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The Chemical Fermentation Process Properties, Bioactive Compounds, and Health Benefits of Fruit Vinegars in Pilot-Scale in Thailand

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Abstract

Vinegar is a liquid product produced from alcoholic and acetous fermentation. Vinegar contains acetic acid and bioactive compounds, which are brewed by liquid-state and solid-state fermentation techniques. This study reviews chemical fermentation process properties, bioactive compounds, and health benefits of fruit vinegars in Thailand. Chemical properties are the alcohol percentage and acetic acid percentage of vinegar. Bioactive compounds include antioxidant activity and total phenolic content, which have the role of antioxidative activity, blood pressure and glucose control, and anti-tumor. However, further studies are needed to find the new fruit raw materials to produce fruit vinegar which have more bioactive compound and more good taste.

Keywords: *Acetobacter pasteurianus*, Antioxidant activity, Fermentation, Fruit vinegar, Wine

Introduction

Nowadays, pollution possesses a high risk in human life. It can be the cause of many diseases, including cancer and hypertension. Therefore, functional food is commonly produced to prevent such diseases. The production of vinegar is generally low in costs because sheap raw materials, like by-products from local fruits and fruit waste, are utilized [1]. Vinegar is rich in nutrients including amino acids, vitamins, sugar, organic acids, polyphenols tetramethylpyrazine, and melanoidins [2]. Recently, the demand for fruit vinegars has increased because it benefits as health food products, which are used to promote different kinds of beneficial effects to humans, such as having antibacterial, antidiabetic effects and lowering cholesterol levels by inhibiting oxidation of low-density lipoproteins (LDLs). Thailand is considered one of the most abundant resources of tropical fruits in the world. The main fruit season in Thailand runs from April to July. There are many fruits in Thailand, e.g., mango, pummelo, and pineapple. The diversity of raw materials to produce vinegar is appealing because of the different nutrients, bioactive compounds, and cultivars. In China, the term vinegar is used to indicate both fermented and artificial vinegar, according to the Chinese National Standard definitions which classified vinegar in 3 grades, depending on the concentration of acetic acid (3.5 - 4.5, 4.5 - 6.0, and > 6.0 %) [1].

Vinegar production

Vinegar is produced from fruits containing sugar by a two-step fermentation, which consists of an alcoholic and acidic fermentation process. The first step is alcoholic fermentation during which hydrolyzed sugar is converted to ethanol and CO_2 under anaerobic conditions by yeasts. The second step is acetic acid fermentation, in which the alcohol content in the first step is converted to acetic acid and water under aerobic conditions by acetic acid bacteria [3]. Vinegar content varies depending on factors, such as the raw material type and the production method [4]. There have been some studies conducted on the functional and chemical properties and possible use of tamarind, cantaloupe, mango, berry, pineapple, banana, and pumelo [5-12].

Varieties of vinegar

Vinegar is produced from sources that contain sugar such as fruits, grains, and honey. Different raw materials contribute to the different physiochemical properties of vinegar products. According to [1], vinegar varieties vary from country to country, some of the vinegars around the world are shown below (**Table 1**).

Table 1 Type of vinegar around the world.

Category	Raw material	Vinegar name	Geographical distribution
Vegetable	Rice	Komesu, Kurosu, Heicu	East and Southeast Asia
	Malt	Malt vinegar	Northern Europe, USA
	Barley	Beer vinegar	Germany, Austria, Netherlands
	Wheat	Black vinegar	China, East Asia
	Sorghum	Black vinegar	China, East Asia
	Tea and sugar	Kombucha vinegar	Russia, China, Japan, Indonesia
	Sugarcane	Cane vinegar	France, USA
Fruit	Apple	Cider vinegar	USA, Canada
	Grape		
	Cooked must	Balsamic vinegar	Italy
	Red or white wine	Wine vinegar	Widespread
	Mango	Mango vinegar	East and Southeast Asia
	Mulberry	Mulberry vinegar	East and Southeast Asia
	Banana	Banana vinegar	Southeast Asia
	Coconut	Coconut water vinegar	Philippines, Sri Lanka
	Plum	Ume-su	Japan
	Kaki	Persimmon vinegar	South Korea
Animal	Whey	Whey vinegar	Europe
	Honey	Honey vinegar	Europe, America, Africa

