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Prospects of Maize (Corn) Wet Milling By-Products as a Source of Functional Food Ingredients and Nutraceuticals

Running head: Maize Wet Milling By-Product Derived Bioactives

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SUMMARY

Maize (corn) consists of distinct parts, germ, endosperm, and pericarp, with different chemical compositions. During the maize wet milling process, the maize is disintegrated into the main product starch and by-products, including corn germ, corn fiber and corn gluten (the technical term for corn endosperm specific proteins and not the same as wheat gluten). These by-products are used as low-value animal feed products. The corn germ contains high amounts of tocols and phospholipids, while the corn gluten is rich in carotenoids and the corn fiber fraction is rich in phytosterols and complex carbohydrates. Each by-product has the potential to serve as a precursor in the manufacture of functional food ingredients or nutraceuticals that have antioxidant, anti-inflammatory, hypocholesterolemic, hypolipidemic and hypoglycemic properties. These food ingredients/nutraceuticals can be obtained through physical, chemical or enzymatic processes. Some nutraceuticals and food ingredients with market potential include corn fiber gum, oil, arabinoxylans, and xylo-oligosaccharides from corn fiber; corn germ oil and phospholipid ester from corn germ; and carotenoids and oligopeptides from corn gluten. This review focuses on current and prospective

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research into the use of corn germ, corn fiber and corn gluten in the production of potentially high-quality food ingredients or nutraceuticals.

Key words: maize (corn); wet milling; by-products; nutraceuticals; functional food ingredients

INTRODUCTION

Maize (*Zea mays* L.), which belongs to the family *Gramineae* and Genus *Zea*, is a staple food in many places worldwide and the third most important crop after rice and wheat (*1*). World maize production is currently 1136 million metric tons (MMT) and in India, it is 30.2 MMT (*2*). Due to its diverse uses as a food crop, animal feed, and important raw material, maize is a crop that is of vital importance around the world. It is used to manufacture various food and industrial products such as starch, animal feed, sweeteners, beverages, oil, glue, industrial alcohol, and fuel ethanol(*3*).

About 20 % of the corn produced in India is used for food purposes, about 47 % for poultry feed, 13 % for livestock feed, 14 % in the wet milling industry for starch and 6 % for export and Industrial non-food products (*4*). The corn is mainly processed into food using dry milling and wet-milling procedures. The wet milling process mainly concentrates on starch and its derivatives from corn. Starch undergoes various physical, enzymatic or chemical modifications in order to obtain various products. Some products are Maltodextrin, dextrin, dextrose monohydrate, sorbitol, liquid glucose, high maltose syrup, dextrose syrup, and anhydrous dextrose. These are used to produce beverages, bakery goods, pastries, meat, soups, sauces or baby food, textiles, dextrins, paper and pharmaceutical products (*5*). In addition to starch, corn steep liquor, corn germ, corn fiber and corn gluten (technical term for corn endosperm-specific proteins) are the by-products obtained. This review article aims to provide current literature on the various studies on the nutraceutical composition of the by-products and the gap existing therein. It also focuses on the opportunities used and prospects for the valorization of by-products to produce value-added components, functional food ingredients and nutraceuticals.

Maize Wet Milling

Maize Wet-milling involves the process of various physical, chemical, biochemical and mechanical operations to separate the components of the maize grain (germ, steep liquor, starch and corn gluten) into valuable products that are far more worthy than the raw grain (*6*). Corn starch and corn germ oil are the main profitable products of the maize wet-milling industry (*7*). With starch (60-70 %) being the main product, the by-products include steep liquor/solubles, corn germ, corn fiber and corn gluten (*6*).

The corn wet-milling industry was started in 1844 in the United States of America by Thomas Kingsford of the Wm. Colgate and Company in Jersey City, NJ, using a new alkali process to extract starch from corn (*5*). At the initial stages, the corn industry discarded the corn fiber, corn germ and protein obtained during the processing. Over time, however, the wet milling process gradually was changed by the non-starch components finding applications in animal feed, oil, polymer and pharmaceutical industries (*5*).

Conventionally, the wet milling process includes grain handling, steeping, separation and product recovery (Fig. 1).

Fig.1

The kernels are cleaned, segregated for quality kernels and conveyed to steeping tanks. In this process, maize kernels are steeped in SO_2 (0.2 %) and lactic acid (0.5 %) solution for 24 to 48 h to facilitate kernel hydration, leaching and induce mechanical stress for efficient separation of the kernel components (*6*).

In the beginning, lactic acid hydrates the kernels and ferments the soluble carbohydrates of the corn germ to produce lactic acid. When SO_2 starts operating, it restricts fermentation and facilitates starch separation from the protein matrix in the endosperm (*6*). Consequently, the components are then sequentially separated through industrial processes to obtain the end products.

