# DETERMINATION OF SOME CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF KIWI VINEGAR PRODUCED UNDER DIFFERENT CONDITIONS

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## ABSTRACT

Vinegar, known as a unique product produced from plant-derived raw materials by two-stage alcohol and acetic acid fermentation, is produced in various countries mainly to add flavor to foods and sauces. Fruit peel, which is an important waste product in the fruit processing industry, has at least as much phenolic content as the fruit. Therefore, in this study, kiwi peels were used as raw materials for vinegar production, as well as kiwi fruit. Fermentation was carried out under aerobic and anaerobic conditions, and the total acidity, pH, total phenolic substance and microbiological properties of the produced vinegar were determined. During the fermentation process, the pH in kiwi vinegar decreased from 3.5 to 2.4 on average. Total acidity was determined as 2.3-6.3 g/100 ml after 3 weeks. It has been determined that both vinegars made from fruit and peel have high phenolic content (3.91.26-431.93 mg GAE/L). As the fermentation progressed, a decrease in the number of *Escherichia coli* and mold was observed with the increase in the total acid content.

***Keywords***: *Fermentation, Kiwi, Vinegar, Asetic acid, Phenolic compound*

## INTRODUCTION

Kiwi is an edible fruit belonging to the genus *Actinidia* **[1].** Kiwifruit has many health benefits such as anti-diabetic **[2]**, anti-inflammatory **[3]**, cardiovascular protective **[4]**, antimicrobial and laxative activity **[5, 6]**. Another important feature of kiwi is that it contains a high amount of vitamin C. The amount of vitamin C in some kiwi varieties is as high as 420 mg/100 g **[7, 8]**. In addition to being consumed directly as a fruit, kiwi can also be used into many food products such as fruit juice, vinegar, jam, wine, jelly **[6]**.

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According to the Turkish Food Codex; vinegar, is defined as a food product obtained by subjecting sugary fruits such as grapes, apples and figs to alcohol fermentation and then acetic acid fermentation **[9]**. According to the TSE 1880 vinegar standard, it is defined as "a unique product produced biologically from agricultural liquids or other substances by two-stage alcohol and acetic acid fermentation" **[10]**. Depending on the vinegar raw material; It can be classified as "grain vinegar" obtained from sorghum, rice, wheat or other grains, and "fruit vinegar" obtained from fermented grape or apple juices **[11]**.

In addition to being used as a flavoring in the food industry, vinegar has been added to foods and sauces as an antimicrobial or used as a disinfectant since ancient times. However, due to the belief in its beneficial effects on health, it has started to be accepted as a potential functional foodstuff in recent years **[12]**.

Fruits and grains are generally used as raw materials in the production of traditionally consumed vinegar **[13]**. Water constitutes about 80% of vinegar, while the remaining 20% is composed of organic acids, alcohols, polyphenols and amino acids **[14]**. As a result of the fermentation process, functional compounds such as organic acids, which are not found in raw fruits or are present in trace amounts, are released that increase the antioxidant capacity of the human diet **[13, 15, 16].**

Vinegar is made by converting sugars to alcohol by yeast and converting the alcohol to acetic acid by bacteria **[12]**. In other words, when producing vinegar from sugary fruits, two completely different fermentations take place. These are alcohol fermentation and acetic acid fermentation, respectively. First, the sugar in the fruit or must is converted into alcohol. This process is carried out by yeasts. The resulting alcohol is then converted to acetic acid by the vinegar bacteria. Before the acetic acid fermentation starts, the alcohol fermentation should be completely finished, that is, there should be no sugar left in the environment **[17].**

Vinegar is the product produced by the oxidation of ethanol to acetic acid under aerobic conditions by acetic acid bacteria, following the ethanol

fermentation of fermentable sugars by yeasts under anaerobic conditions **[18]**. The group of Gram-negative bacteria capable of oxidising ethanol to acetic acid is called acetic acid bacteria (AAB) which includes nineteen genera **[19]**. AAB belong to the *Acetobacteraceae* family are Gram-negative or Gram-variable, non-spore forming, ellipsoidal to rod-shaped cells that can occur in single, pairs or in short chains **[20, 21]**. The main species responsible for the production of vinegar belong to the genera *Acetobacter*, *Gluconacetobacter*, *Gluconobacter* and *Komagataeibacter*. These bacteria have high capacity to oxidise ethanol to acetic acid and high resistance to acetic acid released into the fermentative medium **[19, 22]**.

Vinegar production methods can be grouped under 3 main headings as slow method (traditional method, orleans method, also known as French or pasteur method), quick method (German method), deep culture method (submerged process) **[23]**.