Microorganism for vinegar production

There are 2 fermentation processes to produce vinegar, which are alcoholic and acetous fermentation. Alcoholic fermentation usually depletes most sugars within the first weeks. Sugars are converted into ethanol by the action of yeasts (*Saccharomyces cerevisiae*) under anaerobic conditions, while in acetous fermentation, the acetic acid bacteria are the genus *Acetobacter* and can oxidize ethanol into acetic acid under aerobic conditions.

Yeast is a fungus in a vegetative state that is predominantly reproduced by budding or fission which results in growth that comprises mainly single cells. Yeasts are organisms with a respiratory metabolism that can grow in the presence of oxygen. Many types of yeasts in the absence of oxygen show a fermentative metabolism when metabolizing monosaccharides with 6 atoms of carbon like glucose, fructose, and mannose that were metabolized into 2 molecules of pyruvate in the glycolysis, called glycolysis pathway (Embden-Meyerhof glycolytic pathway. The pyruvate was reduced to ethanol (C_2H_5OH) and carbon dioxide by the enzyme pyruvate decarboxylase and alcohol dehydrogenase [13]. The overall chemical reaction is presented as:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2} + 56 \text{ Kcal/mol}$$

Table 2 shows the most important minor products of fermentation produced by *Saccharomyces cerevisiae* [1].

Table 2 Minor products of alcoholic fermentation produced by *Saccharomyces cerevisiae*, about 100 vol of ethanol produced.

Compound	Amount	Origin
Glycerol	4 - 7 g	Sugar
Succinic acid	0.30 - 0.6 g	Sugar
Acetic acid	0.1 - 1 g	Sugar
Isoamylic alcohol	100 - 300 mg	Sugar and amino acid
Phenylethyl alcohol	10 - 100 mg	Sugar and amino acid

Acetic Acid Bacteria (AAB) are Gram-negative with ellipsoidal to rod-shaped morphologies, being motile due to the presence of flagella. AAB are mesophilic obligate aerobes that can oxidize sugar, sugar alcohol, and ethanol to allow the production of acetic acid. AAB are used as the starter cultures for vinegar production such as *Acetobacter pasteurianus* and *A. polyoxogenes*. In acetous fermentation, the biochemical mechanism for the conversion of alcohol into acetic acid start with the oxidation of alcohol into acetaldehyde in the presence of alcohol dehydrogenase. These dehydrogenase enzymes consist of quinoproteins and flavoproteins, which have pyrroloquinoline quinone and covalently linked flavin adenine dinucleotide as prosthetic groups, respectively. This membrane-bound ADH has pyrroloquinoline as a cofactor and is independent of NAD(P)+, although a cytoplasmic NAD(P)+-dependent alcohol dehydrogenase has also been identified. The latter, however, has a much lower specific activity than the membrane-bound ADH and a higher optimal pH of 6 to 8, which limits its contribution to the oxidation process of ethanol. The ALDH is also located in the cytoplasmic membrane, which is independent of NAD(P)+ and has an optimum pH of between 4 and 5. It is, also, to catalyze the oxidation of acetaldehyde to acetate at lower pH values. Acetobacter strains can further oxidize acetic acid to CO_2 and water through the tricarboxylic acid cycle, called over oxidation [11]. This occurs in the case of ethanol depletion, and seems to be an irreversible change in their metabolism [14]. The overall chemical reaction is presented as:

$$CH_3CH_2OH \xrightarrow{\text{ADH}} CH_3CHO + 2H \xrightarrow{\text{ALDH}} CH_3COOH + 2H$$

The first reaction is catalyzed by alcohol dehydrogenase (ADH) and the second by aldehyde dehydrogenase (ALDH), which is located at the outer surface of the cytoplasmic membrane.