After steeping, the steep water is drained and dried to form condensed steep solubles. Initially, corn kernels are coarsely ground to form a slurry using diameter disk mills containing intermeshed teeth to free the corn germ from endosperm and hull. Following this step, the slurry is pumped through a two-stage hydro cyclone system to recover the corn germ, which is further dried. The germ is usually delivered to oil mills, purified, and sold for human consumption. Residual corn germ meal is used as a part of livestock feed. Once the germ is separated from the slurry, constituting corn fiber, corn starch, and corn gluten, it is finely ground in the plate or single-disk mills. The thus obtained fine slurry is screened for corn fiber by screening and centrifugation, followed by washing and drying. From this de-fibered slurry, the corn starch and corn gluten are separated based on the differences in densities by the disk-nozzle centrifuge. Subsequent starch washing in hydro cyclones further removes the corn gluten. A mill stream thickener dewaters corn gluten which is further passed through a vacuum belt filter and then dried by a ring dryer. Both starch and corn gluten are dried separately (*6,8*).

Industrially, the starch yield is about 60–70 %, corn germ yield is about 7 %, corn gluten constitutes 5–6 %, corn fiber constitutes about 12 % and steep water solubles about 7 % yield (*9*). Starch is the primary product, but the by-product value can significantly affect overall plant economics.

Current uses of maize wet milling by-products

Steep solubles (corn steep liquor) is currently used as a nutrient-rich medium for antibiotic production (*e.g.* penicillin) and used as a feed additive for livestock, aquaculture and poultry. Steep solubles is used as feed additive up to 12 % of the diet dry matter without adversely affecting feed intake (*10*). Corn Steep Liquor (CSL), also known as condensed fermented corn extractives, is a high protein ingredient. It is often used as a corn gluten feed (CGF) constituent but may be sold as 50 % dry solids for cattle feeds or as a pellet binder (*10*). In some cases, corn germ is used directly to feed ruminants like Holstein cows/ mid-lactation dairy cows. Corn Gluten Feed (CGF) is a combination of the hulls and fiber fraction with steep water, corn germ meal and other process residuals. Corn Gluten Meal is the concentrated and dried corn protein obtained after the final separation from starch (*11*). The gluten/ gluten meal is a rich protein source and also contains carotenoids. Corn Gluten Meal (CGM) is the main feed for poultry and fish for pigmentation of egg and the flesh. The corn fiber is recombined with steep liquor and used as poultry feed. The corn germ meal, corn fiber and steep solubles are recombined to produce the co-product called Corn Gluten Feed (CGF) and used as animal feed (*11*). The industrial corn wet-milling by-products are represented in Fig. S1.

Fig. S1

By-products account for 30–40 % of the total product yield, but 20- 25 % of the kernel is processed with no added value, even though corn germ oil and corn gluten meal have a higher value than starch in the US (United States of America) market (*6*). A wide variety of products can be made from wet-milled starch and by-products, while the use of the by-products in human food is limited.

The use of corn wet milling is increasing, mainly to obtain compounds used in industrial ethanol production. Biofuel(ethanol) production from corn in the US and Brazil and the conversion of food into fuel is a cause for concern (*12*). There will be a justification for the biofuel industry if it creates value addition to by-products for functional foods. Bioethanol production from corn in the US is growing rapidly as an alternative to increasing gasoline prices and the national renewable fuels program (*13*). Due to increased bio-ethanol (corn wet-mill based) production, the volume of the by-products has also grown substantially, creating a need for the ethanol industry to find new uses for these by-products and an opportunity for the food ingredient and nutraceutical manufacturers (*13*).

However, adequate research and commercial interest in improving the existing by-products can help identify new industrial uses or nutraceuticals from by-products (*6*). Traditionally these by-products of corn wet milling are mainly used as animal feeds, which command less value. Therefore, specific research is needed to study and improve the use of these by-products. Nutraceuticals and functional food ingredients are highly valued, profitable and offer economic relief to farmers by increasing income. It would benefit the wet-milling industry and consumers by reducing fuel and food costs.

PHYTOCHEMICAL COMPONENTS OF MAIZE WET MILLING BY-PRODUCTS

Maize germ and its co-products

The corn germ makes up 9–11 % of the kernel mass. 80- 84 % of the total kernel oil is located in the corn germ, 12 % in the aleurone layer and 5 % in the endosperm (*9*). Characterized by a 39–47 % lipid content, the corn germ has an 18-19 % protein content and starch content of 8 % (*9,14*). The health-beneficial phytochemicals present in corn germ are antioxidant tocols, cholesterol-lowering phospholipids, phytosterols and policosanols (*15*). Weber found 63–91 % of tocols in the corn germ and tip cap, 3-11 % in the floury endosperm and 6–26 % in the horny endosperm (*16*). In the case of corn dry milling, it has been reported that the germ separated thereby contains 46.3 mg/100 g of total tocols (*17*). The phospholipids content in the corn germ of dissected kernels of high amylose maize (Amylomaize), LG-11 hybrid maize and waxy maize was 1224 mg/100 g, 1453 mg/100 g and 1363 mg/100 g, respectively (*14*). The serum-lipid reducing policosanols content in the corn germ was 1.93–3.71 mg/100 g (*15*).

The wet-milled corn germ was reported to have 11–16 % protein and more than 30 % oil (*18*). The corn germ, derived from lab scale wet-milling, had a total tocol content of 8.5 mg /100 g, including 6.6 mg/100 g of γ-tocopherol and 1.9 mg/100 g of α- tocopherol. However, the tocotrienol content was not present in measurable amounts (*19*). At 16.8 mg/100 g, the phytosterol content in wet-milled corn germ was higher than in wet-milled sorghum germ (*20*).

