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Chapter

Bioconversion of Agricultural and Food Wastes to Vinegar

Debajyoti Saha and Prabir Kumar Das

Abstract

Agricultural residues and fruit/food wastes are a curse to the environment but this can also play an important role in meeting the growing needs for energy, value-added chemicals, and food security problems. Vinegar is an acidic liquid whose major component is acetic acid and consists of different organic acids and bioactive compounds. Vinegar is a substance produced by the acetic acid bacteria *Acetobacter* and *Gluconobacter* that has a 4% acetic acid content. For the efficient biological production of acetic acid, a variety of renewable substrates are used, including agro and food, dairy, and kitchen wastes. This reduces waste and lowers environmental pollution. There are different types of traditional vinegar available all over the world and have many applications. Vinegar can be made either naturally, through alcoholic and then acetic fermentation, or artificially, in laboratories. This chapter emphasizes the production and biotransformation of agricultural and fruit wastes into vinegar and the genetic manipulations done on microorganisms to utilize a wide range of substrates and achieve maximum product titer.

Keywords: agri-food waste, biotransformation, acetobacter, anaerobic fermentation, acetic acid, vinegar

1. Introduction

Acetic acid and water combine to produce vinegar, a liquid consumable, mostly used as food ingredients, flavoring agents, or preservatives. It usually contains 4–8% (v/v) acetic acid, one of the most easily produced mild acids. Depending on the type of vinegar, additional substances may also be present, such as flavorings or synthetic coloring. There are numerous variations, qualities, and price ranges of vinegar available worldwide, starting from inexpensive distilled or synthetic vinegar and ordinary wine/cider vinegar to extremely expensive traditional balsamic goods [1]. Vinegar is one of the most valuable food products due to its adaptability and range of usages, and the global vinegar market has reached a value of US\$ 2.27 Billion in 2021 and is expected to increase by 2.6% up to 2027 [2]. Cider vinegar and normal vinegar are the two main varieties of vinegar available. As compared to regular vinegar, which is manufactured from untreated plant materials like grains, apples, grapes, or sugarcane, cider vinegar is made from fruit juices. In addition to having anti-diabetic properties

and decreasing blood cholesterol levels by reducing the oxidation of low-density lipoproteins (LDLs), cider vinegar is a highly advantageous beverage for consumers.

Vinegar is produced using two basic biotechnological processes: acetous fermentation in the presence of acetic acid bacteria and alcoholic fermentation, which takes place in the presence of yeast, such as *Saccharomyces cerevisiae*. In contrast to acetous fermentation, which turns alcohol into acetic acid, alcoholic fermentation involves the conversion of sugar to alcohol. There are two different ways to make vinegar: the traditional way (Orleans method) and the rapid way (submerged and generator methods) [3]. A schematic diagram of three major different vinegar-making processes is shown in **Figure 1**. However, the method of making it has been seen long ago as a chemical process. The Dutchman Boerhaave noticed that the “mother of vinegar” was a live organism in 1732, as described in the overview of the history of vinegar, although he did not specify how this organism contributed to the acidification process. Due to its tight oxygen requirement, we will refer to this process as “acetification” rather than the more well-known “acetous fermentation.”

There is a wide range of raw materials used in the production of vinegar, from agricultural surpluses and wastes to premium substrates for the most distinctive and valuable vinegars, such as Sherry vinegar (Spain) and Aceto Balsamico Tradizionale (Italy). Up to ten different types of vinegar are listed in the quality standard, including wine vinegar, fruit vinegar, cider vinegar, alcoholic vinegar, cereal vinegar, malt vinegar, malt distillate vinegar, balsamic vinegar (with added grape must), and “other balsamic vinegars,” which include any other agriculturally derived substrate, such as honey or rice. However, worldwide most of the vinegar produced is “white” vinegar, that is, vinegar produced directly from diluted alcohol [4].

It is well known that producing macerated vinegars using various fruit components is one of the potential uses of waste from the fruit business. For the maceration with vinegar, the peels of citrus fruits like orange, lemon, lime, grapefruit, or the complete strawberry have been used numerous times. There are also several instances of vinegar maceration with other fruits like bananas, passion fruits, or apples in the

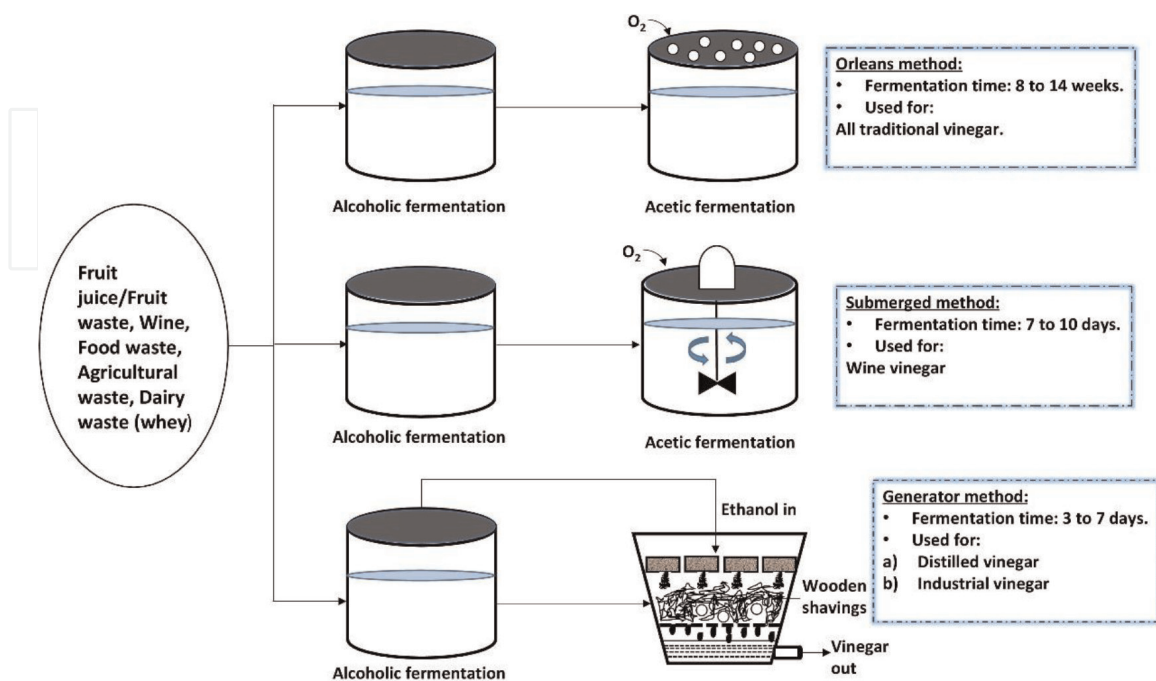


Figure 1. A schematic representation of major three ways of vinegar production from various wastes.

