Taking Ethanol Quality Beyond Fuel Grade: A Review

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Abstract

Ethanol production in the United States approached 15 billion gal/y in 2015. Only about 2.5% of this was food-grade alcohol, but this represents a higher-value product than fuels or other uses. The ethanol production process includes corn milling, cooking, saccharification, fermentation, and separation by distillation. Volatile byproducts are produced during the fermentation of starch. These include other alcohols, aldehydes, ketones, fatty acids, and esters. Food-grade ethanol is generally produced by wet milling, where starch and sugars are separated from the other corn components, resulting in much smaller concentrations of the impurities than are obtained from fermentation of dry-milled corn, where cyclic and heterocyclic compounds are produced from lignin in the corn hull. Some of these volatile byproducts are likely to show up in a distillate. The fermentation byproducts in ethanol could affect human health and cause unpleasant flavours, however, if any of this were to be used for human consumption. There is some interest for improving ethanol quality, since human consumption represents a higher value. Advanced purification techniques, such as ozone oxidation, currently used for drinking water and municipal wastewater treatment, offer possibilities for adaptation in ethanol quality improvement. The development of analytical techniques has enabled the detection of low-concentration compounds and simple quality assurance of food-grade alcohol. This review includes the most recent ethanol production methods, potential ethanol purification techniques, and analytical techniques. Application of such techniques would aid the development of simplified alcohol production.

Keywords: Analytical techniques, Ethanol, Purification, Separation technologies, Volatile byproducts

Introduction

Bioethanol is produced by fermenting sugars from biomass. The United States is the largest bioethanol producer with a production of 14.3 billion gal in 2014 and capacity of more than 15 billion gal/y *(1)*, accounting for more than half of the global ethanol production. Corn is used as the feedstock for about 90% of US ethanol. The second largest bioethanol producer is

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Brazil, with a production of 6.2 billion gal in 2014 *(1)*, using sugar cane as the major feedstock *(2)*. Contributions of various countries to global bioethanol production are given in Table 1.

Biological feedstocks, contain either simple mono- or disaccharides or polysaccharides, such as starch or cellulose that can be converted into simple fermentable sugars to produce ethanol. Feedstock can be classified into three groups based on the carbohydrate complexity:

(i)   sucrose-containing feedstocks, e.g., sugar beet, sweet sorghum, and sugar cane,

(ii)   starchy materials, e.g., wheat, corn, and barley, and

(iii)   lignocellulosic biomass, e.g., wood chips and grasses,

with increasing needs of processing intensity.

In practical terms, despite variable processing needs, one of the most important criteria in choosing feedstocks is availability, which can vary from season to season and depend on geographic location *(2)*. Feedstocks used in different countries are shown in Table 1 *(3,4)*.

Volatile byproducts are produced during ethanol production. Other alcohols, aldehydes, ketones, fatty acids, and esters derive from starch; cyclic and heterocyclic compounds derive mainly from lignin. While ethanol purity is not important in fuel applications, volatile byproducts in ethanol could threaten human health and cause unpleasant flavours for use in food-grade applications. Even when not present at harmful levels, volatile organic compounds (VOCs) are known to contribute to the quality of alcohol, as interpreted as the flavour and odour of alcoholic beverages *(5)*. Some of these compounds contribute unpleasant aromas.

Separation of impurities from ethanol is required to produce the highest-value material. Distillation cannot be relied upon to lead to complete separation of these fermentation byproducts, due to the compounds having similar temperature-vapour pressure profiles or azeotrope formation. To achieve industrial and food-grade quality, with increased value, additional purification is required.

The first difficulty with distillation is the removal of water. Water left beyond the azeotrope in ethanol distillation, for fuel-grade applications, is usually removed by molecular sieves i.e. by adsorption on zeolites as a form of desiccation. However, these zeolites do not remove other organic impurities efficiently. No other techniques are employed in the further purification of commercial ethanol. Some food-grade alcohol is further purified by adsorption on charcoal or activated carbon.

By contrast, there are various additional purification techniques, based on oxidation, gas stripping, coagulation, adsorption and ion exchange that are currently used in drinking and domestic wastewater treatment. Some of these may be adapted advantageously to overcome the shortcomings of distillation of ethanol, and to remove undesirable volatile compounds and impurities from it.

The recent development of improved analytical techniques enables more sensitive analysis of ethanol. The identification analysis of impurities in ethanol has been completed with advanced separation techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC). This paper reviews the most recent ethanol production methods, the application of water and wastewater treatment technologies to ethanol purification, and ethanol analysis techniques. The advanced purification and analysis techniques of ethanol could lead to broader applications of purified ethanol.

Raw materials for ethanol production

Sucrose-containing feedstocks.