Corn germ oil

Corn germ oil is the product of the mechanical expulsion/solvent extraction of oil from corn germ in the wet milling industry. The significant presence of phytosterol in corn germ oil makes it suitable for reducing cholesterol absorption. The phytosterols limit the absorption of cholesterol mainly in the intestinal area and thus effectively prevent cardiovascular diseases (*15*). The tocols (tocopherols and tocotrienols), the dietary antioxidants in corn germ oil, enhance its oxidative stability. These tocol antioxidants protect against cell damage caused by free radicals. They help in maintaining food quality and health. Phenolic acids are about three times higher in cold-pressed corn germ oil than refined corn oil and can be used as functional ingredients (*21*). It is considered beneficial to health mainly due to its poly-unsaturated fatty acids- oleic and linoleic acids. A high oil content (80-84 %) in hybrids can improve the commercial prospects of corn germ oil (*9*).

According to a study, the tocopherols content of oil extracted by Soxhlet extraction using n-hexane from unroasted wet-milled corn germ was 59.4 mg/100 g (*18*). The oil also showed a high γ-tocopherol content of 41.9 mg/100 g followed by 7.92 mg/100 g of β-tocopherol, 6.26 mg/100 g of α-tocopherol and 3.23 mg/100 g of δ-tocopherol (*18*).

The phytosterol content of the same corn germ oil mentioned above was 713.9 mg/100 g with sitosterol content of 504.64 mg/100 g, 155.8 mg/100 g of campesterol and 53.3 mg/100 g of stigmasterol (*18*). In a comparative study, the tocopherol content in corn germ oil was around 28 times higher than in corn fiber oil extracted from corn fiber (*22*).

Defatted corn germ flour

Defatted corn germ flour can be a nutritious component of food. In this context, a flour was prepared from the wet-milled corn germ containing 30 % protein with 5.9 % lysine and a balanced ratio of other essential amino acids. Dried corn germs were first aspirated to remove the hull and flake it. The lipid was removed by solvent extraction and the final product contained significantly low lipid levels by a second solvent extraction of the flakes with 82:18 hexane/ethanol azeotrope via refluxing. The corn germ flakes were then ground in a grinder. The ground flour was analyzed to contain 2 % ash, 18 % starch and 0.6 % lignin. The flour contained high amounts of dietary fiber (22–29 % pentosans and 11–13 % cellulose) (*23*). Defatted protein-rich corn germ flour can be a functional ingredient that is added with other flours to make chapatis or bread.

Phospholipids as a functional ingredient

Corn germ is rich in amphiphilic molecules called phospholipids (*14*). They have hydrophobic fatty acid chains and hydrophilic units that occur in the cell membrane. They are therefore mainly found in all animal and plant-based foods.

The residual corn germ meal left after the corn germ oil extraction is usually discarded but is rich in lecithin. Lecithin is a high-quality additive component, which exhibits advantageous interfacial properties and has aroused increasing interest as a natural emulsifier in the food, pharmaceutical and cosmetics industries (*24*). It comprises a concentrated mixture of phospholipids (PL) such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA) and phosphatidylserine (PS) (*24*). Lecithin is usually extracted through multi-stage solvent extraction (*24*). PC, PE, PI and PS are the significant phospholipids in maize that regulate brain function and are essential for cell membrane function (*15*). Phospholipids reduce lipid levels in the liver by disrupting sterol absorption in the intestinal cavity. The other phospholipid functions include stimulation of bile acid and cholesterol secretion. Phosphatidylinositol and serine reduce blood triglycerides, fatty liver, bipolar disorders and neurodegenerative diseases (*15*).

Since corn germs are rich in phospholipids, they are a potential source for the development of phospholipids as a functional ingredient.

Corn fiber and its co-products

Corn fiber is a by-product yielding high after starch in the corn wet-milling process. It is commonly known as "white fiber," a mixture of fibers of the pericarp (bran) and hull (coarse fibers) and fibers of the corn germ cell wall and endosperm (fine fibers) (*25*). The wet-milled corn fiber consists of complex carbohydrates (Non-starchy polysaccharides), composed of 40 % hemicellulose, 25 % starch, 12 % cellulose, 10 % protein, 3 % oil and 10 % other substances such as phenolic antioxidants, ferulate phytosterol esters, lignin and ash (*26*). The hemicelluloses (arabinoxylans and β-glucans) are reported to have antioxidant activity (*27,28*). Phytochemicals, mainly phytosterols and tocopherols also are present in corn fiber. Corn fiber extract of hexane was investigated to contain a phytosterol level of 19.3 mg/100 g, which was highest among the wet-milled by-products compared to corn germ (16.8 mg/100 g) and corn gluten (4.8 mg/100 g) (*20*). The tocols were extracted using ethanol from wet-milled corn fiber (lab-scale) and the total tocol levels were observed to be 5.4 mg/100 g (*19*). The homologues, γ- tocopherol (1.47 mg/100 g), α-tocopherol (0.73 mg/100 g), α- tocotrienol (1.29 mg/100 g) and γ-tocotrienol (1.98 mg/100 g) were described in the lab-scale wet-milled corn fiber fraction (*19*).