literature [5]. Vinegar can frequently be produced from waste fruit, substandard honey, and other sugar-containing materials that are unsafe to drink or sell. This is how waste cores and peels from fruit dryers and canneries can be used to make money. As a form of agricultural waste, pineapple peels account for about 35% of the total mass of the fruit. These wastes could result in a significant issue if they are released into the environment untreated. Therefore, it is essential to recycle waste raw materials into useful products with higher value-added, or even as raw material for other industries, to use as food or feed after biological treatment, or all three [6].

Acetic acid bacteria (AAB), which are frequently present in the raw materials used in the production of vinegar, are capable of producing vinegar. The majority of the species identified in vinegar belong to *Acetobacter* and *Gluconacetobacter*, two of the officially recognized AAB taxa in the *Acetobacteraceae* family. At low acetic acid concentrations, *Acetobacter* spp. prevail. Species like *Komagataeibacter europaeus* or *Gluconacetobacter intermedus* predominate when acetic acid levels approach 5% (v/v). The optimum method for ensuring the success and evolution of the fermentation is typically thought to employ a mixed culture of a “fast starter” (such as *Acetobacter pasteurianus* or *Acetobacter aceti*) and one with high resistance to acetic acid (such as *K. europaeus*). So, this chapter emphasizes the bioconversion of various food or agro-industrial wastes into vinegar with the help of genetically modified microorganisms [7].

In this chapter, we have shown a wide range of waste resources can be converted into different types of vinegar which have numerous usages. The improved production of vinegar by developing the fermenting microorganism’s genome with the help of genetic and metabolic engineering is also shown in this chapter. So, this chapter will give a comprehensive idea about the bioconversion of various agro and food industry wastes into innumerable vinegar with diverse applications.

2. Feedstocks used for vinegar production

The food sector relies heavily on vinegar as a food preservative. Vinegar can be made from a variety of basic materials, including different fruits and fruit peels. Additionally, a variety of agricultural waste products can be employed as raw materials. Several studies revealed that the saccharification process was significantly impacted by the enzymatic treatment of pineapple wastes.

2.1 Agricultural wastes as feed

Every microorganism requires nutrient media as a source of energy and minerals for utilization in growth and multiplication. When media are fed to microorganisms inside a bioreactor is termed feed. These media mainly consist of one or two carbon sources, nitrogen, amino acid, and salts. The carbon source in the feed is mainly used for cell growth and product formation. C₆ sugars (glucose & fructose), C₅ sugars (xylose), and glycerol are the mainstream carbon sources that are used. Whereas, nitrogen is required for the synthesis of DNA, RNA, amino acids, ATP, and other molecules. These include yeast extract, beef extract, malt extract, ammonium chloride, ammonium sulfate, peptone, and tryptone. Microorganisms that cannot synthesize particular proteins on their own are fed amino acids in the media.

Due to overgrowing population, there is an increase in the demand for food, fuel, and biochemicals. Sugar is a major ingredient/precursor for the production of the

above three and is mainly extracted from sugarcane, sugar beet, corn, and other sources, creating competition between food and chemicals. An alternative way to this precursor is the use of plant fiber waste from agricultural waste. Agricultural wastes include solid and liquid wastes discharged by farm products processing and agricultural production, livestock and poultry breeding, and rural residents [8]. Agricultural biomass wastes are mainly crop stalks, bagasse, leaves, roots, fruit peels, and seed/nutshells, which are mostly discarded, burnt, or leftover in fields, and are composed of cellulose, hemicellulose, and lignin [9].

Microorganisms used in the fermentation process, do not have access to direct consumption of glucose and xylose, mannose and arabinose present in cellulose and hemicellulose respectively due to the presence of a protective layer of lignin. So, the agricultural waste needs to be delignified to release C5 and C6 sugars. Delignification is done by pretreatment and enzymatic hydrolysis of lignocellulosic biomass. Pretreatment can be broadly categorized into physio-chemical pretreatment and biological pretreatment.

Biological pretreatment is considered the most eco-friendly process that is carried out using enzymes [10, 11] and microbes [12]. In this treatment, neither addition of chemical supplementation is required nor toxic by-products are formed during the process. This treatment process is very slow and time-consuming compared to other processes. It requires several weeks for the sufficient delignification of lignocellulosic biomass. A newly developed fungal consortium was used to pretreat rice straw and willow, resulting in saccharification yields of up to 74.2% and 63.6% respectively [13]. After 12 weeks of pretreatment of bamboo culms with white-rot basidiomycete *Punctularia* sp. TUF20056 showed high lignin degradation (>50%) and glucose and total sugar yields were increased to $42.9 \pm 10.9\%$ and $60.3 \pm 16.1\%$, respectively [14]. Corn stalks pretreatment by white-rot fungus *Irpex lacteus* yielded a maximum reducing sugar of 313.5 mg/g (w/w) and overall glucose of 236.9 mg/g after 28 days of pretreatment [15]. *Stereum hirsutum* degraded the lignin of Japanese red pine *Pinus densiflora* by 14.52% after 8 weeks of pretreatment [16].

