Possible ways of bio-refining and utilizing the residual lignocelluloses of corn growing and processing

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Abstract

The events of the past five years showed that the most promising alternative liquid fuel is the bioethanol, which could replace fossil fuels, thus impeding the further acceleration of the global warming. The bioethanol production of the world showed a significant growth, which was basically caused by the increase of corn starch processing and fermentation. However, the growing and the processing of corn results in lignocellulosic by-products like corn stover, corncob, corn fiber, and press cake. The weight of the starch is only ∼25% of the whole corn plant. The further increase of corn growing and processing will increase the production of the lignocellulosic by-products as well.

The idea of "bio-refining" means the fractionation of the biomass with chemical and biotechnological methods, and producing value added products from each fraction. The goal of most researches in the field of lignocellulose utilization is the production of ethanol. Most authors conclude that it is not economical because of the high ratio of the yet unutilizable fractions, like hemicellulose and lignin. But if these fractions could be sharply separated, and converted to valuable products, the whole process of lignocellulose utilization (bio-refining) could be economically feasible. The scope of this study was to review and evaluate all of the presently available methods for separating the fractions of lignocellulosic biomasses, and for utilizing them. Over one hundred publications were reviewed from the fields of biotechnology, food chemistry, and chemical technology to collect all processes for lignocellulose utilization.

Keywords

lignocellulose utilization · bioethanol · bio-refining · corn processing

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1 Introduction

For economic and strategic reasons, several countries have already decided to produce fuel ethanol from biomass during the 20th century. Nowadays environmental problems, like the acceleration of the global warming, caused by the antropogene emission of the so-called greenhouse gases, and also the danger of running out of fossil fuels in the next few decades urge engineers again to find the best renewable energy sources. The causes of the global warming may be, but the phenomena itself is not questionable: since 1860, the five warmest years came after 1994 [1]. The average temperature of the Earth was 0.8˚C higher in 2005, as it was at the beginning of the XX. century (2005 being the warmest year ever since reliable measurements are performed). On the other hand, predictions about the future fossil fuel shortages now seem to agree, that around 2050 dramatic decreases will take place in the global crude oil production [2].

Bioethanol is one of the most promising alternative (renewable) liquid fuels of the near future. Nowadays the majority of bioethanol production is based on maize (*Zea mays*) and sugar cane (*Saccharum officinarum*). Sugar cane production is limited to the tropical climate, so Europe and the United States can not count on this source. Ethanol production from corn starch is possible and reasonable in temperate zones such as the USA and the EU. Every year there is a huge unmarketable (or cheap) corn in the EU and the USA, so the processing of the corn is a convenient and economical way of producing bioethanol, which simultaneously gives a boost for agriculture and landfarming as well. On the other hand, the growing of corn results in vast and increasing amounts of unmarketable or unexpoited by-products, like corn hull (also referred as corn fiber), corncob, corn stover (including cornstalk, and -blade), and press cake (the residue of corn oil extraction). These lignocelluloses need to be taken care of. The US fuel ethanol industry produced 6.2 billion liters of ethanol in 2000, most of which (∼95%) was produced from cornstarch [2]-[6]. In 2005, about 10 billion liters was produced in the States. If this tendency remains, the States is going to outrun Brasil (producing a stable amount of ∼15 billion liters/year) in bioethanol production. This means that the formation of lig-

nocellulosic residues of corn growing and processing is going to increase.

Lignocellulosic biomasses consist of three main components: cellulose, hemicellulose and lignin, and some minor fractions, such as proteins, lipids, and ash [7]. Common lignocellulosic biomasses are: wood, grass, bagasses, waste paper, and the stalks of cereals, which are all available and cheep feedstocks for ethanol production. Huge efforts have been made to utilize these substrates to ethanol production, and developments in production technology have reduced the projected gate price of ethanol from about US$0.95/liter in 1980 to only about US$0.32/liter in 1994 [3],[4]. Technical targets have been identified to bring the selling price down to about US$0.18/liter, a level that is competitive when oil prices exceed US$25/barrel [4].