Raw Material for fruit vinegar production and their phytochemicals

Thailand is located in the tropical zones and its climate is suitable for growing fruit, so a wide variety of fruits are available in all seasons; therefore, Thailand has a numerous of fruits which could produce vinegar, e.g., Tamarind, Cantaloupe, Mango, Mulberry, Pineapple, Banana, and Pomelo. Many fruit species can be used for vinegar production since they have 2 basic attributes (1) to be safe for human consumption (2) to be a direct or indirect source of fermentable sugars.

Tamarind (*Tamarindus indica*)

The dried tamarind pulp of commerce contains 8 - 18 % tartaric acid (2, 3-dihydroxy butanedioic acid-$C_4H_6O_6$, a dihydroxy carboxylic acid) and 25 - 45 % reducing sugars, of which 70 % is glucose and 30 % fructose. Tamarind pulp is rich in minerals such as potassium (62 - 570 mg/100 g), phosphorus (86 -

190 mg/100 g), and calcium (81 - 466 mg/100 g), and iron (1.3 - 10.9 mg/100 g) magnesium content is high (25.6 - 30.2 mg/100 g), as is sodium (23.8 - 28.9 mg/100 g), whereas copper (0.8 - 1.2 mg/100 g) and zinc (0.8 - 0.9 mg/100 g) are low. It also excels in riboflavin and is a good source of thiamin and niacin, but is poor in vitamin A and vitamin C [15].

Cantaloupe (*Cucumis melo*)

Cantaloupes are a low-calorie and low sodium refreshing fruit, enjoyed by people of all ages. Cantaloupe is high in potassium (267 mg/100g), beta-carotenes (2.02 mg/100g), vitamin A content (3,382 IU/100g), vitamin C content (36.7 mg/100g), vitamin K content (2.5 µg/100g) and sugar content (7.86 g/100g) [16].

Mango (*Mangifera indica* L.)

Mango is rich in bioactive compounds, such as proteins (0.36 - 0.40 g/100 g fresh weight) of pulp, vitamin A (0.135 - 1.872 mg/100 g fresh weight pulp), vitamin C (7.8 - 172 mg/100 g fresh weight of pulp), carotenoids (0.78 - 29.34 µg/g fresh weight of pulp), phenolic compounds, dietary fiber (DF), carbohydrates, minerals, and other antioxidants [17].

Mulberry (*Morus indica*)

Mulberry is popularly consumed in the human diet in fresh and processed various forms, such as beverages, yogurts, and jams. The major groups of phenolic compounds present in berries are anthocyanins and phenolic acid. Mulberry contains cyaniding-3-glucoside and cyaniding-3-rutinoside [18].

Pineapple (*Ananas cosmosus*)

Pineapple contains a considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water, and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It contains 10 - 25 mg of the vitamin. Pineapple juice contains ascorbic acid and is a good source of Vitamin C. Ascorbic acid or vitamin C fights bacterial and viral infections which is an effective antioxidant. Several essential minerals exist in pineapples, including manganese, a trace mineral instrumental to the formation of bone. Pineapples minerals also include copper, another trace mineral. It assists in the absorption of iron and regulates blood pressure and heart rate [19].

Banana (*Musa* spp.)

Banana pulp contains various antioxidants, e.g., vitamins, carotenoids, and phenolic compounds such as catechin, epicatechin, lignin and tannins, and anthocyanin. Awak pulp contains total phenolic content of about 0.36 mg of gallic acid equivalent/g fresh weight. Pisang mas contain total phenolic content of about 27 mg of gallic acid equivalent/100g fresh weight, the flavonoid content about 13.7 mg catechin equivalents/100g fresh weight. The antioxidant activities of the banana fruit were 0.59 µmol Fe (Π)/g fresh weight and a 36.8 % DPPH inhibition [20].