The main sucrose feedstocks are sugar cane and sugar beet. Estimated production potential of ethanol from sugar cane and sugar beet is 70 L/ton and 110 L/ton, respectively. Brazil is the largest single producer of sugar cane, with 40% of world production. European countries use beet molasses as their sucrose-containing feedstock *(6)*. The advantages of sugar beet over corn are a shorter cycle of crop production, higher yield, higher tolerance of climate variability, and low water and fertilizer requirements *(2)*. Ethanol production from 5- and 6-carbon carbohydrates is easier, compared to starchy materials and lignocellulosic biomass, since pretreatment of the feedstock is not required. Disaccharides such as sucrose from cane or beets can be broken down easily by yeast cells *(6)*. Starch and lignocellulosic feedstocks get progressively more difficult to depolymerize and thus release simple sugars for fermentation.

Starchy materials.

In North America and Europe, ethanol is currently mainly produced from starch, mainly derived from corn and wheat *(6)*. Starch is a homopolymer of α-1,4'-linked D-glucose with some branch points of 1,6' linkage *(7)*. Ethanol production from starch necessarily requires depolymerization to obtain glucose, the proximate carbon source for the fermentation. Corn — the major feedstock in the United States — is converted into ethanol starting by either dry- or wet-milling. Dry mills produce approximately 90% of U.S. fuel ethanol, with the balance produced by the wet-milling technique *(1)*. Currently, wet mills produce all the alcohol for non-fuel purposes. The main difference between dry mills and wet mills is the focus on coproducts. Dry mills almost exclusively make ethanol and animal feed. A new dry-milling plant has the capacity to produce 2.8 gal ethanol/bu corn, compared with approximately 2.7 gal/bu in wet mills. Wet-milling is more capital and energy intensive. However, it allows the separation of various components from grain before fermentation and thus derives more value from those coproducts.

The wet-milling process separates the grain into its components, including starch, fibre, gluten, and germ. First, corn is steeped in a solution of water and SO_2 to separate germ, fibre, and hull from kernels. Corn oil is extracted from the germ, which is removed from the corn kernel. Corn gluten is very different from that found in wheat, barley and rye, and rather based on the protein zein and this is concentrated in some of the lower-value coproducts in wet milling. The typical high-protein feed products produced are corn gluten meal and corn gluten feed. The wet-milling process uses starch only, after enzymatic saccharification, for fermentation to produce ethanol in continuous fermentation, to produce generally a higher quality alcohol than is produced in dry-grind processing.

The dry-milling process, does not separate the grain is into its components, or only partially in some recent modifications. Corn is ground and mixed with water to make a mash. The mash is cooked, and the starch is saccharified into glucose by alpha- and gluco-amylase addition. Yeast is added to ferment the sugar to ethanol in batch processes. The initial product of fermentation is a mixture of ethanol, water and solids. Ethanol is separated by distillation. The bottom product from distillation, the whole stillage, is centrifuged to separate out most of the solids. These solids are dried to distiller's dried grains (DDGs), sold as an animal feed supplement. The liquid stream, thin stillage, still containing large quantities of organic material, both dissolved and suspended, needs further processing; various possibilities exist here. Current practice is to recycle part of the thin stillage to the fermentation and condense most of the thin stillage to be added to the distiller's grains before drying to make distiller's dried grains with

solubles (DDGS), also sold as animal feed. Additional centrifugation on many dry-grind ethanol plants recovers more oil. Separating corn germ on dry-grind plants technology has been developed more recently: Quick Germ *(8)*, and enzymatically milled germ *(9)* report germs with 30 and 39 %, respectively. Rasmussen et al. *(10)* and Sankaran et al. *(11)* described how various valuable byproducts can be derived from thin stillage through the cultivation of filamentous fungi.

Lignocellulosic biomass.

The current cornstarch-based ethanol production may not be practical in terms of socio-economic impact on agricultural land usage because of the trade-off in grain usage for fuel production vs. food for humans or animals. A potential alternate material for ethanol production is lignocellulosic biomass. Examples include wood, grasses, crop residue, and other agricultural waste, produced in much larger quantities than the starch-bearing seeds. Although the fractional yield of glucose would be lower in cellulosic material than in starch or sugar crops, the huge quantity available makes this an ultimate goal for fuel production The total potential bioethanol production from crop residues and wasted crops is 491 billion L/y *(12)*, which is approximately an order of magnitude higher than the current world ethanol production. Lignocellulosic perennial crops are promising feedstock because of high crop yields, low costs, good sustainability in low quality land, and low environmental impacts *(13)*.

 Comprehensive studies have been conducted on the utilization of lignocellulosic biomass for fuel ethanol production *(13-17)*. The major difference between starch and lignocellulosic biomass is the accessibility of substrates by cellulose enzymes. The salient factors affecting the hydrolysis efficiency of cellulose enzymes are porosity (accessible surface area), crystallinity of cellulose fibre, and content of lignin and hemicellulose *(18)*. The presence of lignin and hemicellulose obstructs cellulose enzymes to reach substrate. This results in a decrease in the efficiency of hydrolysis. Increased porosity, reduced crystallinity, and removal/degradation of lignin and hemicellulose are necessary to improve hydrolysis efficiency and eventually ethanol production. This process will need utilization of C5 sugars to become economical. Additionally, some lignin byproducts will inhibit yeast bioconversions of sugars to ethanol.