Lignocellulosic biomass needs some kind of pretreatment for the cellulose fraction to be enzymatically digestible [2]-[6]. Researches in the field of ethanol production from lignocelluloses did not calculate with the value of other fractions than cellulose. Pretreatments were all optimized for the highest glucose (ethanol) yield in the enzymatic hydrolysis (fermentation) step. Economical calculations supposed that the residual sludge after ethanol production – which mainly consists of lignin – is to be burned and thus energetically utilized [3, 4]. Our idea is to construct and optimize the utilization of the whole lignocellulosic biomass, and not only the cellulose fraction. This idea is called "bio-refining", which means fractionation of the biomass, and producing value added products from each fractions. The goal is obviously to gain the highest profit, what means that the most valuable products should be produced with the less financial outlay. In most lignocelluloses the fractions of hemicellulose and lignin add up to ca. 30 and 20 % (w/w), thus if these fractions were sharply separated, and converted to valuable products, the profitability of ethanol production could be increased[8],[9]. A minor component, called corn fiber oil (CFO) also represent a high value in the corn fiber and in the press cake.

The scope of this study was to review and evaluate all presently available methods for separating the fractions of lignocelluloses, and for utilizing them. Over one hundred publications were reviewed from the fields of biotechnology, food chemistry, and chemical technology to collect the main processes for lignocellulose utilization. It is clear that the organic chemistry is capable of producing technically anything from lignocelluloses, so it would be an infinite discussion if we tried to mention every possible products and reaction pathways. We estimated that the products and reaction pathways which have economic rationality or market potential are already discussed in the literature. This paper does not include new products or pathways, but it is a proof of collecting the results in this field up to date.

2 Cellulose

2.1 Structure and Separation

Cellulose comprises long chains of D-glucose sugars that can be broken apart by a hydrolysis reaction with water when catalyzed by enzymes – known as cellulases – or by acids. However, hydrogen bonds hold the long cellulose chains tightly together in a crystalline structure, impeding breakdown to glucose. Due to its properly arranged structure, cellulose is one of the most persistent materials on earth. It is not soluble in water, organic solvents, and dilute acids and bases. Clean cellulose can be produced by removing other fractions (hemicellulose and lignin) from the fibrous material.

2.2 Utilization

It is commonly assumed that cellulose should be used for the production of fuel grade ethanol via enzymatic saccharification and fermentation. However, cellulose fibers need some kind of pretreatment in order to be enzymatically digestible. Pretreatments can affect hemicellulose-cellulose and lignin-cellulose interactions, increase pore size of the fibers, and reduce cristallinity, thus making the cellulose more susceptible to enzymic action. All of the presently available pretreatment methods – except physical methods [10] – result in a liquid fraction, which includes a part of the hemicellulose and the lignin, and a fibrous (unsoluble) fraction, which consists of the cellulose and residual matters.

The pretreatment method, which results in the highest glucose yield in the enzymatic hydrolysis step, is by now the steam explosion [11]-[17]. Steaming [18],[19] or steam explosion (with or without catalyst) is an extensively investigated pretreatment method. An advantage of steam pretreatment is that it is one of the very few pretreatment processes that have been tested in pilot scale. Reaction temperature ranges from 160 to 260°C, and residence times from 10 sec to 1 h. After SO_2 catalyzed steam explosion, >60% of the hemicellulose can be removed, while the fibrous cellulose becomes ≥95% digestible.[11, 12] A drawback of steam explosion is that hemicellulose and lignin fractions degrade significantly during treatment.

The most convenient way of producing ethanol from the pretreated cellulose is undoubtedly the simultaneous saccharification and fermentation, due to small product inhibition, small risk of infection, and simplicity [20]-[24]. The fermented ethanol must be distillated and dewatered to be a suitable fuel for otto-engines. However, other solvents than ethanol can be produced via fermentation after enzymatic hydrolysis. By using special microorganisms in the SSF step, a mixture of acetone, butanol and ethanol can be produced ("ABE-fermentation") [25, 26]. Since the beginning of the ABE process (the first part of the XXth century), many solvent producing clostridial strains have been described. Currently, the solventogenic clostridia are classified into four groups of species. The best known groups are *Clostridium acetobutylicum* and *C. beijerinckii*, all growing at 30-37°C [26]. *C. acetobutylicum* has the advantage of

fermenting both glucose and xylose. Possible alternative products of solventogenic bacteria are: acetic- and butyric acid, propanol, isopropanol, 1,2-propanediol, and some other solvents [26]. The three major factors that hamper the economic viability of ABE fermentation are: (1) the cost of the substrates, (2) the low product concentration (~20 g/l due to solvent toxicity), and (3) the high product recovery costs (distillation has been used)[26]. Other strains, like *Clostridium thermocellum* and *Klebsiella pneumoniae* can ferment butanediol from lignocelluloses [21]. It is also possible to produce hydrogen from the glucose solution by heterotrophic or by photoheterotrophic microorganisms[26]-[30]. The major advantage of energy from biohydrogen is the lack of polluting emissions, since the utilization of hydrogen, either via combustion or via fuel cells, results in pure water. As yet the technical feasibility of the fermentations has not been demonstrated, and therefore no biohydrogen production processes are currently operative on a commercial basis.