Dietary fibers are the in-soluble non-starchy polysaccharides present in corn fiber that help in the physiological processes of the grain (*29*). The total dietary fiber content was 52.6–73.5 g/100 g in wet-milled corn fiber (*30*). Based on previous studies, corn fiber is a potential source of arabinoxylans, a typical dietary fiber (*25*). The soluble fiber components of corn are arabinoxylans, fundamentally situated in pericarp cell walls, where they support the kernel structurally and functionally. These arabinoxylans have arabinose side chains esterified with phenolics like ferulic acid and exert antioxidant properties (*29*).

Corn fiber gum

Corn fiber gum (CFG) is an alkaline extract of corn fiber comprised of arabinoxylan (hemicellulose) with high solubility and low viscosity (*7,25*). Corn fiber gums could contain less than 5 % of proteins, phenolics (ferulic and p-coumaric acid) and lipids combined (*27*). The hemicellulose of corn fiber has properties similar to gum arabic and is often expensive and scarce. Hemicellulose was extracted with hot alkali, $NaOH/Ca(OH)_2$ and bleached with hydrogen peroxide. CFG extracts were obtained as both acid-soluble and acid-insoluble fractions (*25*). CFG is an excellent emulsifier with improved physicochemical and nutritional properties due to the presence of phenolic acids, lipids and proteins (*7*). Additionally, it can be an adhesive, thickener, stabilizer and film former. Therefore, the application of CFG can be studied for its viability in expanded snacks, bakery goods, beverages, specialty foods, edible coatings, nutritional supplements and the development of functional foods for its health benefits.

Dietary fibers are the main constituents of corn fiber and aid in the proper functioning of the digestive system by acting as a stool bulking agent (*29*). Arabinoxylans (both soluble and insoluble) can improve colon function, prevent diabetes mellitus, cardiovascular diseases, some cancers and immunological disorders (*7*). They have a robust prebiotic effect, reduce gut infections, prevent colon cancer and increase intestinal short-chain fatty acids known to reduce blood cholesterol (*7,29*). Arabinoxylans have been used to produce functional gluten-free bread with improved properties (*7*).

Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are potential prebiotics, partially hydrolyzed, water-soluble xylan fragments obtained from corn fiber by enzymatic or high-temperature treatment (*7*). Microbial xylanases are used for the enzymatic degradation of corn fiber. In contrast, hydronium ions and organic acids are used for high-temperature treatment at 160–220 °C to partially hydrolyze heteroxylan polymers and yield soluble hydrolysates of XOS (*31*). Preliminary studies on the use of commercial XOS structurally similar to corn fiber in older Japanese men showed an increase in bifidobacteria concentration after three weeks on XOS (*32*). In addition to its prebiotic activity, the XOS may exhibit antioxidant activity based on the attachment of ferulic acid ester moieties to the solubilized oligosaccharides (*7*).

Ferulic acid

Ferulic acid is reported to have many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, anti-thrombotic and anti-cancer activities (*33*). Due to its antioxidant and antimicrobial properties, ferulic acid can be an excellent preservative. An expensive chemical process is used to produce commercial ferulic acid with environmental concerns, and therefore a need for natural sources to obtain the product exists. Corn bran contains good amounts of ferulic acid (30 g/kg) compared to rice bran oil (10–20 g/kg) and can therefore be an alternative source of ferulic acid (*7*). Unfortunately, ferulic acid in corn bran is necessarily bound to the cell wall, thus posing major challenges in its isolation from the corn bran. Experiments on the extraction of bound ferulic acid from corn bran have been demonstrated. After a more aggressive alkali (NaOH) treatment of corn bran to break the ferulate crosslinks, a high amount of ferulic acid was released into the solution (*34*). As a result, the corn gum and ferulic acid are co-solubilized and the ferulic acid is separated by precipitating corn gum in ethanol (*34*). Another study demonstrated that thermal and enzymatic treatments release ferulic acid from corn bran (*35*). Ferulic acid from corn fiber is an attractive opportunity to transform the corn wet milling industry.

Xylitol

Xylitol is a naturally occurring five-carbon sugar alcohol derived from xylose by reduction of the carbonyl group. It is used as a low-calorie sweetener in the food industry as it is helpful for people with diabetes and has bulking properties similar to sucrose (*7*). It does not cause tooth decay, is slowly absorbed and has a lower glycemic index (*36*). The chemical hydrogenation of xylose produces the major part of xylitol, usually obtained from the wood hydrolysate. Corn fiber can be a potential starting material as it has a xylose content (~200 g /kg) similar to that of hardwoods (*7*).

In an experiment employing yeast, xylose is initially hydrolyzed with dilute acid or enzyme treatment in a cost-effective step. The resulting syrup is then fermented by a yeast called *Candida tropicalis* and then treated with activated charcoal to remove xylitol inhibitors to increase xylitol yields (*37,38*).