There are different approaches to the pretreatment of lignocellulosic material to reach aforementioned aims. One is physical-chemical and the other enzymatic. Chemical treatment includes strong alkaline or acid hydrolysis and heat treatment *(19)*. Enzymatic treatment has been commercialized by companies such as Novozymes, Diversa, and Dyadic. Experimental approaches include *in-situ* production of enzymes by the cultivation of fungi *(20)*. Four plants that are pioneering cellulosic ethanol production are just coming into operation. These are owned by Iogen, POET, DuPont and Abengoa. The economics of this approach is yet to be proven.

Ethanol purification

Ethanol byproducts.

Numerous yeast varieties are used in industrial ethanol production. Some strains have advantages over others, such as a faster specific rate of ethanol production, improved yield, sugar stream used, and/or enhanced ethanol tolerance *(21)*. No matter which yeast strain is used, however, byproducts are inevitable. Additionally, byproduct formation depends on the purity of

the starch feedstock; thus in the case of ethanol purification from lignocellulose, additional impurities are expected. Indeed, byproducts of ethanol production can be listed as starch-derived and lignin-derived. Of course, it is expected that lignocellulosic material will be in much lower concentration in the wet-milled grain product used for food-grade alcohol production.

Starch-derived byproducts.

Acetaldehyde is the most significant byproduct of ethanol production. Its concentration changes depending on the fermentation process or aging. Atmospheric oxygen is the stoichiometric reagent in the slow oxidation of ethanol to acetaldehyde. Formation of acetaldehyde in the presence of excess residual ethanol invites condensation products, such as 1,1-diethoxyethane and ethyl vinyl ether. Further autoxidation of acetaldehyde produces acetic acid and additionally invites ethyl acetate.

Volatile fatty acids and alcohols of different carbon chain lengths are also byproducts derived through biological processes from starch. Volatile fatty acids and ethanol form esters as condensation byproducts. Examples of possible starch-based byproducts are summarized in Table 2 *(22)*.

Lignin-derived byproducts.

Lignin is an integral part of the cell walls of plants that helps hold cells together and provides macroscopic plant rigidity. It is an amorphous cross-linked polymer of heterogeneous structure. The principal repeating unit is a propylphenol-derived polyol. The precise lignin composition is unique to each plant. The degree of polymerization is difficult to measure due to fragmentation during extraction *(23)*. Ethanol production typically partially dissolves or fragments lignin. The primary byproducts from lignin are non-volatile and water-soluble. They derive from *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol *(24)*. Of more concern to product quality are the cyclic and aromatic compounds such as phenol, benzoic acid *(25)*, and styrene *(26)*. The schematic of the ethanol byproduct production process is described by Onuki *(27)* and Onuki et al., *(28)*.

Ethanol purification techniques

Ethanol, as initially produced, contains various kinds of impurities derived from the fermentation process. Some of them merely cause unpleasant odours or flavours, while some are toxic and of health concern. It is necessary to remove the impurities to make value-added ethanol products for food, pharmaceutical, and industrial use. The main approaches for ethanol purification are illustrated in Figure 1.

Distillation.

Distillation is the most commonly used industrial separation technique in ethanol (including food-grade alcohol) production. Distillation separates two or more compounds by utilizing the difference of their volatilities. The degree of separation obtained in a single distillation step depends on the initial composition of the mixture and the difference of boiling points at the operating pressure, which is atmospheric for commercial ethanol distilleries. Distillation for ethanol fuel production is simply about water removal, but food-grade alcohol

distillation also aims at quality improvement by eliminating organic impurities, with no concern about water. A series of distillations or distillation plates is typically required to achieve high separation of each component from ethanol-water mixtures containing other minor impurities. The initial fermented mix is vaporized in a distillation tower. The vapour is condensed, obtaining more concentrated ethanol. Repeated vaporization and condensation through 20 to 24 cycles results in 95.6% ethanol content by weight, with the remaining component being largely water. The highest achievable concentration of ethanol with multiple distillation is 95.6%. At this concentration, the ratios of ethanol to water in the liquid and vapour phases are the same, and further separation cannot be obtained. This is called the azeotropic limit. Many other compounds form azeotropes with ethanol, and thus also cannot be quantitatively removed when the aim is purification for human consumption *(29,30)*.

An azeotrope is a mixture of two or more compounds in which liquid-vapour phase equilibrium coefficient (K_i) of each compound equals unity (i.e., when boiling) and the corresponding mole fractions in each phase are the same. The following equation describes the equilibrium between vapour and liquid phases as can be derived from Raoult's Law of partial pressures.

$$y_i = K_i x_i$$
(1)

where
x_i = equilibrium composition of liquid
y_i = equilibrium composition of vapour
K_i = liquid-vapour phase equilibrium coefficient.