Pomelo (*Citrus grandis*)

Pomelo, also known as pummelo, shaddock or Chinese grapefruit, is commonly classified as common (or white) or pigmented (or pink). Pomelo contain total sugar in pink type (76.45 mg/mL) white type (77.96 mg/mL). Ascorbic acid in pink type (0.22 mg/mL) white type (0.39 mg/mL). Citric acid in pink type (14.15 mg/mL) white type (0.14 mg/mL). Pomelo contain malic acid in pink type (0.75 mg/mL) white type (1.94 mg/mL), succinic acid in pink type (0.05 mg/mL) and white type (0.39 mg/mL) [21].

Methods of fruit vinegar production [7]

The fruits were used for the production of vinegars via a 2-stage (alcoholic and acetous) fermentation process. The fruits were crushed and mixed with water at a ratio of 1:1 to prepare the juice. After adjustment of the pH to 4.0 - 4.5 and sugar content up to 20 - 25 ºBrix, the juice was pasteurized for 30 min at 60 ºC. Alcoholic fermentation was conducted for 5 - 7 days at room temperature under static conditions in plastic vessels containing 3 L of the juice inoculated with *Saccharomyces cerevisiae* at a ratio of 0.75 % (v/v). Preparation of yeast inoculum was carried out by mixing 5 g of yeast powder with 60 mL of warm water. At the end of the fermentation process, the obtained wine was separated from the sediment by allowing it to settle in glass bottles, followed by pasteurization for 30 min at 60 ºC and clarification for 45 days at 10 ºC. Prior to the acetous fermentation, the alcohol content of the obtained wine was adjusted to 4 - 10. Acetous fermentation was performed for 15 days under the aforementioned conditions in glass vessels containing 135 mL of the wine inoculated with *Acetobacter pasteurianus* TISTR 521 at a ratio of 10 % (v/v). Sampling was performed at given time points to collect the 2-stage fermented vinegars by allowing them to settle in microtube and storage at 4 ºC in microtubes before the analyses (**Figure 1**).