Vanillin

Vanillin is an essential flavor and aroma compound used in the food, pharmaceutical and cosmetic industries. It is obtained from properly cured vanilla (*Vanilla planifolia* Andrews) pods occurring at a level of 10–30 g/kg whole beans. Vanillin is also found in agricultural products, including pine, tobacco and citrus fruits (*7*). In corn bran, vanillin is present at a level of 55 g/100 g (*39*). The demand for vanillin exceeds its production from vanilla beans. Many researchers have turned to the microbiological conversion of ferulic acid to vanillin (*40*).

A procedure involving *A. niger* was used to obtain vanillin from ferulic acid obtained from autoclaved corn bran (*40*). Recently, one process used pressurized subcritical water (low polarity water) to convert ferulic acid to vanillin by breaking its aliphatic double bond (*39*). It is also known that corn fiber contains sufficient amounts of ferulic acid and, therefore, can be used for vanillin production.

Corn fiber oil

Corn fiber oil is a unique oil extracted from finely ground corn fiber, usually using hexane, ethanol, or supercritical CO_2 (*41*). It was estimated that the total phytosterol content in corn fiber oil was about 7939 mg/100 g compared to 840 mg/100 g of corn germ oil (*42*), but the yield of corn fiber oil is about 2–3 % using hexane (*41*). The corn fiber contains starch, hemicellulose and cellulose, which must be removed by treatment to improve corn fiber oil yield. After treatment with dilute acids and enzymes, the phytosterol content in corn fiber increased from 19.8 mg/g to 1256.2 mg/g (*26*).

Corn fiber oil has recently been of interest due to high ferulate phytosterol esters, the most predominant being sitostanyl ferulate (*42,43*). Additional animal studies have confirmed its cholesterol-lowering effects (*43,44*).

Policosanols

Policosanol is a mixture of long-chain primary aliphatic alcohols mainly rich in sugarcane. These compounds were identified and first approved as dietary supplements in Cuba and are commercialized in the Caribbean and South American countries (*45*). As mentioned earlier, the pericarp fraction of the wet-milled corn fiber contains policosanols. The corn pericarp has a policosanol content of 72.7–110.9 mg/kg (*46*). Physiologically they improve health by reducing blood lipid levels and platelet aggregation (*15*). Octacosanol (C28), Triacontanol (C30) and Hexacosanol (C26) are primarily reported policosanols that contribute to the lowering of serum cholesterol levels. The distillers' dried grains with solubles (DDGS) obtained after fermentation of dry milled corn by-products contain policosanols and can be converted into health-promoting functional ingredients (*15*).

Several dietary supplements are commercially available in the US market containing policosanol, usually derived from sugarcane (*15,46*). Numerous scientific studies indicate that the daily consumption of 1–20 mg of policosanols effectively reduces insulin resistance, total blood cholesterol, LDL-cholesterol in older adults (>75 years) (*15*). Further research is needed to determine the policosanol components in corn fiber.

Corn gluten (proteins) and its co-products

Corn gluten/corn gluten meal (CGM) (corn protein) is a significant corn wet milling by-product containing at least 60 % protein and is rich in health-promoting carotenoids (*10*). Corn protein is not the same as wheat gluten which causes celiac disease. Corn contains albumins, globulins, prolamines (zein protein) and glutelin proteins (35 %), with zein protein contributing more than 50 % of them. A mixture of zein protein and glutelins, known industrially as corn gluten, are endosperm-specific (*47*). CGM contains adequate quantities of sulfur-containing amino acids- methionine and cysteine- involved in synthesizing intracellular antioxidants (*10*). The hydrophobic amino acid composition of Leu, Ala and Phe makes CGM proteins a good source of bioactive peptides (*48*). However, due to its imbalanced amino acid composition and low water solubility proteins, CGM is mainly marketed as a feedstock or discarded but not used for human food purposes (*49*).

Nevertheless, corn gluten meal hydrolysis can provide peptides with antioxidant properties and can therefore be revalorized in food or pharmaceutical products (*48,49*).

Low phytosterol levels of 4.8 mg/100 g were detected in the wet-milled gluten (*20*). Phytochemically, yellow corn contains 74–86 % of carotenoids (primarily xanthophylls) in the endosperm trapped in the gluten matrix (*1*). The CGM contained around 195–491 mg/kg xanthophylls (lutein, zeaxanthin, cryptoxanthin), whereas the starting corn material has only 40.1 mg/kg xanthophylls (*10,50*). The carotene content of CGM was 49–73 mg/kg while it was 22 mg/kg in the starting corn material (*10*). These studies indicate that xanthophylls and carotenes are concentrated in the CGM obtained from the corn wet milling process. Zeaxanthin is a significant component in cooked corn compared to other foods such as spinach, lettuce and parsley (*51*).

CGM is characterized by high protein and energy content making it a potential high energy source of nutrition.