Under azeotropic conditions, equilibrium composition of liquid and equilibrium composition of vapour becomes equal and thus K_i value of each compound =1 *(31)*:

$$y_i^{az} = x_i^{az} \text{ and } K_i^{az} = 1$$
(2)

where
x_i = equilibrium composition of liquid.

Distillation utilizes the difference between equilibrium coefficients of liquid and vapour to separate each compound in one mixture, so under azeotropic conditions, further separation cannot be achieved. Ethanol and water form a so-called positive azeotrope, in which the non-ideal variation from Raoult's law results in the azeotropic boiling point being the lowest of any mixture of the two compounds.

Health and quality concerns in food-grade alcohol.

Ethanol tends to form azeotropes with compounds whose boiling points (*bp*) are close to its own (78.3° C), including benzene (*bp* = 80.2° C), butyraldehyde (*bp* = 75.7° C), thiophene (*bp* = 84.1° C), and many more as listed in Table 3 *(32)*. The consequence of this for producing high-quality alcohol is that it is not possible to remove any of these impurities quantitatively by simple distillation and another process must be used. Many of these compounds are of health concern. For instance, benzene causes aplastic anemia and acute myelogenous leukemia *(33)*, butyraldehyde causes pathologic changes in the respiratory tissues *(34)*, and thiophene causes degeneration of granule cells in the cerebellum *(35)*. Isopropanol can lead to nausea and abdominal pain, and affect liver and kidneys; n-pentane causes narcosis and is to be avoided

(36). Of course, concentrations of these compounds in multiple-distilled alcohol are low, so the concern is rather about taste and smoothness of the product. In fact, even pure ethanol in excess can cause health problems such as cirrhosis of the liver *(37).*

While none of these organic compounds is formed at concentrations within even an order of magnitude of the ethanol, the point remains that they are exceedingly difficult to impossible to remove by distillation alone. Additional purification techniques have to be employed to remove these azeotrope-forming compounds, as in the sections to follow.

Adsorption with activated carbon.

Activated carbon (AC) is commonly used to remove trace organic compounds from water. AC has a large, hydrophobic surface area, ranging between 300 m^2/g and 2000 m^2/g, that enhances adsorption of many organic compounds. Activated carbon can be made from various kinds of organic materials such as wood, fruit kernels, peat, lignite and coal. Gradually heating these materials to about 700° C with exclusion of oxygen volatilizes small hydrocarbons. The remaining char particles are heated with mildly oxidizing gases, water or CO_2 at high temperature (800° C < T < 900° C). Surface oxidation cause development of a porous structure internally in the char particles, in a process called activation *(38).* The internal structure of AC consists of macropores (>25 nm), mesopores (1 nm < D <25 nm), and micropores (<1 nm) *(38).* As a result, molecules of a wide range of sizes are effectively adsorbed. Smaller molecules can diffuse easily and adsorb most readily in the smaller pores. The mesopores are important for easy flow of fluids to the micropores. Because the AC surface is non-polar, the less polar the compound, the more it is adsorbed, particularly when AC is used to purify a polar compound such as ethanol or water. Ethanol, with only two carbons per HO group remains a very polar compound, but of course is less so than water. Thus, separations are still possible, but adsorption or organics is less favourable from ethanol than from water. The "polarity" of a molecule is used here as a general term as is common in organic chemistry, and does not correspond precisely to physical measurements, such as dielectric constant or dipole moment.

The adsorbability of major organic compounds on AC from aqueous vapours is summarized in Table 4 *(39).* The adsorbability of compounds will change in ethanol solutions (generally decreasing), but trends are expected to hold. Table 4 provides data useful to compare compounds in the same chemical groups. For example, for alcohols, the adsorbability increases as their hydrocarbon chains become longer, as the effective polarity of the molecule decreases with increasing carbon/OH ratio. Hexanol and higher alcohols are hardly soluble in water. Also, long hydrocarbon chains increase molecular mass, which contributes to an increase in the adsorbability through hydrophobic effects and increased London dispersion forces with the AC. Compared to their alcohol analogs, aldehydes are adsorbed slightly less efficiently from water, but the trend with carbon chain length remains the same. As shown in Table 4, acetates have relatively high affinity coefficients. Although acetaldehyde is difficult to remove with AC, it is possible to remove it by prior oxidation to acetic acid. Aromatic compounds show low polarity and low solubility. Therefore, their adsorbabilities are relatively high.

Adsorption with activated alumina.

Activated alumina (Al_2O_3) is another type of adsorbent that is made from aluminium hydroxide. Its typical surface area is 200 to 300 m^2/g *(40,41).* The main applications of activated

alumina are water removal from a gas stream or liquid samples *(42-44)*, refining of petroleum *(45)*, and fluoride ion removal from water *(46-48)*.

Adsorption with silica gel.