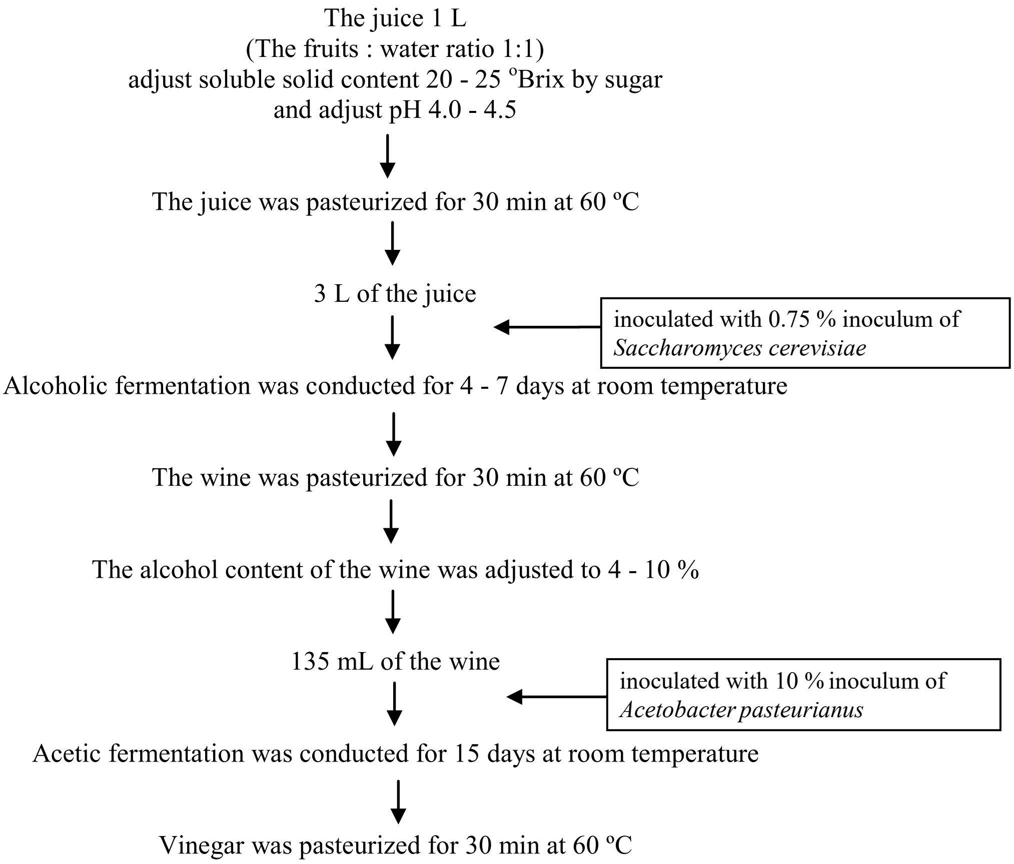

Figure 1 Diagram of fruit vinegar production.

Change of alcohol and acetic acid content using alcoholic and acetous fermentation process of fruits vinegar

A study by [7] reported that after 8 days of alcoholic fermentation, the total soluble solid content from 22 ^{o}Brix decreased due to its consumption by yeast. The Namdokmai cultivar has the highest alcohol percentage (14.82 ± 0.11 %v/v). In 15 days of acetous fermentation, the initial concentrations of alcohol content was 7 %v/v, the final acetic acid was produced from its culivar was 4.88 %v/v.

A study by [8] reported that the mulberry wine was produced for 7 days, the total soluble solid content begins at 22 ^{o}Brix. Finally, its wine has an alcohol percentage (11.37 ± 0.03 v/v). In 15 days of acetous fermentation, the initial concentrations of alcohol content were 7 %v/v, the final acetic acid was produced from its vinegar was 3.96 %v/v.

Similarly reported by [22] the data showed about the production of the red plum vinegar, start with the alcohol content was 6.49 ± 0.34 %v/v. In 15 days of acetous fermentation, the acetic acid was 4.04 ± 0.19 %v/v (**Tables 3** and **4**).

Table 3 Percentage of alcohol content in wine.

Raw material	Cultivar	Detection method	Initial total soluble solid	%(v/v) Alcohol content	Period (Day)	Reference
Tamarind	Si Thong	GC-FID	22	9.33	7	[5]
	Pra Kai Thong			8.40		
	Si Chom Phu			9.34		
	Yak Fak Dab			9.38		
Cantaloupe	Aka	HPLC	20	14.20 ± 0.59	7	[6]
	Amy			14.34 ± 0.44		
	Honeyworld			14.75 ± 0.55		
	Kimoji			12.27 ± 0.84		
Mango	Namdokmai	HPLC	22	14.82 ± 0.11	8	[7]
	Kalon			9.14 ± 1.41		
	Keaw			12.01 ± 0.18		
	Chokanan			9.31 ± 0.05		
	Mahacharnok			10.56 ± 0.05		
Berry	Mulberry	HPLC	22	11.37 ± 0.03	7	[8]
	Cranberry			5.72 ± 0.08		
	Raspberry			11.49 ± 0.04		
	Blackberry			11.73 ± 0.01		
Pineapple	Homsuwan	GC-FID	20	12.55 ± 0.61	5	[9]
	Huamui			11.32 ± 1.83		
	Puttavia			11.41 ± 0.68		
	Tradsrithong			12.44 ± 0.68		
Banana	Khai Pra Tabong	HPLC	25	6.66 ± 0.00	5	[10]
	Nak			9.54 ± 0.01		
	Hin			6.92 ± 0.00		
	Phama Heak Kuk			8.28 ± 0.00		
	Gros Michel		18	12.04 ± 0.02	3	[11]
Pumelo	Thong Dee	GC-FID	22	13.25 ± 0.45	5	[12]
	Takoy			13.52 ± 0.21		
	Tubtim Siam			15.24 ± 0.50		
Red plum	Ju-li	HPLC	24	6.49 ± 0.34	5	[22]

Table 4 Percentage of acetic acid content in vinegar.