Carotenoid supplements

Functional carotenoid supplements or ingredients can potentially be extracted from CGM. These carotenoids are usually extracted from samples by mechanical/physical method assisted (maceration/ microwave/ ultrasound) solvent extraction (hexane/ acetone/ ethanol) or supercritical fluid (CO_2) extraction or soxhlet extraction (*52*). Carotenoids are excellent natural antioxidants for maintaining food quality and human health (*15*). Carotenoids promote eye health, prevent cancer and are anti-aging. Lutein and zeaxanthin are the carotenoids in the macula of the retina necessary for sharp and detailed vision. Studies have shown that they protect the eye from phototoxic damage, age-related macular degeneration and age-related cataract formation (*53*). Lutein also shows inhibitory effects during cancer promotion as a chemopreventive and suppressive agent (*54*). Extraction and utilization of carotenoids, mainly lutein and zeaxanthin, from corn gluten as dietary supplements or food ingredients is an exciting prospect as it is a rich and cheaper source.

Corn protein hydrolysates and bio-active peptides

Corn gluten meal (CGM) contains diverse proteins, including albumins, globulins, glutelins and prolamins (zein protein). It is reported that the bioavailability of proteins of CGM can be enhanced remarkably by enzymatic hydrolysis. As a result of this process, hydrolysates are obtained containing small peptides, especially dipeptides and tripeptides, that can be absorbed more efficiently than the intact proteins or the free amino acids (*49*). These protein hydrolysates are antioxidative and can effectively inhibit lipid oxidation in foods (*55*). Additionally, they can be used as food additives and to make edible coatings and packaging films.

Bioactive peptides are encoded specific peptide fragments within the primary structure of proteins that remain inactive and have potential health benefits (*56*). After the protein hydrolysis, bioactive peptides are released that can modulate human metabolism, and also treat chronic diseases with discrete potency and fewer side effects such as toxicity. Enzymatic hydrolysis is predominantly used for the production of protein hydrolysates. Alcalase is used for example, to produce bioactive peptides (*57*). Additionally, the integrated utilization of multiple enzymes or enzymes linked to other techniques was standardized for use in the hydrolysis process of CGM (*58*). After hydrolysis, the isolation and purification procedure involves membrane separation (Ultra-filtration or Nano-filtration) or column chromatography (FPLC, size-exclusion and others). The peptides obtained are later characterized by SDS-PAGE, MIR or MS techniques (*59*).

Recently, corn peptides, a novel food derived from CGM through enzymatic hydrolysis or microbial fermentation, have been recognized for their various bioactive properties, including antioxidant activity (*49,60*), improvements in lipid profiles and the ability to accelerate alcohol metabolism and protect against alcohol-induced liver injury (*61*). Corn peptides are distinguished as small in size, readily absorbable and safe for consumption. They have been reported to have many inherent bioactive properties such as anti-inflammatory, antioxidant, anti-hypertensive, hepatoprotective, alcohol metabolism-facilitating, anti-cancer, antimicrobial and DPP-IV (dipeptidyl-peptidase IV, EC 3.4.14.5) inhibitory activities (*48,49,58,60*). Corn peptides from Corn Gluten Meal were investigated to show anti-hypertensive activity with strong ACE (Angiotensin Converting Enzyme) inhibitory activity (*62*). Many studies have examined the anti-obesity effects of corn protein hydrolysates and enriched peptides (*49*). Some scientists in China have also worked on the anti-obesity activity of corn peptides on obese rats (*49*). In addition to these functions, some corn peptides also possessed antimicrobial or metal-binding activities (*63*). Antimicrobial peptides could find exciting applications in the field of food safety. For example, laboratory tests have shown that they protect fresh meat by inhibiting bacterial growth and blocking bacteria from adhering to meat surfaces (*64*).

Phytochemicals from the by-products mentioned above are usually associated with antioxidant and antiradical activities, anti-mutagenesis, anti-carcinogenesis, antimicrobial, anti-inflammatory activities, anti-lipidemic and hypocholesterolemic properties. These phytochemicals may be partially degraded during storage, milling, and processing. These aspects have to be examined to study the effects of processing on the nutraceutical quality of the food ingredients. Nevertheless, the maize wet mill by-products are rich in nutraceuticals. Nutraceuticals are nutritional supplements containing concentrated bio-active components from a specific food, presented in a non-food matrix, to promote health using dosages that may exceed those obtained from regular food (*65*). Table 1. below shows the phytochemical/ nutraceutical composition of corn wet milling by-products.

It can be seen that the studies on phytonutrients in maize wet mill by-products are scarce and therefore worth investigating.

Table 1

The bio-active components are intrinsic to by-products: corn germ is rich in phytosterols and tocopherols; corn gluten is high in carotenoids and proteins, and corn fiber is high in phytosterols and dietary fiber, as mentioned above.

A nutraceutical is indicated to have a physiological benefit or protect against chronic diseases. Nutraceuticals have demonstrated beneficial effects in combating oxidative stress, chronic diseases, and cancer.

Some functional food ingredients containing nutraceutical compounds have been developed from corn wet milling by-products. Table 2 shows the nutraceuticals and functional food ingredients from corn and their beneficial effects.

Table 2

This table demonstrates that the corn wet milling by-products are rich in phytochemicals and can be transformed into functional food ingredients or nutraceuticals. Functional food ingredients/nutraceutical supplements are available from various expensive sources, such as the carotenoids from marigold flowers, policosanols from sugarcane or beeswax. However, the by-products of the corn wet milling process are an alternative source for manufacturing nutraceuticals and functional food ingredients.

