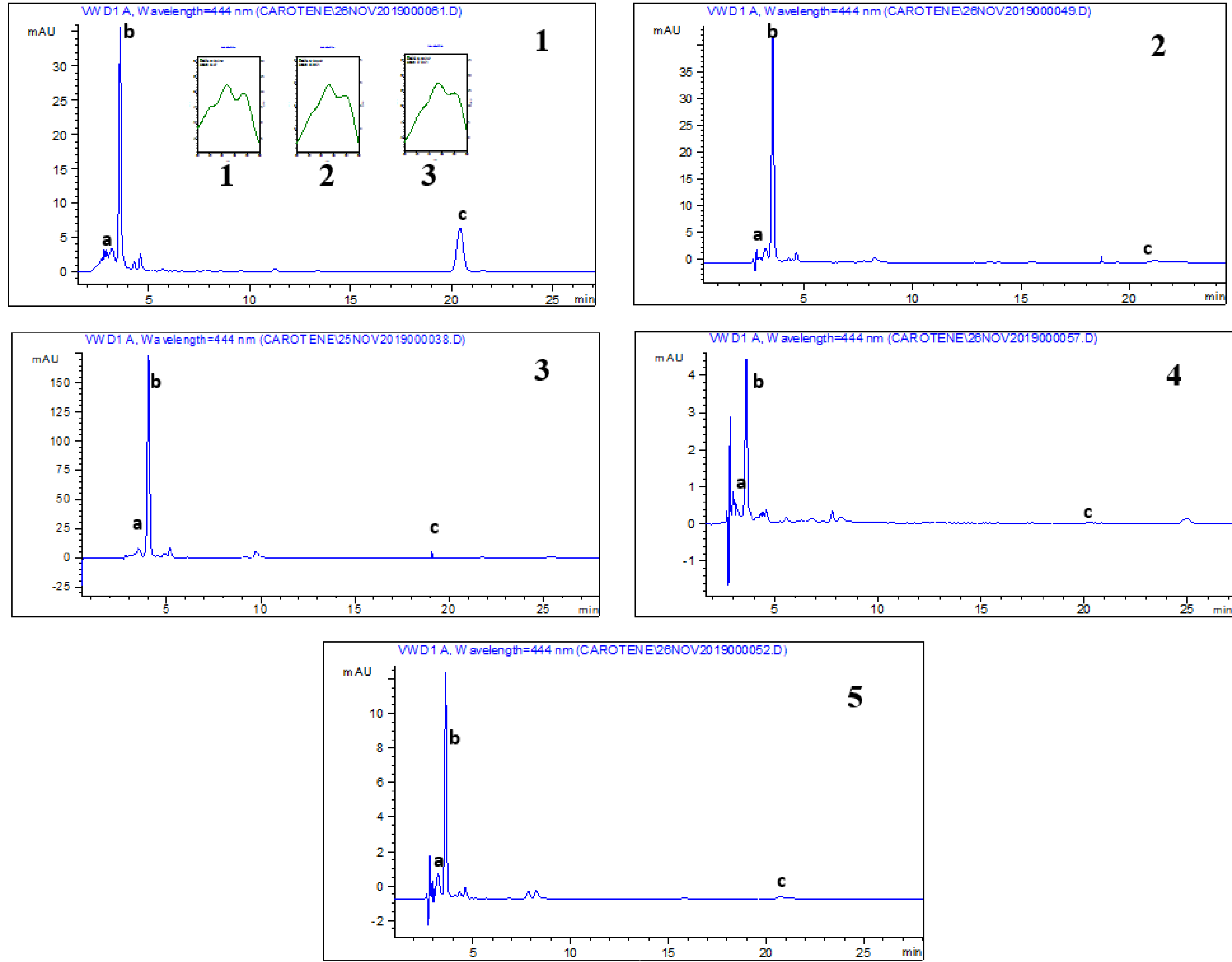


Figure 4

HPLC Chromatograms of 1. Std. Mix (insert shows the spectrums of 1-lutein, 2-zeaxanthin, and 3- β-carotene), 2. Corn Fiber 3. Corn Gluten 4. Corn Germ and 5. Whole Corn flour extracts of carotenoids showing peaks of a. Lutein b. Zeaxanthin and c. β- Carotene

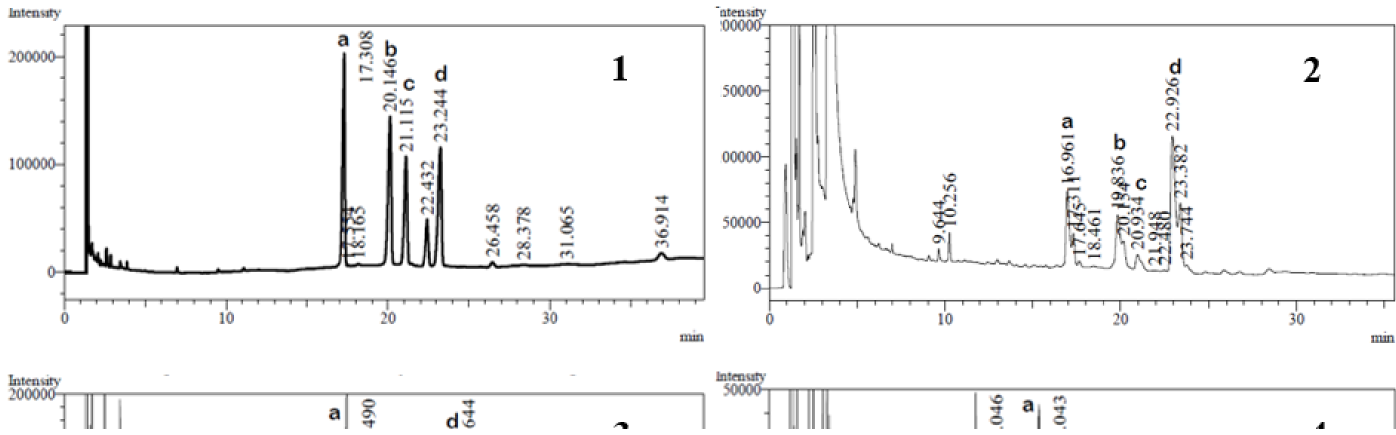


Figure 5

GC Chromatograms of 1. Std. Mix, 2. Corn Germ, 3. Corn Fiber, 4. Corn Gluten and 5. Whole Corn flour extracts, showing peaks of a. Internal std. b. Campesterol, c. Stigmasterol, & d. β-Sitosterol