Raw material	Cultivar	Detection method	Initial %alcohol content	% (v/v) Acetic acid content	Reference
Tamarind	Si Thong	Titratable acidity	8	5.18 ± 0.63	[23]
	Pra Kai Thong			5.40 ± 0.16	
	Si Chom Phu			4.65 ± 0.71	
	Yak Fak Dab			5.34 ± 0.06	
Cantaloupe	Aka	HPLC	7	6.73 ± 0.14	[6]
	Amy			7.31 ± 0.07	
	Honeyworld			6.31 ± 0.04	
	Kimoji			6.57 ± 0.09	
Mango	Namdokmai	HPLC	7	4.88 ± 0.00	[7]
	Kalon			6.96 ± 0.08	
	Keaw			5.47 ± 0.26	
	Chokanan			5.72 ± 0.04	
	Mahacharnok			4.90 ± 0.01	
Berry	Mulberry	HPLC	7	3.96 ± 0.00	[8]
	Cranberry			5.01 ± 0.01	
	Raspberry			4.74 ± 0.00	
	Blackberry			4.72 ± 0.04	
Pineapple	Homsuwan	Titratable acidity	10	2.74 ± 0.28	[9]
	Huamui			2.82 ± 0.26	
	Puttavia			2.72 ± 0.52	
	Tradsrithong			3.09 ± 0.04	
Banana	Khai Pra Tabong	HPLC	6	2.27 ± 0.00	[10]
	Nak			3.49 ± 0.00	
	Hin			3.23 ± 0.00	
	Phama Heak Kuk			3.24 ± 0.00	
	Gros Michel		8	5.13 ± 0.05	[11]
Pumelo	Thong Dee	Titratable acidity	4	5.46 ± 0.25	[12]
	Takoy			4.83 ± 0.70	
	Tubtim Siam			5.16 ± 0.17	
Red plum	Ju-li	HPLC	6.49	4.04 ± 0.19	[22]

Bioactive compounds

The bioactive compounds in vinegars consist of organic acids, polyphenols, melanoidins, and tetramethylparazine. Bioactive compounds can act as antioxidants, enzyme inhibitors, and inhibitors of gene expression.

Acetic acids

Acetic acids in vinegar come from fermentative production. Acetic acid was the main organic acid in vinegars, which occurred during the step of alcohol and acetous fermentation. Acetic acid can penetrate the cell membrane of microorganisms and cause bacterial cell death which was the most effective organic acid against *Escherichia coli* 0157:H7 [2,24].

Polyphenols

The phenolic compound in vinegar exhibit a high antioxidant activity which is capable of reducing oxidative stress in the body, blood pressure control, prevention of cardiovascular diseases [2]. Several studies that the raw materials of fruit vinegars contain polyphenols. It is important to determine the contents of polyphenols, as they play an important role in antioxidant activity. It is worth mentioning that [7] reported that mango vinegar which was produced from Mahacharnok cultivar had the highest antioxidant activity is showed in **Table 5** (91.00 ± 2.37 %). In a previous study, [25] founded the volatile compositions in mango vinegar which had antioxidant activity, such as Furaneol, Methionol, and Acetoin. The pineapple vinegar produced from Homsuwan cultivar had the highest total phenolic content (332.34 ± 4.87 mg/mL) [9]. According to [26] revealed that L-lysine, mullein, and gallic acid were significantly more concentrated in the pineapple vinegar.