There are some other ways of utilizing the cellulose. Modified celluloses like ethylhydroxyethlycellulose (EHEC), carboxymethylcellulose (CMC), hydrolxyethylcellulose (HEC), hydroxypropylcellulose (HPC), methylcellulose (MC) and nitrocellulose (NC) are widely used in the industry [31]. Industrial applications are mostly based on the solubility of the derivatives in organic solvents or water, and also on the thickening, emulsion stabilizing, water retenting, film forming, and adhesive features of these substances. CMC, EHEC, and MC are used in the food and cosmetic industries. EHEC, HEC, and MC are used as paint thickeners, while MC and HPC are used in the pharmaceutical industry [31].

3 Hemicellulose

3.1 Structure and Separation

Hemicellulose is an amorphous chain of a mixture of sugars. The collective term "hemicellulose" is defined [32] as "those polysaccharides soluble in alkali that are associated with cellulose of the plant cell wall." Hemicelluloses are polysaccharides with degree of polymerization (DP) up to 200, associated in plants with cellulose and lignin. The hemicellulose differ from cellulose by having a composition of various sugar units (usually including D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, 4-O-methyl-D-glucuronic acid, D-glucuronic acid, D-galacturonic acid), shorter molecular chains, and by carrying side-groups, like acetate and methylate [32].

Enzymes and chemical reagents can separate the hemicellulose fraction as well. Enzymatic hydrolysis of hemicellulose is a promising method, which requires no pretreatment, reagents, and subsequent neutralization. Structure and kinetics of hemicellulases, like xylanase, mannanase, galactosidase, esterase are deeply experimented [31] - [33]. However, industrial competitiveness of these enzymes is unpredictable due to the lack of information on their productivity.

Dilute alkali pretreatment solves 40-70% of the hemicellulose

fraction in the form of dextrines (oligo- and polysaccharides) [34]-[43]. S. Curling reports a slight improvement in hemicellulose yield using potassium hydroxide rather than sodium hydroxide [44]. He recommends that the lignin should be removed before extraction by hydrogen peroxide, because it increases the purity of the yielded hemicellulose. According to S. Curling [44] and P-Y Pontailer [45], it is beneficial to mill the raw material before the extraction, or to perform the extraction in a twin screw extruder. If the pH of the supernatant is set to 4-4.5, hemicellulose A precipitates, and thus it can be separated by filtration. By adding two volumes of 95% ethanol to the supernatant, hemicellulose B precipitates in the form of corn fiber gum (CFG). That part of the original hemicellulose, which is not soluble in 4% NaOH (after 2 hours at room temperature), is called Hemicellulose C [43]. According to P-Y Pontailer[45] the extract should be centrifuged before precipitation. He recommends acetic acid and ethanol to have a good yield of precipitation. The precipitate has to be filtered, dried and grinded. Another method of producing solid hemicellulose is dry spraying the solution, which is cheaper than alcoholic precipitation.

Autohydrolysis[46] and wet oxidation [47] are deeply investigated pretreatment methods. Many authors state that by removing the lignin fraction the yield of hemicellulose extracion significantly increases. The lignin can be removed by wet oxidation, hydrogen peroxide treatment or organic solvent extraction. The wet oxidation process treats the substrate with oxygen and water at elevated temperature (170-240˚C) and pressure. Enzymatic digestibility of cellulose is often good (~85%), but wet oxidation converts the hemicellulose and lignin fractions to oxidized components (mainly low-molecular weight carboxylic acids and carbon dioxide), which can not be easily processed. After wet oxidizing wheat straw (temperature: 170˚C, reaction time: 5-10 minutes, oxygen pressure: 10 bar), 82% of the original hemicellulose, lignin, and non cell wall materials (proteins, pectins, etc.) is removed [47]. 55% of the dissolved hemicellulose is not detected as carbohydrates, thus it is oxidized to other substances. Only 60% of the original hemicellulose is recovered after pretreatment at 185˚C. When using black oak as a substrate, 50% of the lignin is solubilized or degraded at already 140˚C. Steaming or steam explosion is also capable of separating a part of the hemicellulose. The problem is the same as at the previous methods: the increasing severity results in the decrease of the pentose yield. The severity, which is satisfactory to produce digestible cellulose fraction is too high to obtain good pentose yield.