Silica gel (SiO_2) is an adsorbent made from the neutralization of sodium silicate with mineral acid *(45)*. The surface area of silica gel ranges from 300 to 700 m^2/g *(49,50)*. Although it is common as desiccant *(51-53)* and an adsorbent to separate organic compounds from one another, it is not common in water or wastewater treatment.

Adsorption with molecular sieves.

Molecular sieve is a class of porous material with crystalline structure, in contrast to AC, activated alumina, and silica gel, which have amorphous structures. Sieves have a uniform pore distribution and a definite pattern structure *(45)*. They can be made from various materials such as carbon *(54-56)*, titanium silicate *(57-59)*, and aluminophosphate *(60-62)*. The most common molecular sieve is based on anhydrous aluminosilicate zeolite *(63,64)*. For ethanol treatment, dehydration after distillation is completed with molecular sieves selected for their high selectivity for water derived from uniform appropriate pore diameters *(65-67)*.

Pervaporation.

Pervaporation (PV) is a liquid-liquid separation technique used to separate mixtures where distillation is inapplicable due to azeotrope formation, heat-sensitivity, and similar *bp* problems *(68-70)*. A membrane can separate a mixture of two or more liquids by applying a vacuum on one side. This causes a gradient of chemical potential, resulting in the penetration of the mixture into the membrane and evaporation from the other side. The separation by PV is governed by the difference in solvents sorption affinity and diffusion coefficients in the membrane *(70)*. Sun et al. *(71)* obtained a separation factor of 153 with a flux 231 kg h^{-1} m^{-2} in an aqueous ethanol solution by a H-ZSM-5 filled chitosan membrane. Leppäjärvi et al. *(72)* obtained fluxes through a thin, supported, high-silica MFI zeolite membrane of hydrophobic character of 2 to14 kg h^{-1} m^{-2} with ethanol/water separation factors of 4 to 7, respectively. Additionally, Chovau et al. *(73)* have reported on the influence of fermentation byproducts on ethanol purification by PV.

Ozonation.

Ozonation is not a purification method, per se, but rather it is a very inexpensive and clean way to oxidize many organic compounds whose products can be more easily removed by one of the other purely physical methods. It is commonly used in the treatment of water. Ozone, O_3, is an allotrope of oxygen — made *in situ* from O_2 — that is much less stable than O_2, the diatomic species. The gas has a pale blue colour and a unique sharp odour. It is also a powerful oxidant. Under many real-world conditions, oxidation occurs both directly from ozone and from other reactive oxygen species (ROS), mainly hydroxyl radicals (HO•) that are produced as a result of its chemistry *(74)*. Ozone can be used to oxidize various organic and inorganic compounds contained in ethanol, regardless of their boiling points. The oxidation has dramatic impact on the boiling points, and thus separability, of these components. Carbon-carbon double

bond scission is the net result of the most common ozonolysis reaction, resulting in compounds that are more volatile and may be efficiently removed by gas stripping. Other reactions result in compounds with higher boiling points than previously, allowing for more efficient separation by distillation. Initial oxidation can also increase biodegradability and reduce toxicity of certain impurities, making subsequent biological purification steps more effective or directly removing a hazard. For example, Brooke et al., *(75)* reported that microcystin, a toxin derived mainly from *Microcystis* cyanobacteria, can be completely detoxified by O_3 treatment.

Also, it is reported that the efficiency of membrane bioreactors (MBR) can be improved by ozonation *(76)*. Sludge can be ozonated, and the reduction in its production was observed in wastewater treatment. There was no adverse effect on the biological performance of mineralization and nitrification by sludge ozonation. Ozone has also been used within activated sludge for selective disinfection to enhance the settleability of the biomass (*77,78*) and for selective oxidation to reduce toxicity and increase biodegradability *(79)*.

It was pointed out under adsorbability that oxidation of many organic compounds leads to substances that adsorb more readily. Ozone is particularly suitable to achieve such oxidation. Selective oxidation of impurities is used in the purification of vodka *(27,80)*.

Moreover, ozonation has cost advantages relative to other oxidative methods. Ozone treatment operates under atmospheric pressure and does not oxidize the alcohol under controlled reaction rates. The half-life of O_3 is relatively short, requiring neither extra heating nor any other additional treatment to remove residual ozone. Its only long-term residual product is molecular oxygen (O_2), which is both ubiquitous in all solutions kept under air, and obviously non-toxic. Thus, ozonation does not leave harmful residuals in a treated sample and readily decomposes in the headspace.

Ozonation - reaction mechanisms.

It is archetypal for ozone to undergo cycloaddition reactions with unsaturated hydrocarbons, resulting in carbon-carbon scission after several spontaneous subsequent steps and this is the dominant mechanism by which ozone is effective for ethanol purification. Another potential reaction mechanism is where hydroxyl radicals are produced during reactions between aqueous hydroxide ion and O_3. Only one hydroxyl radical per ozone molecule is produced, thus halving the electron acceptor number. However, the hydroxyl radical is significantly more reactive with a wider range of electron donors, and thus considerably less selective in its reactivity. In fact, hydroxyl radical would be expected to react with ethanol itself and introduce new impurities. Thus, indirectly, ozone can oxidize both saturated and unsaturated compounds. However, under proper conditions, direct oxidation of alcohols, aldehydes, ethers, and other saturated compounds is slow and indirect oxidation can be minimized. This represents a selectivity for oxidizing certain of the ethanol impurities selectively. For a more extensive discussion, see Bailey *(81)*.