Vinegar is produced very slowly with slow method. But the quality of the vinegar produced is quite high. The fermentation occurres at 28-30°C. Since the density of acetic acid produced is higher than alcohol, it accumulates at the bottom of the container. Vinegar mother, which is an important formation, is a gelatinous structure and on the surface of vinegar.

Ma et al., **[6]** reported that products obtained by kiwi fermentation such as vinegar and wine contain more nutrients than products such as fruit juice. It has been stated that the nutrients in the kiwi dissolve better with fermentation. In addition, kiwi fruit and its products contain high amounts of phenolic substances and therefore are a high source of antioxidants **[24, 25].**

In this study, slow method was used for vinegar production. The aim of this study was to produce vinegar in aerobic and anaerobic conditions by using the kiwi fruit and its peels, which contain high levels of nutrients and active ingredients, and to determine some physical, chemical and microbiological properties of the produced vinegars.

## MATERIAL AND METHODS

### Sample and Sample Preparation

Kiwi fruits were purchased from a local market. After washing, the fruits were separated from their skins and diced and collected in three different 500mL jars that had been sterilized in an autoclave (15 min at 121°C). One of the jars containing the kiwi fruits was covered with thin cheesecloth (aerobic, K1), the second jar was closed with parafilm (K2), and the third jar was sealed with a lid (anaerobic, K3). Kiwi peels were collected in a separate jar and covered with cheese cloth (KK). The jars were filled with water with the head space at the top after adding the fruit and shells, closed as described above and left to ferment in the incubator at 22°C. All jars except the jar closed with the lid were mixed every 2 days. Thus, in the study, 4 different kiwi samples were studied. Samples were taken from the vinegars at 7, 15 and 21 days of fermentation and analyzed. The samples were prepared on the day the vinegars were made.

### Determination of Total Acidity

Determination of total acidity in kiwi vinegar samples was carried out with the help of titrimetric method **[26]**. A 20 mL sample was taken from the vinegar sample and made up to 100 mL with distilled water. Then, 20 mL of the mixture was taken into a flask and 1-2 drops of phenolphthalein indicator was added to it. The solution was titrated with 0.1 N NaOH until the pH was 8.1. Total acidity in kiwi vinegars was calculated as % acetic acid.

### pH Measurement

A sample of kiwi vinegar was taken into the beaker and pH was measured at room temperature using a pH meter (Mettler Toledo S220) probe. Before each measurement, the pH meter was calibrated with calibration solutions.

### Total Phenolic Content

Determination of the total phenolic content was performed spectrophotometrically according to the Folin-Ciocalteu method **[27]**. Before analysis,

2 mL of Folin Ciocalteu reagent (diluted with water at a ratio of 0.5:5) and 1.6 mL of 20 g/100 mL sodium carbonate were added to 4 mL diluted vinegar samples that were filtered through a cellulose acetate membrane filter. Samples were kept in the dark for 90 minutes. 4 mL of water was used as control samples. UV/Vis spectrophotometer (PG INSTRUMENTS-T60+) absorbance measurements were performed at 765 nm. Gallic acid solutions prepared at different standard concentrations (100-2000 mg/L) were used in the calibration curve. The results are expressed as mg gallic acid equivalent (mg GAE/L vinegar).

**Microbiological Analysis**

Samples for microbiological analysis were taken in 7, 14 and 21 days. To determine the microbiological properties of kiwi vinegar, 25 mL of vinegar sample was taken into a sterile stomacher bag and diluted 1:10 with peptone water (PW, 0.1%, pH 6.3 ± 0.2) under aseptic conditions. The number of microorganisms in 1 mL of vinegar was determined. Enumeration of mesophilic aerobic bacteria in vinegar samples were performed on Plate Count Agar (PCA, casein peptone 5.0 g/L, yeast 2.5 g/L, D glucose 1.0 g/L, agar-agar 14.0 g/L); total coliform on Chromocult Coliform Agar (peptone 3.0 g/L, NaCl 5.0 g/L, $NaH_2PO_4$ 2.2 g/L, $Na_2HPO_4$ 2.7 g/L, sodium pyruvate 1.0 g/L, tryptophane 1, 0 g/L, sorbitol 1.0 g/L, tergitol-7 0.15 g/L, chromogenic mixture 0.4 g/L, agar-agar 10.0 g/L), acetic acid bacteria (AAB), HS in Hestrin-Schramm medium, (2% D-glucose, 0.5% peptone, 0.5% yeast extract, 0.27% $Na_2$ $HPO_4$ , 0.115% citric acid, cycloheximide 50 mg/L for prevention of moulds, pH 5.0), ($CaCO_3$)-ethanol medium (% 0.05 D-glucose, 0.3% peptone, 0.5% yeast extract, 1.5% $CaCO_3$, 1.2% agar, 1.5% ethanol); lactic acid bacteria (LAB) Man Rogosa and Sharp Agar (MRS agar, pH 6.2 ± 0.2, Merck) and yeast-mold counts were performed on Yeast Extract Glucose Chloramphenicol Agar (YGC, pH 6.6±0.2, Merck). The media were incubated at the following temperature and time: PCA at 37°C for 48h; MRS at 37°C for 48h; YGC agar at 25°C for 72h. For the determination of acetic acid bacteria, 0.1 mL sample were added to HS agar, previously incubated at 30°C for 3 days in HS medium, incubated at 30°C for 3 days . Cream-beige colored, smooth-edged, sticky colonies with a diameter of 2.5-3 mm were selected and inoculated in $CaCO_3$-ethanol medium at 30°C for 3 days. Colonies that dissolved $COCO_3$ and became transparent were considered as acetic acid bacteria.