Physio-chemical pretreatments are conventional physical/mechanical, chemical, and combined pretreatment. Conventional physical/mechanical pretreatment includes steam explosion, milling, chipping, etc. Other than steam explosion all other physical pretreatment methods are solely used for size reduction of biomass, which alone is found to be ineffective for delignification, depolymerization, and crystallinity reduction. So, chemical pretreatment like acid treatment, ammonia-assisted treatment, organosolv pretreatment, alkaline, alkaline peroxide treatment, and liquid hot water treatment is used for further processing of biomass. When rice straw was pretreated with 12% w/v NaOH at 0°C for 3 h and concentrated H₃PO₄ at 50°C for 30 min, glucose yield was 163.5 and 192.3 g/Kg, respectively [17]. It was observed that 0.61 M H₂SO₄ catalyzed liquid hot water pretreatment (143.2°C, 38.4 min) of pineapple leaves resulted in 91% glucose yield [18]. After pretreatment of oil palm, empty fruit bunch in 1.5% maleic acid at 176.5°C for 70 min resulted in 45% cellulose and 20% hemicellulose yield [19]. Hydrolysis of barley straw was carried out with 2% H₂SO₄ at 180°C for 30 min resulting in a maximum glucose yield of 28.2 g/L [20].

2.2 Fruit and food waste as feed

Increased use of processed fruit products globally is increasing fruit processing waste (FPW) by up to 20 to 50% [21]. These wastes include rotten & discarded fruits, leaves, peels, pulps, and pomace, which are generated during harvesting,

transporting, extracting/processing, and consuming. FPW is dumped in landfills or incinerated which causes various environmental problems like leaching and a bad odor due to its high moisture content, high organic matter content, and low pH [22]. But these are found to be a good source of bioactive compounds like anthocyanins, phenolics, alkaloids, glycosides, and volatile oils [23, 24]. These can be even used for the production of volatile fatty acids [25, 26], biogas [27–29], and bioenergy (bio-ethanol, butanol & electricity) [30–34].

Some FPW also needs to be pretreated to release reducing sugars, to be easily available to microbes. Mango and banana being the two most important food crops of India [35], generates a large amount of waste, which could be used to produce renewable fuels and fermentation products. To access their sugar contents the researchers treat banana peels, leaves, stems, pith, and mango peels in different acid/alkaline pretreatments [36, 37]. Microwave-assisted 1% NaOH pretreatment of mango peels for 2 min resulted in 0.704 g/g of reducing sugar yield where cellulose and hemicellulose were 44.2% and 26.2%, respectively [38]. Pretreatment of banana leaf in 0.1 N H₂SO₄ at 45°C resulted in 524.483 mg/g of reduced sugar yield [39], while microwave-assisted acid pretreatment in 5% H₂SO₄ at 180°C for 30 min resulted in the cellulose of ~37% and hemicellulose of ~48% from banana leaf and pith [37]. Nguyen et al. did subsequent hydrothermal pretreatment at 95°C for 30 min and enzymatic hydrolysis with 2% cellulase and 0.125% Tween-20 on low-grade longan fruits wastes which yielded 240.396 g/L of reducing sugar [40]. Date palm cellulosic wastes under 2 N NaOH treatment at 30°C yield 31.56 mg/mL of glucose [41]. Apple juice has become a part of the diet in most households, this high demand and production of apple juice create a large quantity of waste in the form of pomace which is high in sugars. Pretreatment of apple pomace with 0.5% CaO yielded high glucose and fructose of 40 g/l and 25.9 g/l respectively but the yield was lower when treated in dilute sulfuric acid [42], whereas when treated in 2% HNO₃ at 120°C yields 53.1 g/l of total sugar [43].

2.3 Dairy waste as feed

A rise in the demand for milk and milk products has led to the huge growth of the dairy industry in the world [44]. The dairy industry produces a variety of products which includes- milk, milk powder, cream, butter, cheese, yogurt, fermented milk products, and many more, which generates a lot of solid and liquid wastes. These wastes are detrimental to water bodies and the environment because of their high organic content. Dairy wastewater contains suspended and dissolved solids, trace and soluble organic components, lactose, nutrients, fats, sulfates, chlorides, etc. and it is normally characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) [45], which makes wastewater treatment challenging and expensive [46]. An alternative way to handle this waste is to use them as raw material for the production of other industrial products or energy generation. Whey, a liquid by-product from cheese and casein manufacturing is the most abundant pollutant in dairy wastewater. It is composed of lactose (45–50 g/L), soluble proteins (6–8 g/L), lipids (4–5 g/L), and mineral salts (8–10% of dried extract) like NaCl, KCl, calcium phosphates and lactic acid (0.5 g/L), citric acid and group B vitamins [47]. It also shows high chemical oxygen demand (COD; 50–70 g/L) and biological oxygen demand (BOD; 27–60 g/L) [48]. Other than using whey as feedstock for animal feeding, nowadays it is being fermented by different microbes for the production of value-added products ranging from whey drinks (from lactic and acetic fermentations), vinegar, and biopolymers like

polyhydroxyalkanoates (PHAs) and bacterial cellulose (**Figure 2**). Whey vinegar production includes sequential alcoholic and acetic fermentation by consuming lactose and producing ethanol which is further consumed to yield acetic acid. It has been reported that vinegar from whey fermentation contained acetic acid in the range of 4–5% and tasted mellow acidic. **Table 1** enlists some of the whey vinegar production conditions, strains involved and acetic acid content.

3. Waste to vinegar production

According to Food and Agricultural Organization, the production of fruit and vegetables went up by 54% and 65% respectively between 2000 to 2019 [84], but one-third (~1.3 billion) of the production is lost as waste [85] of which 14% is lost during harvest and retail [86].

Nowadays, vinegar is being produced from nonconventional substrates like onions, discarded fruits, peels, pulp, pomace & agricultural wastes. When standard apples and grapes were fermented with *S. cerevisiae* and *A. aceti* NCIM 2094 at 30°C and pH 5.0 yielded maximum acetic acid of 6.75% (apple) and 6.80% (grape) after 13 days [59]. Vinegar gets its unique aroma and flavor from acetic acid and other fermentation products in vinegar like organic acids, esters, ketones, and aldehydes [87], which are produced during the fermentation and aging process, where acetic acid acts as the precursor for the formation of these products [88]. Vinegar produced from agricultural and fruit wastes is also found to be rich in those volatile compounds. Pineapple's (*Ananas comosus*) peels and core is discarded as waste, which was pretreated and subjected to fermentation with *S. cerevisiae* for 7–10 days under aerobic conditions at 25°C and then with *A. aceti* for 30 days at 32°C and pH 3.0 to obtain 5% acetic acid and also contained higher alcohols, esters, several aldehydes and ketones (including acetoin, 2,3-butanediol, ethyl acetate, and 2-phenyl ethanol) as the major