A convenient way of separating the hemicellulose fraction is dilute acid treatment.[48]-[62] 0.5 – 3.0 % (w/w) sulphuric or hydrochloric acid can be used as solvents. Dilute acids remove hemicellulose, while cellulose and lignin remain in the solid fracion. The kinetics of hemicellulose removal are reasonably known and modelled, and the severity parameter has been particularly effective in correlating performance over a wide range of temperatures, times, and acid concentrations [4]. Dilute sul-

phuric acid pretreatment of wood and corn residues at 160˚C resulted in 88-94% digestible cellulose (retention time: 10 min, acid: 0.5% v/v) [48]. Note that – depending on the substrate – 13-25% of the original glucan was solubilized during pretreatments. Hemicellulose recoveries in the liquid fraction were 80-90%. A part of the lignin was also solubilized: as a function of the substrate, 6-26% of klason lignin was removed during pretreatments. By using lower temperature (120˚C) and long residence times, it is possible to achieve ∼100% yield of pentose sugars, while no lignin solubilization is observed (reaction time: 90-120 min, acid: 2% w/w, substrate: corn stover) [49]. In this case, fibrous cellulose becomes only ∼60% digestible, thus it needs further pretreatment before enzymatic hydrolysis. At 95˚C reaction temperature, 75% of the hemicellulose can be removed after 60 minutes (using 2% w/w acid), while only 0,9% of the glucan, and none of the lignin is hydrolysed [50]. By using concentrated acids, cellulose can also be brought to solution, thus a mixture of pentose and hexose sugars is produced. This requires more chemical reagents, and an adequate microorganism, which can ferment the mixture. Xylose, glucose and glucose-xylose mixture can be fermented by yeast (1400, pLNH33) with yields of 80, 86 and 84% [63].

S. Curling reports that it is also possible to extract the hemicellulose by microwave extraction, but it is found to be ineffective [44].

3.2 Utilization

There are three main approaches in the possible utilization of the hemicellulose:

1 producing bioethanol,

2 producing food industrial products (f. e. xylitol),

3 producing biopolymers,

4 producing other chemicals (bio-surfactants, adhesives, pharmaceuticals).

The xylose rich dilute acid hydrolysate can be fermented to ethanol by yeasts, bacteria, fungi, or by genetically engineered microorganisms [64]-[66]. The highest ethanol yield reported (0.54 g ethanol/g xylose) is produced by recombinant *Eschericia coli* (KO11),[64] when the hydrolysate is fermented after detoxification (over-liming with Ca(OH)$_2$. (*E. coli* KO11 carries the genes *pdc* and *adh* B from *Zymomonas mobilis* integrated into the chromosome, and also some other genetic changes were carried out to minimize by-product formation). However, the fermentation of the hemicellulose sugars in industrial scale is still a challenge, and faces many difficulties.

Catalytic hydrogenation or microbial conversion can convert xylose to xylitol – a five-carbon sweetener. Xylitol can be further oxidized, and thus trihydroxy-glutaric acid, a souring additive is produced, which can replace citric acid. *Candida sp.* 11-2 (identified by the American Type Culture Collection as a strain

of *Candida tropicalis*) is a xylitol producing yeast and yields 0.57 g xylitol/g xylose [63]. The substrate (corn cob) have to be treated with 10% ammonium hydroxide solution to gain a fermentable hydrolysate. The authors conclude[63] that inhibitors like acetate or phenolics inhibit xylose utilization. The inhibition can be relieved also by treating the hydrolysate with an anion exchange resin column. Another application to the dilute acid hydrolysate could be the production of crystalline pentose sugars. Due to their lower absorbtion in the human digestive tract, and lower sweeting capacity relative to glucose, cleansed xylose is a potential additive of diabetic foods.