## RESULTS AND DISCUSSION

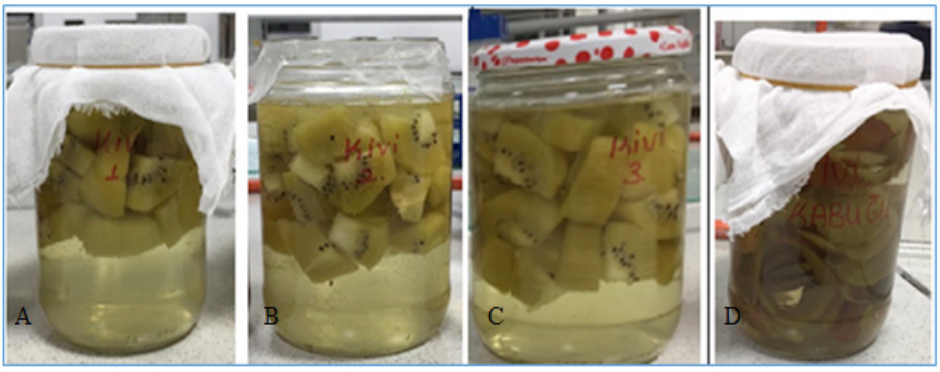
### Determination of Total Acidity

Change in total acidity values (acetic acid g/100 mL) in samples taken in 1, 7, 14, and 21 days in kiwi fruit vinegars produced is shown in Table 1.

**Table 1.** Total acidity values of kiwi vinegar samples

| Kiwi samples | Total acidity (g/100ml) | | |
|---|---|---|---|
| | Days | | |
| | 7 | 14 | 21 |
| K1 | 2.1 | 3.2 | -ND |
| K2 | 2.1 | 4.2 | 6.3 |
| K3 | 2.1 | -ND | 2.3 |
| KK | 2.1 | 4.2 | 5.3 |

K1: Vinegar covered with thin cheesecloth (aerobic), K2: Vinegar covered with parafilm, K3: Vinegar covered with an airtight lid (anaerobic). KK: Vinegar from kiwi peels covered with cheesecloth (aerobic), ND: not detected




F**igure 1.** Kiwi vinegars prepared in different conditions. A) Vinegar covered with thin cheesecloth (aerobic, K1), B) Vinegar covered with parafilm (K2), C) Vinegar covered with an airtight lid (anaerobic, K3). D) Vinegar from kiwi peels covered with cheesecloth (aerobic, KK).

It is expected that the amount of acid produced in vinegar during the fermentation process will increase depending on time. It is seen that the total acidity values of kiwi vinegars prepared under different conditions increased during the 21-day fermentation period (Table 1). This is due to the growth of microorganisms that produce organic acid, mainly acetic acid **[28]**. Yeasts convert fermentable sugars to ethanol under anaerobic condi-

tions. Acetic acid is produced by using this ethanol by acetic acid bacteria under aerobic conditions. Acetic acid bacteria not only produce acetic acid, but also improve the flavor of vinegar **[29]**. The kiwi vinegars prepared under different conditions are shown in Figure 1. The least increase in acidity occurred in vinegar with the lid completely closed (anaerobic condition), and the total acidity at the end of 21 days was determined as 2.7 g/100 ml in this vinegar. The acidity level of kiwi vinegar may vary depending on the production method. As a matter of fact, in another study, the amount of acetic acid in kiwi vinegar prepared under different ambient conditions was found to be 6.28 g/100 mL **[30]**.