CONCLUSIONS

When corn is wet-milled, the starch is obtained at a 60-70% yield with by-products (corn germ, gluten, and fiber) at a yield of more than 30%. These by-products are currently used as low-quality feed for poultry, farm animals, pigs, and fish. Research studies confirm that the by-products of wet milling are rich in phytochemicals, mainly tocols, phytosterols, phospholipids, carotenoids, phenolic compounds, and arabinoxylans/fiber. Among the by-products, the corn germ is rich in tocols; corn fiber is high in phytosterols and dietary fiber, whereas corn gluten(protein) is high in carotenoids and proteins. Functional foods are produced from by-products of corn wet milling. Few functional foods rich in phytochemicals are corn germ oil from corn germ, corn fiber oil and gum from corn fiber, protein hydrolysates from corn gluten. These functional foods are reported to have beneficial health effects like cholesterol-lowering, cardio-protective, hepato-protective, anti-carcinogenic and prebiotic properties. The by-products can be upcycled to high-quality nutraceutical sources. However, more scientific information about the phytochemical content of corn wet milling by-products is required. Sufficient research is also required on the effects of technological interventions such as thermal, physical, and enzymatic treatments on the nutraceutical quality of corn wet milling by-products to enable the development of functional food ingredients or dietary supplements. Overall, these by-products can establish a lucrative platform for industrial corn wet milling, the bioethanol industry, and farmers to transform the agricultural sector economically.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

All supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

Deepak T S - data collection, data analysis and interpretation, performing the analysis, drafting the article. Jayadeep A - conception or design of the work, data analysis and interpretation, critical revision, final approval of the version to be published.

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Table 1. Proximate and phytochemical composition of corn wet milling by-products

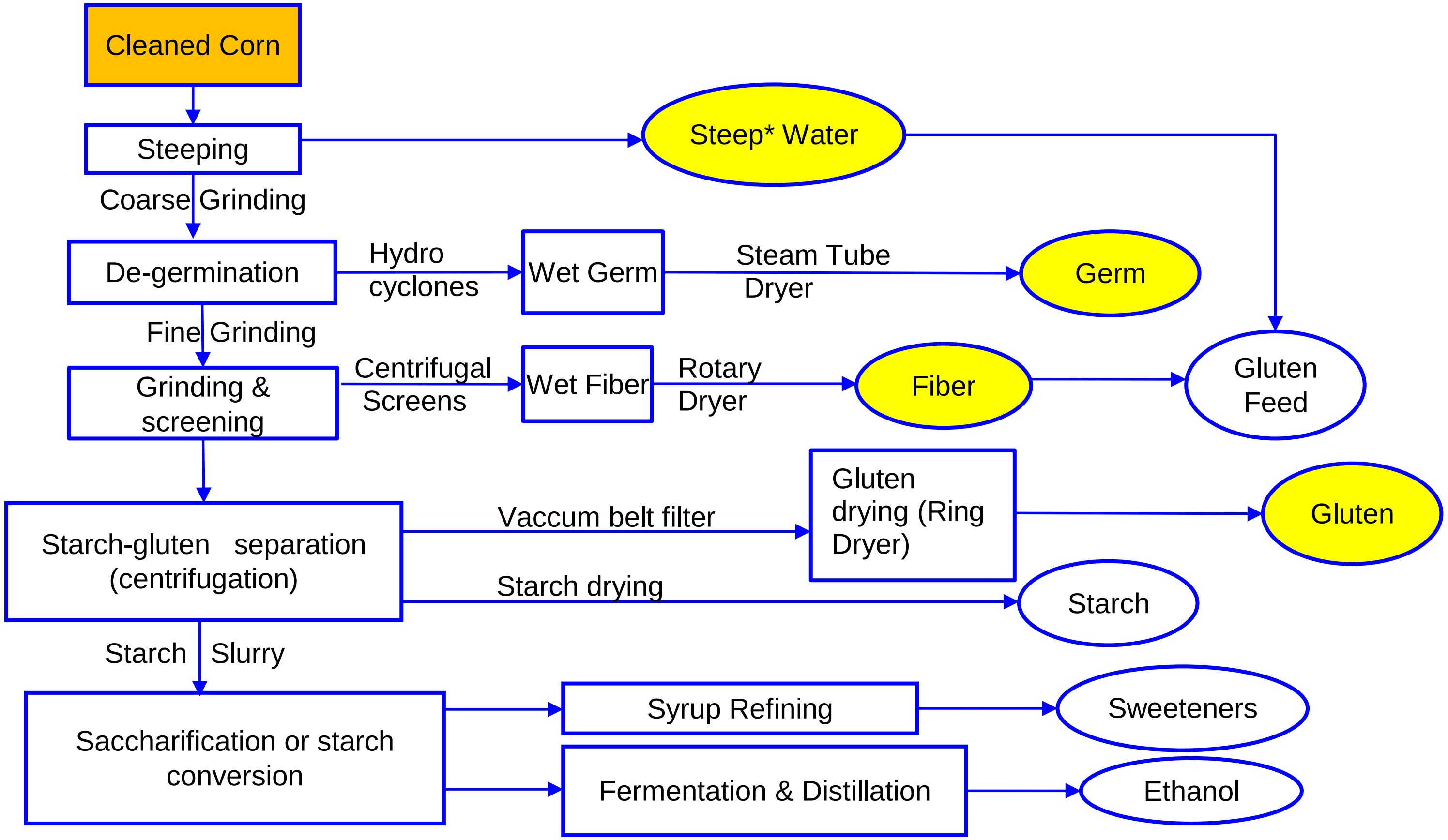
Nutrient	Corn	Gluten meal	Germ	Fiber	References
w(protein)/%	7–13	60–75	20–30	10–13	(*9,10,50,66–68*)
w(starch)/%	67–73	12–20	19–25.4	15–20	(*9,10,50,66–69*)
φ(oil)/%	2–6	1–6.5	1–20[a], 40–50 %[b]	1.72–3.68	(*9,10,50,66,70,71*)
w(ash)/%	1.4	1.1–4.6	1.6–4.3	6–20	(10,12,50,66–68)
w(dietary fiber)/%	12.19–12.80	4.65	NR	52.6–73.5	(*30,69,72*)
w(phenolics as GAE)/(mg/100 g)	239.2–327.7	NR	NR	NR	(*73*)
w(xanthophylls)/(mg/100 g)	4.01	19.5–49.1	NR	NR	(*10,50*)
w(carotenes)/(mg/100 g)	2.2	4.9–7.3	NR	NR	(*10*)
w(vitamin-E (tocols))/(mg/100 g)	6.69	7.85	8.5	5.46	(*19,73*)
w(phytosterols)/(mg/100 g)	88.01	4.8	16.8	19.3	(*20*)