B. Estrine[67] draws the attention to the carbohydrate-based surfactants. He states that the global market volume of surfactants in 2003 was 12 M t, in which the proportion of non-ionic surfactants was 4.8 M t. This means a huge potential market for the bio-surfactants, which is scarcely expoited, since the present production is only 200,000 t/a carbohydrate surfactant in the world. As usual, the USA was the fastest in supplying the demand: 40% of the bio-surfactants is produced in the States. In the case of these substances the monosaccharide (which can be any of those conforming the lignocellulose matrix) is bound by a glicosidic group to a (fatty acid derived) fatty alcohol. Thus the produced molecule consists of a hydrophilic „head" and a lipophilic „tail", which is the case at any commercial surfactants (like soaps and emulsifiers) as well. B. Estrine investigated the influence of monosaccharide and fatty alcohol type. He concludes that pentoses have more advantages. The alkyl pentosides have a lower degree of polimerization vs alkyl hexosides and the pentoses have a higher reactivity with fatty alcohols, which results in a lower reaction temperature (< 90°C vs > 110°C). According to the author the length (carbon atom number) of the fatty alcohol determines the field of use of the bio-surfactant. Surfactants containing fatty alcohols with 14-22 carbons are suitable as emulsifiers, with 8-14 carbons as washing or foaming surfactants and with 4-8 carbons as solubilizers, plasticizers, etc. Some examples of the broad range of existing (but yet not exploited) markets of these substances: cosmetics, agrochemical, petrochemical, environmental industries. These substances are biodegradable, not irritant (especially for skin).

The hydrolysate of the dilute alkali pretreatment can be used for the production of soluble biopolymers. Such biopolymer is the corn fiber gum (CFG), which is made from corn fiber, the residue of corn milling processes [35]-[43]. Applications where hemicellulose polymers have been used are: cationic biopolymers, hydrogels, and long-chain alkyl ester derivatives. Natural gums like konjac glucomannan, guar gum, and locust bean gum are obtained from plant sources, and can be used as food additives [32]. However, CFG could rather be applied as an additive or a raw material of the plastic industry. P-Y Pontalier [45] produced films from dry sprayed hemicellulose and investigated the mechanical properties of different films, but mentions no certain use of the film.

4 Lignin

4.1 Structure and Separation

Lignin is a phenolic polymer that accounts for 15-36% of lignocellulosic biomass. It serves several functions in the extracellular matrix of plants; it gives the cell wall mechanical support, also serving as a barrier against microbial attack, and it acts as a water impermeable seal for the xylem vessels of the plant vasculature. Numerous reagents are capable of separating the lignin from lignocellulosic biomass. 2.9M NH_4OH removed 80-90% of the lignin from corn cob [22]. 98% of the base could be recovered under vacuum at below 60˚C, but there was a significant loss of polysaccharides during pretreatment. The AFEX (Ammonia Fiber/Freeze Explosion) treatment can also remove lignin to some extent [68]-[70]. Lignin is considered to be a potential adsorber of cellulases, thus it decreases cellulase activity. Removing the lignin fraction can result in less enzyme load needed. Cellulase load of 1 IU/g dry substrate after AFEX treatment is enough [68]-[70]. Alkaline peroxide treatment is also capable of removing the lignin, but in this case the lignin is oxidized to water soluble substances.[71] Enzymatic degradation of lignin is also in the focus of research and development studies [72]-[74]. The problem with the enzymatic degradation is that it produces small molecular weight, water soluble substances, which are not easy to be utilized. Furthermore, enzymatic degradation of lignin is slow, and it does not enhance enzymatic digestibility of the polysaccharides.

The most convenient way of producing a clean lignin fraction is the organic solvent extraction [25, 50, 75]. The most selective solvents of lignin are butanol and propanol [50]. Butanol removes 90% of the lignin (temperature: 160˚C, time: 1h, solvent:water = 7:3 vol, catalyst: 0.5% H_2SO_4), while no reducing sugars are detected in the solvent. Phenol also does not remove any sugars, but the lignin solution rate is only 80%. Ethanolamine removes 94.5% of the lignin fraction, but it also solves 13% of the polisaccharide fractions. Results of aspen chips delignification show that an aqueous solution of 50% (v/v) monoethanolamine – after 24 hours reaction time – removes 91.2% of original lignin content, but 19% of original hexosans, and 9% of original pentosans are also transferred to the liquid phase [25].

However, it is also possible to produce the lignin at the end of the bio-refining plant. If the lignin fraction is not separated at the beginning of the technology (and it is not broken down during the lignocellulose refining) then it can be found at the end of the process. The residual sludge contains the residual lignin, cellulose, hemicellulose, and some other components (proteins, lipids, nucleotic acids, pectins, etc.).