## pH Measurement of Vinegar

pH values in samples taken in 1, 7, 14 and 21 days in kiwi fruit vinegars produced is shown in Figure 2. The pH in kiwi vinegars decreased from 3.5 to 2.4 on average. As a result of microbial activity, the amount of acetic acid in the vinegar increases and therefore the pH of the vinegar decreases. These results showed that the amount of acid in the vinegar increased and the production of vinegar continued in a positive way. In a study, the average pH level of kiwi vinegar produced was found to be 3.41 **[31].** It was determined that the pH values measured in the study of kiwi vinegars were compatible with the literature.

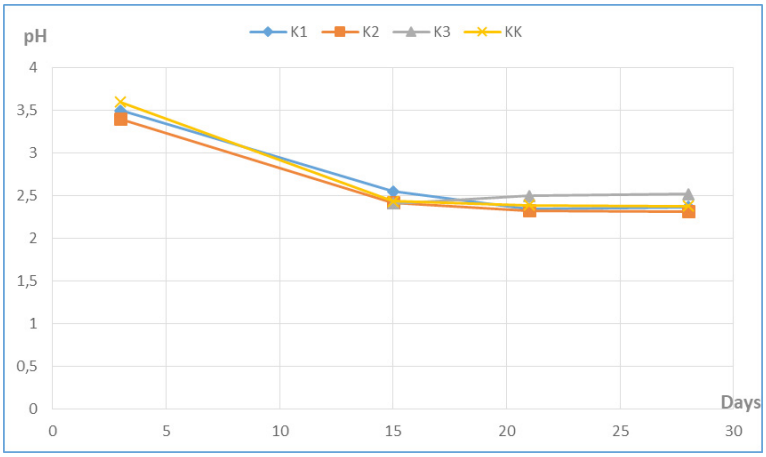



**Figure 2.** The pH of kiwi vinegars during fermentation

## Total Phenolic Content of Vinegar

It was determined that the total phenolic content of the vinegars decreased during the 21-day fermentation period. For example, the amount of phenolic content in the K1 sample, which was 489.6 (mgGAE/L) at the beginning, decreased to 426.37 (mgGAE/L) after 21 days. It was determined that the vinegar with the highest phenolic content among the vinegar samples was K1 coded kiwi vinegar (426.37 mgGAE/kg) covered with cheesecloth. Fruits, vegetables and the foods produced from them are important for human health due to their rich polyphenolic content **[30]**.

**Table 2.** Total phenolic content of kiwi vineagars

| Kiwi samples | Total phenolic content (mgGAE/L) | | |
|---|---|---|---|
| | Days | | |
| | 7 | 14 | 21 |
| K1 | 489.60 | 479.93 | 426.37 |
| K2 | 417.26 | 425.26 | 391.26 |
| K3 | 413.04 | 498.71 | 393.37 |
| KK | 439.93 | 446.93 | 431.93 |

K1: Vinegar covered with thin cheesecloth (aerobic), K2: Vinegar covered with parafilm, K3: Vinegar covered with an airtight lid (anaerobic). KK: Vinegar from kiwi peels covered with cheesecloth (aerobic),
GAE: gallic acid equivalent

Kiwi is also known to be a good source of polyphenols **[31].** In one study **[6],** more phenolic compounds were found in kiwi vinegar and kiwi wine compared to kiwi juice. The reason for this is that the vinegar is in contact with the fruit juice for a longer time in the fermentation process and acetic acid bacteria metabolize the polyphenols and help dissolve the nutrients **[6].**

In another study, total phenolic content was measured in kiwi, apple and palm vinegars and the highest phenolic content (754.50mgGAE/L) was found in kiwi vinegar **[33]**. It has been reported that the phenolic content of vinegars is affected by the product used and the production method **[32]**. In another study, the total phenolic content of kiwifruit ranged from 58.45 to 152 mg GAE/100 g FW **[31]**. In another study, the total phenolic com-

ponent contents of different Actinidia cultivars differed as 41.67 ± 5.69 to 710.00 ± 9.54 mg gallic acid/100 g fresh weight **[8]**. In the study, in which antioxidant capacity was largely associated with polyphenol and vitamin C levels, it was stated that the difference in antioxidant capacity was due to the difference in *Actinidia* species and cultivars.