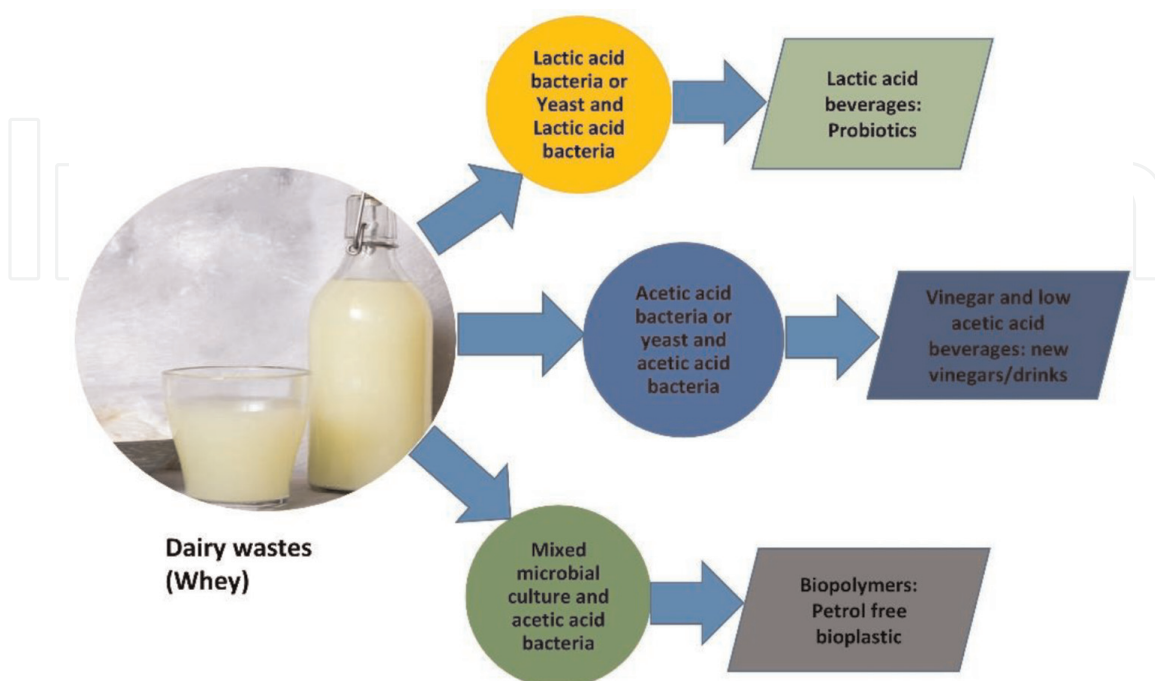


Figure 2. Whey to value-added products through microbial fermentation.

Product	Substrate/pretreatment	Strain	Type of Bioreactor/ mode of operation	Yield of acetic acid	References	
Traditional Vinegar	Rice vinegar	Glutinous rice (Kiaw Ngu). Tanekoji preparation (starch hydrolysis) using spores of <i>Aspergillus oryzae</i> before fermentation.	<i>Acetobacter pasteurianus</i> TISTR 102	Solid state fermentation	6.68% (w/v)	[49]
	Rice wine		<i>Acetobacteraceti</i> WK	100-L internal Venturi injector bioreactor. Semi-continuous	65 g/l	[50]
				Loofa sponge immobilization in 60 L reciprocating shaker	56 g/l	[51]
	Rice wine		<i>Acetobacter</i> sp.	8 L Acetator. Submerged fermentor	7.8% (w/v)	[52]
Wine vinegar	White table wine		<i>A. pasteurianus</i> CECT 824	Batch and semi-continuous	60 g/l and 115 g/l	[53]
	Cashew wine		<i>A. aceti</i>	50 L reactor. Batch	4% (w/v)	[54]
Cider vinegar	Apple juice		AAB, LAB	Wooden barrels	5% (w/v)	[55]
Black vinegar	Kurozu (unpolished rice). Koji preparation (starch hydrolysis) using <i>Aspergillus oryzae</i> or <i>A. luchuensis</i> .		Not required	54 L of ceramic pots. Batch.	By The Japanese Agricultural Standard System is 1.5–8% (w/v)	[56]
Cocoa vinegar	Cocoa pulp liquid		<i>A. aceti</i>	Batch	1.64%	[57]
Chinese vinegar	Cereals and their brans		Koji: <i>Aspergillus</i> , <i>Rhizopus Monascus</i> and <i>Saccharomyces cerevisiae</i> , <i>Hansenula anomala</i> . AAB	Solid state fermentation	3.5–6%	[58]
Fruit waste vinegar	Substandard apples & grapes	Juice extracted from the fruits & treated with potassium metabisulfite	<i>A. aceti</i> NCIM 2094	500 ml conical flask	6.75% (apple) & 6.80% (grapes) (w/v)	[59]
	Strawberry vinegar	Surpluses of strawberry	—	—	5% (w/v) and vinegar yield of 66.2 ± 2.5%	[60]