Note that there is already an abundance of lignin residues produced as a by-product of pulp and paper industry. The size of this resource can be appreciated by considering that the total amount of lignosulfonates and Kraft lignin together outweigh the sum of all man-made polymers in the Unites States.[76]

Given the relatively low cost, high abundance, and renewable nature of this resource, it is not surprising that many attempts have been made to develop higher value products from lignin [77]-[79]

4.2 Utilization

Economic calculations of ethanol production from lignocelluloses assume that the lignin fraction is incinerated at the end of the process [3, 4]. Cogeneration of heat and electricity from renewable feedstocks like lignin is beneficial, since in terms of the EU law, the power suppliers must buy the "green current" at a determined price [80]. However, lignin degradation to value added products may be economically more feasible than incineration. Several methods of oxidative or hydrolytic degradation and catalytic hydrogenation of lignin to phenols and related compounds exist. Lignin degradation processes which are based on liquid-phase chemistry require the consumption of various chemicals and involve tedious separation steps. Gasification or pyrolysis in spite, require no reagents and they offer a relative simple way of degradation and separation [81]-[86]. Gasification in the presence of steam results in a mixture of CO and H_2, while gasification under pressure produces higher yield of methane. Carbon-monoxide and hydrogen are catalitically convertible to methanol, which can be used as a fuel in otto-engines. Pyrolysis reactions need certain time to be complete, depending on reaction temperature. Above 750˚C, detention time of less than 5 seconds is enough for the pyrolysis to be complete in terms of product yields [81]. Depending on the substrate, and the reaction parameters, different pruduct yields are reported. At 650˚C reaction temperature a total volatiles yield of around 60 wt% is reported [81]. The fraction of solid residue (char) seem to decrease significantly at temperatures between 400-700˚C [82]. Above 780˚C the char yield remains constant at 14 wt%. The lignin pyrolysis gas yield increases monotonically with temperature to an apparent asymptote of about 36 wt % at around 880˚C. As temperature increases, tar yield goes through a maximum of about 53 wt % at 580-680˚C and then declines to an essentially asymptotic yield of about 47 wt % at 880˚C, probably due to secondary cracking to light volatiles [82]. Pyrolysis gas of lignin consists of approximately 53% CO, 11% H_2O, 11% CO_2, 9% CH_4, 5% CH_3OH, 4% HCHO, 2.5% C_2H_4, 2.5% CH_3CHO. Complete composition of tar from lignin pyrolysis has not been reported yet. However, the yield of some valuable phenolic compounds seems to reach the maximum at 620˚C [83]. Tetralin vapor addition increased the yield of these compounds from 2 wt % to 3.6 wt % at this temperature. The most common phenolic compounds are p-, m-, o-cresol, phenol, 2,5- and 2,6-xylenol. Yields of all of these compounds reach the maximum within the range of 600 and 650˚C. Tetralin vapor addition increases the yields of all components significantly [83].

5 Lipids

Lipids are minor components in lignocelluloses. The oil extracted from corn fiber is of special interest due to its cholesterol-lowering effect in humans. Corn fiber is a residue of corn dry and wet milling processes, and has several beneficial health effects [87]-[109]. Depending on the process, corn fiber adds up to ∼10% of the total processed maize corn. The composition of corn fiber (produced by a wet milling process) is the following: 20% starch, 14% cellulose, 35% hemicellulose, 11-14% proteins, 8% lignin, 2.5% lipophylic extractives, 2% acetyl groups, 1% ash (based on dry weight) [8]. Corn fiber oil (CFO) can be extracted from corn fiber by organic solvents or super-critical fluid extraction (SFE). The amount of CFO and the rate of the different components of CFO depends on the extraction and fiber pretreatment conditions.

CFO is the only cholesterol-lowering edible oil product on the US market, which contains three different classes of natural cholesterol-lowering phytonutrients: free phytosterols, phytosterol fatty acyl esters, and ferulate phytosterol esters (FPE) [96]. These components significantly reduce levels of „bad" low-density-lipoprotein cholesterol (LDL-C) in humans, and thus they can be used as valuable nutraceuticals. CFO has greater levels of these phytosterols compared to other vegetable oils. The FPE fraction in corn fiber is also unique from several other points of view. First, the main phytosterol components in CFO are sitostanols, i. e. fully saturated stanols. These substances – due to their increased solubility and bioavailability – are more effective in terms of cholesterol-lowering ability than the more commonly occurring unsaturated phytosterols. In fact, CFO appears to be the richest source of natural stanols (and stanol esters) ever reported [96]. Second, the stanol from corn fiber oil is esterified with ferulic acid, which is an antioxidant that might have additional health benefits. CFO also contains γ-tocepherol and carotenoids, both with important antioxidant properties.

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