Ozonation - non-reactive compounds.

In addition to compounds that are only slowly treated by ozone, its stable reaction products are also counted in this non-reactive compounds group. A subsequent purification treatment such as UV radiation and physical adsorption or stripping is required to remove these compounds. The major ozonolysis byproducts are summarized in Table 5 *(38)*.

Onuki et al. *(82-84)* showed that the use of ozonation, AC and stripping with gases (air,

N$_2$, and CO$_2$) can be very effective in removing ten major organic impurities from corn-based ethanol.   The research had the aim to remove impurities after distillation. A 40 mg/L O$_3$ treatment resulted in >56% and >36% removal of styrene and 2-pentylfuran, respectively, without significant generation of byproducts. A 60 g/L AC treatment with 270 min adsorption time resulted in 84%, >72%, and >78% removal of ethyl hexanoate, ethyl octanoate, and ethyl decanoate respectively. CO$_2$-based stripping removed 65%, >82%, and >83% acetaldehyde, ethyl vinyl ether, and 1,1-diethoxyethane respectively. A combination of three approaches effectively removes 8 organic impurities. Further improvement in food-grade ethanol purification with ozonation, followed by adsorption and solid catalysis was shown by Cai et al. *(80)*.

Techniques used for ethanol analysis

Gas chromatography.

A summary of main analytical techniques for ethanol analysis is illustrated in Figure 2.GC is a useful analytical technique for volatile and semivolatile compounds, typically requiring submicrolitre liquid samples or gas samples. It is a physical method in which the solvent and analytes are separated on the basis of volatility and affinity to a particular column coating. Samples are directly injected or thermalized of a solid absorbent. Recent developments in GC instrumentation provides a substantial decrease in analytical time and improvements of analytical sensitivity and selectivity. By optimizing extraction conditions for VOCs and using gas chromatography with quadrupole mass spectrometry detection (GC-qMSD), Rodrigues et al. *(85)* successfully identified 44 compounds in white wine, 64 in beers, and 104 in whiskeys. Some compounds were found to occur commonly in all kinds of alcoholic beverages. GC-qMSD provides some basic structural information based on fragmentation patterns in the mass spectrum, but the latter can also be used in a "fingerprint" mode for comparison to libraries of known compounds. GC with flame ionization detector (GC-FID) is used for quantification of the major toxic volatile compounds in alcoholic beverages including methanol and acetaldehyde *(86)*. It does not provide any structural information, but like all GC methods, retention times are characteristic for a given compound under fixed conditions. Gas chromatography with olfactometry (GC-O) is a technically developed sensory evaluation system to enable the evaluation of odour qualitatively and quantitatively can be used for the odour evaluation of alcoholic beverages *(87)*.
Newer extraction techniques have been developed for analysis of ethanol. Campo et al., *(88)* developed a method for the quantitative determination of aroma-bearing ethyl esters in wine and other alcoholic beverages using solid-phase extraction (SPE) and multidimensional GC-mass spectrometry (MDGC-MS). SPE is a powerful pre-concentration technique due to its robustness, cleanness of extraction, reusability of the adsorbent, environment friendliness, easy handling, and easy application for automation *(89-91)*. Pino et al. *(92)* developed a method for the quantitative determination of higher fatty acid ethyl esters in white rum aroma using solid-phase microextraction (SPME) and GC-FID. Onuki et al. *(28)* developed SPME-based quantification for ten major fermentation by-products in industrial ethanol. Many studies have shown that SPME is a suitable technique for the analysis of volatile and semi-volatile compounds in alcoholic beverages *(93, 94)*. A comprehensive review of vodka analyses methods was published by Wiśniewska et al., *(95)*. A comprehensive review of applications of gas chromatography to the analysis of spirit-based alcoholic beverages was published by Wiśniewska et al.,*(96)*

High-performance liquid chromatography.

High-performance liquid chromatography (HPLC) is performed on liquid samples that by pushing them through a solid phase column at high pressure and separating exclusively based on affinity to the column. Different detection methods can be used, such as UV absorption, fluorometry, and mass spectrometry. Refractive index-type detectors are used for monitoring ethanol production. Among the advantages of HPLC are no limitation by volatility or heat sensitivity of sample compounds. HPLC is a suitable analytical technique for a liquid sample such as ethanol, in which the impurities are the analytes. Loukou and Zotou *(97)* determined biogenic amines in alcoholic beverages by HPLC with fluorometric detection. HPLC in general is the most common determination technique of biogenic amines in wine and beers (*98-100*). Still, derivatization with reagents is in many cases required when the unmodified analyte is not amenable to the chosen or available detection method *(101,102)*. Yarita et al. *(103)* used HPLC-FID to determine ethanol in alcoholic beverages. Nascimento et al. *(104)* identified 10 aldehydes in 75 kinds of alcoholic beverages by HPLC with UV detection. You et al. *(105)* determined aldehydes in alcoholic beverages using HPLC with fluorescence detection and mass spectrometry. Aldehydes were derivatized for accurate quantification *(106-108)* similarly to Zhu et al. *(109)*.