Product	Substrate/pretreatment	Strain	Type of Bioreactor/ mode of operation	Yield of acetic acid	References	
Marula vinegar	Marula (<i>Sclerocarya birrea</i>) fruit processing wastes.	<i>A. aceti</i> ATCC 15973	50 ml falcon tubes. Surface fermentation.	57 g/l	[61]	
Shea vinegar	Shea (<i>Vitallera Paradoxa</i> Gaernt) pulp from shea nut processing industry.	Naturally inoculated by AAB	23 L gallons. Batch.	68.5 ± 0.3 g/l	[62]	
Onion vinegar	Discarded onions	<i>Acetobacter orientalis</i> MAK88	5 L glass fermenter. Semi-continuous	4.35% (w/v) and productivity of 17.73 g/(l-d) was achieved	[63]	
		<i>A. pasteurianus</i>	Charcoal pellet bioreactor.	37.9 g/l	[64]	
		<i>Acetobacter tropicalis</i> KFCC 11476P	120 L pilot scale fermenter. Fed-batch.	0.67 g/g.l.h	[65]	
Pineapple vinegar	Pineapple (<i>Ananas comosus</i>) peels and core. Enzymatic pretreatment	<i>A. aceti</i>	Batch.	5% (w/v)	[66]	
	Pineapple (<i>A. comosus</i>) peels	<i>A. pasteurianus</i> FPB2-3	Simultaneous alcoholic & acetic fermentation	6.5% (w/v)	[67]	
	Pineapple (Smooth Cayenne) peels.	<i>Propiniobacterium acicipropionici</i>	500 mL of Erlenmeyer flask. Batch	4.81–6.15% (w/v)	[68]	
Amla vinegar	Amla (<i>Emblica officinalis</i> Gaertn) fruit food processing waste.	<i>A. aceti</i> NRRL 746	—	5.7% (w/v)	[69]	
Apple vinegar	Apple pomace. Enzymatic pretreatment.	<i>A. aceti</i>	Batch	61.4 g/100 g dry matter	[70]	
Olive oil vinegar	Olive oil mil wastewater	<i>Acetobacter</i> sp.	—	5.6% (w/v)	[71]	
Agricultural/ Forestry waste vinegar	Fermented pepper leaf	Wongi-1 pepper (<i>Capsicum annum L.</i>) leaves.	<i>A. aceti</i> KACC 11978	Batch	46.1 ± 2.22 g/l	[72]
	vinegar	Corn cob	<i>A. aceti</i> NCIM 2116	—	3.91% (w/v)	[73]

Product	Substrate/pretreatment	Strain	Type of Bioreactor/ mode of operation	Yield of acetic acid	References
Wood vinegar	Pruned apple tree branches. Pyrolysis at 500C for 30 min.	—	—	27 mg/ml	[74, 75]
	Chinese fir (<i>Cunninghamia lanceolata</i>) Pyrolysis at 350C.	—	—	34.09%	[76]
	Rubberwood logs. Pyrolysis at 400 C	—	—	5.56% (w/v)	[77]
	Chinese fir wood sawdust Pyrolysis at 260C	—	—	72.4% vinegar yield.	[78]
Paper sludge	Fermentation of paper sludge by co-culture of 3 bacteria.	<i>Clostridium thermocellum</i> , <i>Clostridium clariflavum</i> and <i>Moorella thermoacetica</i> (1:1:1)	—	3.7 g/l	[79]
Dairy waste vinegar	Sweet cheese whey	<i>Kluyveromyces marxianus</i> Y102 and <i>Acetobacter aceti</i> DSM-3508	200 L fed-batch fermenter with biomass recycling.	acetic acid: 11.55 g/L	[80]
	Sweet cheese whey and food-grade lactose	<i>Kluyveromyces fragilis</i> CECT 1123 and <i>A. pasteurianus</i>	250 ml flask.	5%	[81]
	Whey solution was saccharified with Rapeseed meal-Koji/Wheat bran-Koji	<i>Aspergillus oryzae</i> , <i>Zymomonas mobilis</i> and <i>A. pasteurianus</i>	—	4%	[82]
	Whey	<i>Acetobacter pomorum</i> IWV-03	250 ml flask	5.6%	[83]

^{1,2} data not available.

Table 1.
 Different types of vinegar produced from various waste resources.

components, along with significantly low levels of off-flavors. L-lysine, mellein, and gallic acid [66]. A red-colored vinegar composed of a total of 37.9 g/l acetic acids, was fermented from discarded onion juice by aerobic oxidation of ethanol using *A. pasteurianus* within 20 h of fermentation [64]. Fed-batch fermentation of discarded onion juice using *Acetobacter tropicalis* KFCC 11476P showed specific acetic acid productivity of 0.67 g/g.l.h [65] after 2 days of fermentation while the specific acetic acid productivity was 20.5 l/h when fermented by *A. pasteurianus* CICC 20001 [89], with flavonoids and polyphenols contents of 3 mg/ml and 976 µg/mL, respectively. Pulse electric field and ultrasound pretreated date palm (Sukkari variety) when subjected to alcoholic and acetic acid fermentation by *S. cerevisiae* and *A. aceti* GDMCC 1.15, respectively, yielded 30.96 ± 13 mg/ml acetic acid and increased levels of phenolics, flavonoids, and free amino acids than untreated dates [90]. Low-quality Zahdi date palm was used to produce vinegar by 2 step fermentation process, which contained 6.62% (w/v) acetic acid [91]. *Cordyceps militaris*, a popular medicinal fungus is discarded after use, which contaminates the environment, so Liu et al. [74, 75] enzymatically hydrolyzed freshly harvested *C. militaris* obscure solid medium with 0.5% the cellulase, 0.2% α -amylase, and 0.5% glucoamylase at different temperatures. The hydrolysate was then fermented for vinegar production by *A. pasteurianus* for 8 days at 30°C which yielded 28.58 g/l acetic acid and the contents of flavonoids and phenolics were 2.08 times and 67.5 times higher than rice vinegar, respectively [92]. A list of various types of vinegar produced from different feedstocks is shown in **Table 1**.

Bio-oil produced from pyrolysis of agricultural, forestry, and urban pruning residues is composed mainly of water (80–90%) and about 200 organic compounds, among which acetic acid is the main component [93]. A higher percentage of acetic acid is derived from the pyrolysis of Pine, poplar, oak, bamboo, and rice husks. One of the popular vinegar, wood vinegar, is also produced from agricultural residues mainly wood. The most typical types of biomass employed are hardwood chips and sawdust, although a range of biomass types, including wood, crops, agricultural leftovers, or diverse materials of biological origin, can be used to make wood vinegar. A common name for wood vinegar is pyroligneous acid or wood acid. It is an alcoholic beverage made when biomass is pyrolyzed under strictly monitored conditions. Wood pyrolysis is a technique that thermally breaks down biomass at temperatures between 200 and 600°C in an inert environment without oxygen, producing a variety of products like solid char (biochar, charcoal), tar, non-condensable gases, and wood vinegar [74, 75]. Among the pyrolysis products, wood vinegar makes up more than 20% of the yield. This means that after treatment, 1000 kg of feedstock will yield over 200 kg of wood vinegar [94]. A schematic diagram of wood pyrolysis for vinegar production is shown in **Figure 3**.