NR=not reported, [a]oil in germ meal, [b]oil in germ

Table 2. Health benefits of phytochemical-rich corn wet mill by-products and their functional food ingredients

Phytochemicals	Health benefits	By-products and co-products rich in phytochemicals	Functional food ingredients from the by-products	References
Phytosterols: sterols and stanols (*e.g.* β-sitosterol, stigmasterol, campesterol)	Reduces cholesterol absorption in the intestine, prevents cardiovascular diseases, reduces oxidized low-density lipoprotein (LDL) levels, reduces colon tumors, prevents osteoarthritic-degradation.	1. Corn bran/fiber a. Fiber oil 2. Corn germ a. Germ oil	Fiber oil (rich in phytosterols) Cold-pressed corn germ oil	(*15,20,27,42,43,74*)
Tocols/tocochromanols (tocopherols and tocotrienols) (α, β, γ, δ)	Cell protection from free radicals, strengthens the immune system by T-lymphocytes, prevents cardiovascular diseases.	1.Corn germ a. Germ oil 2. Corn fiber a. Fiber oil	Germ oil Gamma tocopherol rich Fiber oil	(*15,22,75*)
Carotenoids: xanthophylls (lutein, zeaxanthin), β-carotene	Prevent cancer, protect eye health, prevent cardiovascular diseases and strengthen the immune system.	Corn gluten meal	Not available	(*10,53,54*)
Policosanols: octacosanol, triacontanol, hexacosanol, dotriacontanol	Reduces blood lipid levels and platelet Aggregation.	Corn fiber	Not available	(*45,46,76*)

Phospholipids: phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine	Hypocholesterolemic, cardio-protective, hepato-protective, hypolipidemic and anti-carcinogenic. Phosphatidyl choline is beneficial in brain and mental development, by neural transmission and can treat neurological disorders.	Corn germ	Phospholipid based emulsifiers (lecithin) from maize are in the market, patented by Cargill company (*e.g.* Nestle-NAN).	(*14,15,75*)
Phenolic compounds Simple phenolics (ferulic acid)	Potent antioxidants that prevent inflammation. Ferulic acid bound to soluble corn fiber gum is delivered to the colon, to prevent colon inflammatory diseases.	Corn fiber	Corn fiber gum, xylo-oligosaccharides	(*27,77*)
Arabinoxylans (copolymers of arabinose and xylose)	Maintaining colon health and resist the absorption of cholesterol in the colon. They are strong prebiotics to maintain gut health by increasing lactobacillus and bifidobacterium population.	Corn fiber	Corn fiber gum and xylo-oligosaccharides Corn arabinoxylans-functional gluten free bread	(*77–79*)
Protein hydrolysates: peptides	Anti-hypertensive, hepatoprotective, anti-inflammatory, increases alcohol metabolism, antimicrobial.	Corn gluten meal	Corn bio-active oligo-peptide extracted from non-GMO corn and spray dried-powder (smartPEP)	(*49,60–62,80*)



*Certain grades of steepwater may also be a final product

Fig. 1. Industrial corn wet milling process (yellow color represents the main by-products) based on Rausch *et al.* (*6*)

Supplementary material

a. b. c. d.

Fig. S1. Industrial corn wet milling by-products: a. corn germ, b. corn gluten, c. corn fiber, d. corn steep liquor