Infrared spectroscopy.

Infrared spectroscopy (IR vibrational spectroscopy) is one of the most common classic spectroscopic analysis techniques for organic compounds. Originally thought of as a method to identify key functional groups in compounds, it became possible to use the vibrational spectrum as a fingerprint for identifying compounds against libraries in the advent of the computer age. In the case of analysis of ethanol, it can provide very rapid and low-cost sample analysis *(110)*. Virtually no sample preparation or time-consuming chromatography is required. Certain typical impurities, notably the carbonyl-containing aldehydes and acids, can be observed easily and quantified. Thus IR is suitable for routine quality assurance analysis of alcoholic beverages when screening for particular identifiable impurities *(111,112)*. Lachenmeier *(113)* used Fourier Transform infrared (FTIR) spectroscopy in combination with multivariate data analysis to determine the quality of alcoholic beverages.

Conclusions

Recent ethanol production methods, purification techniques adapted from water and wastewater treatment to ethanol, and ethanol analysis techniques were reviewed. Volatile byproducts are generated during any kind of ethanol production process. The separation and analysis of volatile by-products are crucial for quality enhancement and value-addition to ethanol. Many purification techniques for water and wastewater treatments are applicable as new purification techniques of ethanol. The purification techniques that do not rely on volatilities of impurities for separation, such as ozonation and physical adsorption, have potential to overcome disadvantages of distillation or exist as complementary additional purification steps. The recent development of separation techniques, such as GC and HPLC, has improved the detection capabilities of impurities in ethanol and enabled the development of pure vodka. This allows the determination low concentration impurities responsible for flavour or toxicity. IR could make the

quality assurance of ethanol more economic and faster. Whether ethanol is targeted for fuel, beverage, or other applications, relevant purification and analysis techniques are crucial for quality enhancement and value addition.

Acknowledgements

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Table 1. 2014 World fuel ethanol production.

Country	Production level ('000 gal/year)	Primary feedstock
United States	14,300	Corn
Brazil	6,190	Sugar cane
Europe	1,445	Sugar beet, wheat, corn
China	635	Corn, wheat
Canada	510	Corn, wheat, barley
Thailand	310	Cassava, sugar cane, rice
Argentina	160	Corn, sugar cane
India	155	Sugar cane
Rest of World	865	
Total	24,570	
Source: (Adapted from *1*)		Source: (*3*) and (*4*)

Table 2. Possible starch-based byproducts of ethanol (adapted from *22*).

Compound name	Compound name	Compound name
Ketones	**Fatty acids**	**Esters**
Diacetyl	Acetic acid	Ethyl formate
	Propionic acid	Ethyl acetate
Aldehydes	Butanoic acid	Propyl acetate
Acetaldehyde	*i*-Butanoic acid	2-Methylpropyl acetate
	Pentanoic acid	3-Methylbutyl acetate
Alcohols	*i*-Pentanoic acid	Ethyl hexanoate
n-Propanol	Hexanoic acid	Hexyl acetate
2-Methylpropanol	Octanoic acid	Ethyl lactate
i-Butanol	Decanoic acid	2-Methylpropyl hexanoate
2- and 3-Methylbutanol	Dodecanoic acid	Ethyl octanoate
2-Phenylethanol		3-Methylbutyl hexanoate
Alcohol		Ethyl decanoate
n-Propanol		Ethyl phenyl acetate
2-Methylpropanol		Ethyl dodecanoate
		Ethyl tetradecanoate

Table 3. Azeotropes of ethanol (boiling point =78.4 °C) (Adapted from *31, 32*).

2nd Component	Boiling point of component (°C)	Boiling point of mixture (°C)	EtOH by weight*
water	78.4	78.1	4.5
Azeotropes with selected esters			
ethyl acetate	77.1	71.8	69.2
methyl acetate	57.0	56.9	97
ethyl nitrate	87.7	71.9	56
isopropyl acetate	88.4	76.8	47
Azeotropes with selected hydrocarbons			
benzene	80.2	68.2	67.6
cyclohexane	80.7	64.9	69.5
toluene	110.8	76.7	32
n-pentane	36.2	34.3	95
n-hexane	68.9	58.7	79
n-heptane	98.5	70.9	51
n-octane	125.6	77.0	22
Azeotropes with selected other solvents			
methyl ethyl ketone	79.6	74.8	60
acetonitrile	82.0	72.9	43.0
nitromethane	101.3	75.95	26.8
trahydrofuran, (@100 kPa)	65.6	65.4	3.3
thiophene	84.1	70.0	55.0
carbon disulfide	46.2	42.4	92

*in binary azeotropes, when only one fraction is given, it is the fraction of the second component (*32*).