4. Genetic engineering and metabolic engineering

Acetic acid bacteria and some other microorganisms can produce acetic acid, the main component of vinegar from different biological routes like oxidation of ethanol, oxidation of acetaldehyde (decarboxylation of pyruvate), and hydrolysis of acetyl-CoA (from the reduction of pyruvate). A schematic metabolic pathway for the production of acetate from different sugars has shown in **Figure 4**. Researchers focused on the engineering of these microbes and manipulating their metabolic pathways and enzymes to overcome the limitations of acid stress, flux distribution, use of a single carbon source, and lower production yield. Fermentative oxidation of ethanol to acetic acid is dependent on two sequential reactions of membrane-bound

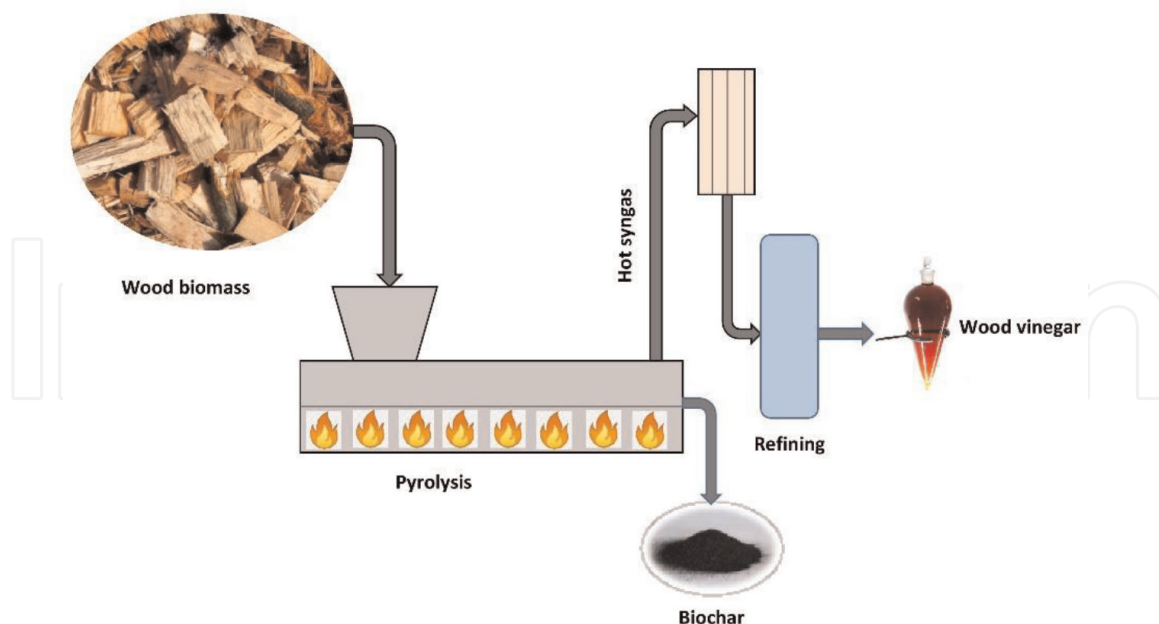


Figure 3.
 A schematic diagram of wood pyrolysis for the production of wood vinegar.

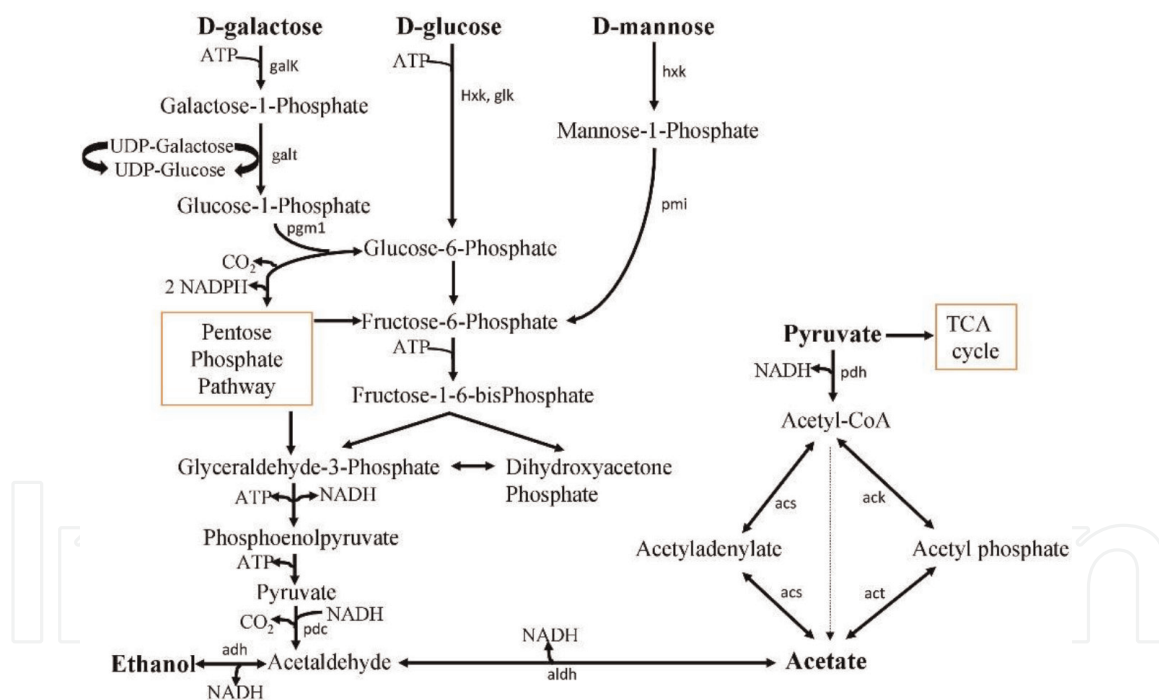


Figure 4.
 Metabolic pathway of acetate production. First alcoholic fermentation by yeast under anaerobic conditions followed by aerobic oxidation of ethanol by acetic acid bacteria.

pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (ALDH), both of which are localized on the periplasmic side of the inner membrane [95, 96]. Thus, an increased yield of acetic acid from 52.23 g/L to 61.42 g/L was observed when the *A. pasteurianus* strain was engineered to overexpress the endogenous pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) genes comprising *adhAB* [97]. ATP-binding cassette (ABC) transporter systems found in both prokaryotes and eukaryotes are membrane proteins that harness

Organism	Engineering strategy	Effect/Yield (acetic acid)	References
<i>Acetobacter aceti</i> IFO3283	Expression of groESL operon (GroES and GroEL molecular chaperones)	Improved tolerance to heat, ethanol, and acetic acid	[101]
	Expression of dnaKJ operon (DnaK and DnaJ proteins)	Improved tolerance to heat and ethanol.	[102]
<i>Acetobacter pasteurianus</i> JST-S	Overexpression of adhA and adhB of Pyroquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH)	61.42 g/l	[97]
<i>A. aceti</i> 10-8S2	Overexpression of aatA, an ATP-binding cassette (ABC) transporter	111.7 ± 3.3 g/l	[98]
<i>A. aceti</i> 10-8S2	Overexpression of Acn (aconitase)	105 g/l	[103]
<i>A. pasteurianus</i> AC2005	Overexpression of UvrA (nucleotide excision repair protein)	85 g/l	[104]
<i>Gluconacetobacter intermedius</i> NCI1051	Disruption of quorum sensing gene ginI	1.24 fold increased yield than parental NCI1051	[105]
<i>A. pasteurianus</i> NBRC3283	Expression of GrpE with dnaKJ operon	Improved tolerance to heat, ethanol, and acetic acid	[106]
	Expression of clpB molecular chaperon		[107]
<i>A. pasteurianus</i> G-40	Mutated fabG encoding oxidoreductase	15.5% increased yield of acetic acid	[108]

Table 2.

Genetic and metabolic engineering for improved stress tolerance and increased acetic acid yield.

the energy of ATP hydrolysis to import nutrients inside cells and export toxic compounds out of cells. Overexpression of aatA, an ABC transporter, in *A. aceti* not only lets it withstand higher acid concentrations (acetic acid, formic acid, and propionic acid) but also increased acetic acid yield up to 111.7 ± 3.3 g/l [98]. Microorganisms like *S. cerevisiae*, *Clostridium saccharoperbutylacetonicum* were engineered to be acetic acid tolerant by overexpressing RCK1 [99] and downregulation of sigI [100] genes, respectively. There are reports of expressing molecular chaperons and nucleotide excision repair proteins to make AAB strains tolerant to acetic acid and high yielding. Genetically modified strains with improved acetic acid production and tolerance to various factors are listed in **Table 2**. Certain strains like *Acetobacterium woodii* and *Clostridium aceticum* can utilize one-carbon compounds, such as carbon monoxide and carbon dioxide, in the presence of hydrogen to produce acetic acid [109, 110]. Researchers are highly interested in utilizing this capability to use waste gases as feedstock for environmental remediation, introduce/express those genes in other AABs and for sustainable production of acetic acid.

5. Applications of vinegar

Vinegar contains different organic acids and bioactive compounds which makes it useful in diverse fields. It is used as a preservative and household cleaning reagent for medicine. A detailed and specific application of various types of vinegar is enlisted in **Table 3**.

Vinegar name	Composition	Major application	Reference
Medicinal use			
<i>Anti-infective</i>			
Date vinegar	—	Shows in vitro antimicrobial activity against <i>Pseudomonas aeruginosa</i> .	[111]
Oxymel	Clarified honey:4 parts & white wine vinegar: 1 part	<ul style="list-style-type: none"> popular gargle for sore throat. used as a vehicle for expectorant preparations, such as ipecacuanha, squill, etc., and in fever drinks. 	[112]
Fruit vinegar	Acetic acid: 0.1% NaCl: 5%	inhibits the growth of foodborne pathogens in vitro, including those of <i>Escherichia coli</i> O157:H7, <i>Salmonella enteritidis</i> , <i>Salmonella typhimurium</i> , <i>Vibrio parahaemolyticus</i> , <i>Staphylococcus aureus</i> , <i>Aeromonas hydrophila</i> and <i>Bacillus cereus</i>	[113]
<i>Eucommia ulmoides</i> leaves vinegar	Acetic acid: 45.5 mg/ml	Exerted antibacterial activity against <i>Bacillus subtilis</i> .	[114]
<i>Daphne genkwa</i> processed vinegar	—	Anti-herpes activity	[115]
Wood vinegar (apricot & olive seeds)		Shows anti-inflammatory, anti-bacterial (<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>Enterococcus casseliflavus</i>) and anti-fungal (<i>A. niger</i> , <i>P. tracheiphilus</i> , and <i>C. parasitica</i>)	[116]
<i>Antitumor effect</i>			
<i>Kurosu</i> or Japanese black vinegar	Acidity: 4.2%	Inhibited the proliferation of cancer cells	[117]
Red grape vinegar	—	reduction of dysplasia and carcinogenesis in hamsters	[118]
Coconut water vinegar	—	Possess anti-tumor effect on 4 T1 breast cancer cells.	[119]
Soybean-boiled extracted vinegar	—	Shows tumor-suppressing effect in vivo (mice) and apoptosis-inducing activity in vitro (U937 cells) analysis.	[120]
Pineapple vinegar	Acetic acid: 6–8%	Delays cancer progression	[121]
<i>Effect on cardiovascular system, blood glucose and obesity</i>			
Honey vinegar syrup	20% vinegar in honey syrup	increased fasting insulin level and decreased total cholesterol level in the intervention group	[122]
Tomato vinegar	Acidity: 5.6%	Decreased body and visceral fat weight and also reduced hepatic triglyceride and cholesterol levels.	[123]
Persimmon vinegar	Acidity: 5.2%	Reduced hepatic triglycerides and total cholesterol	[124]
Pineapple peel vinegar	0.8 ml vinegar	High potency in restoring the Gonadosomatic Index on diabetic rats	[125]
Apple cider vinegar	Different acidity and dilution	Shows potential impact on glycemic control, hyperlipidemia and control on body weight in type 2 diabetes patients	[126–132]