Table 5 Antioxidant activity and total phenolic content in vinegar

Raw material	Cultivar	%Inhibition	TPC (Mg/L)	Reference
Tamarind	Si Thong	32.57 ± 0.41	n.d.	[23]
	Pra Kai Thong	11.86 ± 0.11		
	Si Chom Phu	20.47 ± 0.64		
	Yak Fak Dab	47.47 ± 0.25		
Cantaloupe	Aka	53.78 ± 0.75	n.d.	[6]
	Amy	44.47 ± 1.10		
	Honeyworld	21.26 ± 4.87		
	Kimoji	9.00 ± 0.30		
Mango	Namdokmai	88.30 ± 5.32	71.46 ± 0.19	[7]
	Kalon	68.51 ± 2.66	82.47 ± 0.29	
	Keaw	65.73 ± 3.09	65.72 ± 2.58	
	Chokanan	86.37 ± 4.60	117.81 ± 2.48	
	Mahacharnok	91.00 ± 2.37	83.08 ± 0.57	
Berry	Mulberry	25.25 ± 2.76	190.11 ± 22.05	[8]
	Cranberry	30.89 ± 7.46	250.02 ± 24.19	
	Raspberry	74.43 ± 0.74	181.37 ± 13.51	
	Blackberry	72.62 ± 4.72	167.50 ± 2.20	
Pineapple	Homsuwan	67.54 ± 0.74	332.34 ± 4.87	[9]
	Huamui	84.62 ± 1.74	172.34 ± 0.29	
	Puttavia	89.82 ± 0.83	140.24 ± 0.00	
	Tradsrithong	78.60 ± 0.33	128.96 ± 0.10	
Banana	Khai Pra Tabong	47.44 ± 5.03	243.98 ± 3.35	[10]
	Nak	68.04 ± 1.49	102.23 ± 0.69	
	Hin	69.30 ± 8.09	198.26 ± 1.61	
	Phama Heak Kuk	80.59 ± 2.30	115.92 ± 9.40	
	Gros Michel	73.2 ± 0.11	68.01 ± 0.21	[11]
Pumelo	Thong Dee	57.75 ± 3.75	n.d.	[12]
	Takoy	33.65 ± 4.04		
	Tubtim Siam	55.31 ± 0.01		

n.d. = not detected

Health benefits of vinegar

According to [24], bioactive compounds affect physiological or cellular activities, resulting in beneficial health effects. Bioactive compounds are claimed to have the ability to modify the risk of disease rather than prevent diseases. In the previous studies [27], tomato vinegar has powerful anti-visceral obesity properties in HFD-induced obese rats. The intra-abdominal deposition of visceral adipose tissue is known as a general type of obesity that is associated with conditions such as type 2 diabetes mellitus, hyperlipidemia, hypertension, and coronary heart disease. They found that consuming tomato vinegar regularly can reduce the total visceral fat and the epididymal adipocyte size. According to [28] study examined the effects of vinegar on markers of type 2 diabetes in at-risk adults. The participants (n = 14) were ingested by 750 mg acetic acid as a vinegar drink or a control pill (40 mg acetic acid) twice daily at mealtime. Blood glucose (fasting and 2 h postprandial) was recorded daily. Fasting blood collected at weeks 0 and 12 was analyzed for insulin and glycated hemoglobin. The average change in fasting glucose was reduced in the vinegar group versus control group (0.91 ± 0.27 versus 0.26 ± 0.17 mmol/l) ($p = 0.05$). Ok *et al*. [29] have reported that supplementation of acetic acid and pomegranate vinegar (PV) contributed to lowering both plasma and hepatic triglyceride levels Interestingly, in their study the effectiveness of PV in reducing plasma triglyceride was favored more at a low dose when compared to a high dose. This could be due to various properties of diverse chemical compounds present in PV. Several studies have showed vinegar has a potential to ameliorate obesity, diabetes, cardiovascular disorders, cancer and microbial infections. Daily intake of a drink containing 15 mL vinegar (750 mg of acetic acid) was reported to improve lifestyle-related diseases, such as hypertension, hyperlipidemia, and obesity [30]

Conclusions

Vinegar is a well-known fermented food produced by different raw materials. This paper has summarized the chemical fermentation process properties, bioactive compounds, and health benefits of fruit vinegars in pilot-scale. Thailand has a lot of fruits that can produce vinegars, including Mango, Mulberry, Pineapple, Banana, and Red Plum. The findings of this study are expected to shed light on the effort for improving people's wellness through vinegar consumption.

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