Table 4. Amount adsorbed, k (mg/g), of selected organic compounds from aqueous solution at very low equilibrium concentration (1 mg/L) on CAL activated carbon (Pittsburgh Activated Carbon Co., grade CAL activated carbon) (adapted from *39,* and original three sources from the same research group).

Adsorbate	k (mg/g)	Adsorbate	k (mg/g)
Alcohols		**Ethers**	
1-Propanol	0.745	Diethyl ether	5.14
1-Butanol	3.20	Dipropyl ether	19.4
1-Pentanol	10.5		
1-Hexanol	25.6	**Carboxylic acids**	
2-Methyl-1-propanol	2.75	Propionic acid	2.59
2-Butanol	2.49	Butyric acid	7.04
2-Methyl-2-propanol	1.48	Valeric acid	19.2
3-Methyl-1-butanol	9.58	Hexanoic acid	42.3
2-Pentanol	9.89	**Glycol ethers**	
3-Pentanol	6.67	2-Ethoxyethanol	2.17
2,2-Dimethly-1-prapanol	3.66	2-Butoxyethanol	22.0
2-Methyl-2-butanol	6.91	2-Hexyloxy)ethanol	68.0
Cyclopentanol	4.69		
Cyclohexanol	7.93	**Aromatics**	
2-Methyl-1-butanol	8.98	Chlorobenzene	101
3-Methyl-1-butanol	4.76	Benzoic acid	77.2
Aldehydes		**Phenols**	
Acetaldehyde	0.229	Phenol	37.7
Propionaldehyde	0.663	*o*-Methoxyphenol	130
Butyraldehyde	3.15	*o*-Cresol	90.3
Valeraldehyde	8.35		
		Sugars	
Acetates		D-(+)-Xylose	0.162
Methyl acetate	1.78	D-(-)-Arabinose	0.132
Ethyl acetate	3.60	L-(+)-Rhamnose	0.587
Propyl acetate	12.0	D-(+)-Glucose	0.185
Butyl acetate	26.5	D-(+)-Mannose	0.104
Isopropyl acetate	7.04	D-(-)-Fructose	0.0940
Isobutyl acetate	11.0	D-(+)-Galactose	0.202
		(+)-Maltose	19.8
Ketones		(+)-Sucrose	18.3
Acetone	0.484	(+)-Lactose	20.9
2-Butanone	4.66		
2-Pentanone	7.43	**Others**	
2-Hexanone	16.7	1.4-Butanediol	0.978
Cyclopentanone	6.71	1,2-Butanediol	1.41
Cyclohexanone	9.96	1,4-Dioxane	1.59

Table 5. Major ozonolysis byproducts (adapted from *38*; fermentation raw material is corn mash).

Chemical group	Compound name
Aldehydes	Formaldehyde
	Acetaldehyde
	Glyoxal
	Methyl glyoxal
Acids	Acetic acid
	Formic acid
	Oxalic acid
	Succinic acid
Aldo- and ketoacids	Pyruvic acid
Others	Hydrogen peroxide

Feedback from reviewer

General comments:

Some confusion between biofuel ethanol production and food grade ethanol production. Overall, manuscript is much improved.

Specific Comments:

Distillation section P 6-7; The emphasis of water removal only refers to biofuel use. All Food grade alcoholic beverages contain water. I suggest the authors divide the Distillation section into food grade and fuel grade ethanol to make this clear. Separation is not practical as it would result in substantial repetition. However, a few sentences have been added to also address distillation as used for making food-grade alcohol.

Page 7; lines 268-272; The health concerns of these by-products also need a separate subtitle. The authors emphasizes these toxic issue and they need to make it easy for the reader to find. Also, I thought ethanol had some health issues too. Should these be stated also? Subtitle added and also included ethanol health problems.

Page 10; line 402; The last sentence should read "For a more extensive discussion, see Bailey (1982) (80)." Done

Page 10; Line 410-418; For all the listed compounds removed by ozone only acetaldehyde is listed in Table 4. Are there any additional examples from the compounds listed in Table 4? Unfortunately, not.

Page 11; GC and HPLC are defined on page 3. There is no need to repeat them here. Full words removed.

Table 1; page 20; Argentina is missing a primary feedstock. The original source did not have this, but the info has been added.

Table 2; page 21; The row lines are confusing for a table that is three columns. Remove the row line and add column lines (editor – do not worry about this comment as the typesetter will set the table and they do not like column lines – editor)

Table 4; page 23; Remove the row lines and add a column line. (editor – do not worry about this comment as the typesetter will set the table and they do not like column lines – editor)

Editor – please add comma after the title of the paper rather than period on all of the references