Vinegar name	Composition	Major application	Reference
Vinegar mix	Apple cider vinegar, garlic, ginger, lemon and honey mixture	Anti-hyperglycemic effect on postprandial glycemia in non-diabetic females	[133]
Rice vinegar	—	Acetic acid content reduced blood pressure and renin activity in spontaneously hypersensitive rat	[134]
Nypa palm vinegar	—	<ul style="list-style-type: none"> • Enhanced anti-glycemic effect compared to metformin. • Enhanced insulin level up to 79.8% • Significant anti-glycemic effect. On adult male sprague-dawley rats	[135]
Pomegranate vinegar	Vinegar: 5–13%	Increased lipolysis and fatty acid oxidation.	[136]
Date vinegar	Acetic acid: 2.89 g/g	improved the concentration of lipid and inflammatory biomarkers	[137]
<i>Gastric effect</i>			
Natural vinegar	Acetic acid: 5%	Showed protective effect against stress-induced gastric ulceration in male albino rats	[138]
Apple cider vinegar	2 ml/Kg/day	suppresses the hepatotoxicity and mutagenic activity of nicotine	[139]
<i>Effect on Nervous System</i>			
Grain vinegar	0.1 ml nasal injection of vinegar, acidity: 4.2%	Effective treatment for persistent hiccups.	[140–142]
Oak wood vinegar	Vinegar: 2%	Showed excellent alcohol oxidation by lowering blood alcohol concentration and was also effective in hangover improvement.	[143]
Onion vinegar	Vinegar: 8.8	Improved recovery of focal ischemic brain injured rat, with increased regional cerebral blood flow	[144, 145]
Apple cider vinegar	Vinegar: 0.7% along with Zn with high-fat diet	<ul style="list-style-type: none"> • Higher antioxidant potential • provide a protective effect against Alzheimer's disease like neurological diseases 	[146, 147]
Highbush blueberry (<i>Vaccinium corymbosum</i> L.) vinegar	Vinegar: 120 mg/Kg for 7 days	<ul style="list-style-type: none"> • Facilitated cholinergic activity by inhibiting acetylcholinesterase activity. • Enhanced antioxidant enzyme activity. on Scopolamine-Induced Amnesia Mice Model.	[148]
Vinegar-baked Bupleuri Radix	—	<ul style="list-style-type: none"> • Better antidepressant effects. • Significantly regulated the levels of neurotransmitters in the hippocampus and frontal cortex on a rat model of chronic unpredictable mild stress-induced depression 	[149]
<i>Kurozu or Japanese rice vinegar</i>	Vinegar: 0.25%	Improved cognitive dysfunction in the senescence-accelerated P8 mouse.	[150]
<i>Mulberry vinegar</i>	—	prevents neuroinflammation by regulating the NF-κB signaling pathway and glial activation in C6 glial cells	[151]

Vinegar name	Composition	Major application	Reference
Herbicidal & Pesticidal use			
Coconut shell wood vinegar	Vinegar: water- 1:10	96% of striped mealy bugs <i>Ferrisia virgate</i> were killed.	[152]
	Vinegar: water- 1:50	85% of termite workers, <i>Odontotermes sp.</i> were killed.	
Wood vinegar	Vinegar: 2–5%	A decline in omnivore nematode in a wheat field	[153]
	Vinegar: 1%	Decline in weeds and increased grain yield index.	[154]
Pyrolyzed apple tree branch wood vinegar	—	Broadleaf weed was controlled	[154]
	—	Controlled annual bluegrass	[74]
Wood vinegar <i>Ulmus</i> spp. tree pruning waste	—	Broadleaf weed was controlled	[75]
Wood vinegar (pistachio, cypress, and almond)	—	reduced the mycelial growth of the fungus-like organism <i>Ovatisporangium sp</i>	[155]
Wood vinegar (<i>Toona sinensis</i>)	Vinegar: 2–8%	strong termiticidal activity against <i>Coptotermes curvignathus</i> .	[156]
Extractant use			
Wood vinegar	3:1 (vinegar:water)	used to extract chromium, copper, and arsenic from chromated copper arsenate-treated wood	[144, 145]
	sodium humate: wood vinegar liquid: biochar powder- 1:1:1.5	Enhances the remediation of Pb (II) ions	[157]
‘-’ data not available.			

Table 3.
 A list of different types of vinegar and their specific applications.

5.1 As preservatives

Vinegar is mostly used as a food preservative in many countries. It is used for the pickling of fruits and vegetables and in the preparation of salad dressings, mayonnaise, and other food condiments [158].

5.2 As herbicides and pesticides

To protect crops and plants from weeds and insects different eco-friendly approaches are being taken so that a better yield of crops is achievable and it will not cause damage to the environment. It has been experimentally observed that vinegar especially wood vinegar is a remarkable herbicide when used in a dilute concentration [74, 75, 159, 160]. Wood vinegar contains some benzene derivatives that act as good germicidal and antifungal agents [161–163].

5.3 As extractant

Vinegar is used as an extraction agent of chromium, copper, and arsenic from chromate copper arsenate-treated wood using wood vinegar [144, 145].

5.4 As medicinal

Vinegar finds its place as a good anti-microbial agent, anti-oxidant agent, and in the prevention of various diseases like diabetes, cardiovascular disease, obesity, cholesterol, and cancer. The use of vinegar as a medicine dates back to the time of Hippocrates [129]. It has been shown that fruit vinegar containing 0.1% acetic acid can inhibit the growth of food-borne pathogens invitro.

6. Conclusion

The increasing population has led to an increase in demand for food, which has ultimately resulted in the increased generation of agricultural waste, fruit waste, and food processing waste. These wastes are a burden to the environment and cause pollution. To mitigate these wastes, bioconversion of these C-rich wastes is done. They are fermented or pyrolyzed to produce valuable chemicals, bioactive compounds, and fuels. The main component of vinegar is acetic acid and researchers are targeting to increase its yield by using genetic engineering tools and engineering metabolic pathways of microorganisms that generate acetic acid. Exploring wastes as substrate not only reduces the environmental problem but also solves food security problems leading to a future bioeconomy.


Author details

Debajyoti Saha and Prabir Kumar Das*

Biochemical Engineering Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam, India

*Address all correspondence to: prabir.kumar@iitg.ac.in; prabir.1350@gmail.com

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