**Microbiological Analysis**

The findings of the microorganisms examined in kiwi vinegar are given in Table 3. Mesophilic aerobic bacteria, lactic acid bacteria (LAB), total coliforms and the number of yeasts were investigated in kiwi vinegars. It was determined that the number of bacteria and yeast in vinegars decreased as fermentation progressed. It was determined that the total number of bacteria at the beginning was between 2.17 and 4.99 log cfu/mL. At the end of the 21st day of fermentation, the total bacterial count was found to be between 1.00 and 2.47 log cfu/mL. Similarly, it was determined that the number of lactic acid bacteria decreased, while it was between 4.87 and 5.39 log cfu/mL at the beginning, it decreased to 2.00 - 3.70 log cfu/mL at the end of 21 days.

While the yeast-mold count in the samples was 2.00 - 2.77 log cfu/mL at the beginning, it decreased by 1 log cfu/mL in vinegars under aerobic conditions at the end of 21 days. It was determined that vinegar in anaerobic condition increased to 2.65 log cfu/mL. It was thought that the increase (4.38-5.32 log cfu/mL) observed on the 15th day in kiwi vinegars produced under aerobic conditions was caused by the molds formed on the surface of the vinegars.

**Table 3.** Microbiological analysis of kiwi vinegars

| Type of microorganisms | Incubation days | Number of microorganisms (log CFU/ml) | | | |
|---|---|---|---|---|---|
| | | K1 | K2 | K3 | KK |
| Mesophilic aerobic bacteria | 7 | -ND | 4,47 | 4,11 | 4,99 |
| | 15 | 4,38 | 4,62 | 3,04 | 5,36 |
| | 21 | 1,00 | 1,00 | 2,47 | 2,00 |
| Lactic acid bacteria | 7 | 4,87 | 4,32 | 4,57 | 5,39 |
| | 15 | 3,87 | 4,14 | 3,81 | 3,69 |
| | 21 | 2,00 | 3,47 | 3,70 | 2,53 |

| | | | | | |
|---|---|---|---|---|---|
| Yeast and moulds | 7 | 2,72 | 2,77 | 2,00 | 2,00 |
| | 15 | 3,04 | 4,38 | 3,72 | 5,32 |
| | 21 | 1,00 | 1,00 | 2,65 | 1,00 |
| Total coliforms | 7 | 2,38 | 4,32 | 4,27 | 4,39 |
| | 15 | 3,00 | 4,47 | 2,00 | 5,27 |
| | 21 | 1,00 | 1,00 | 1,00 | 1,00 |

K1: Vinegar covered with thin cheesecloth (aerobic), K2: Vinegar covered with parafilm, K3: Vinegar covered with an airtight lid (anaerobic). KK: Vinegar from kiwi peels covered with cheesecloth (aerobic), ND: not detected, CFU: colony forming unit

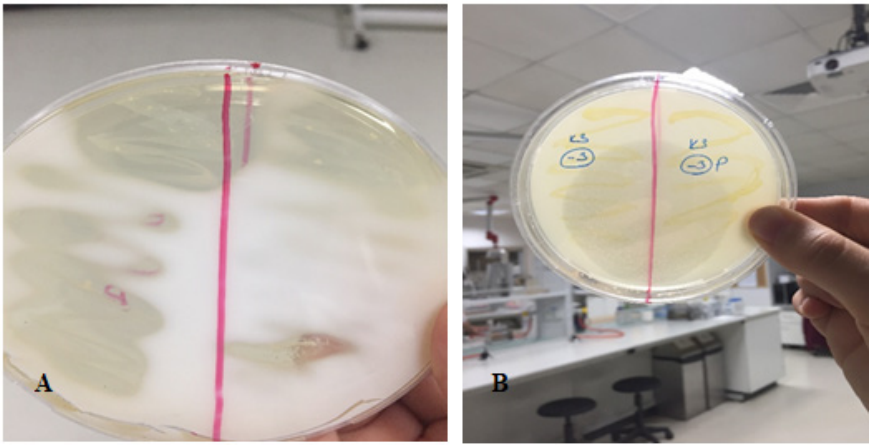



**Figure 3.** A) Acetic acid bacteria positive on medium, B) Asetic acid bacteria negative on medium.

The number of coliforms detected in kiwi vinegars ranged from 2.38 log cfu/mL to 4.39 log cfu/mL, and decreased to 1.00 log cfu/mL as fermentation progressed. The presence of acetic acid bacteria, which is known to be responsible for acetic acid production, was found only in the KK-coded kiwi peel sample. The presence of acetic acid bacteria was detected by the dissolution of $CaCO_3$ and the formation of transparent areas around the colony as seen in Figure 3. The presence of acetic acid bacteria was not found in other kiwi samples, and only yeast colonies were detected on the medium. It was thought that the high number of total aerobic bacteria and coliforms seen at the beginning of fermentation in kiwi vinegars was caused by the water, fruits and equipment used. It was determined that as the fermentation progressed, detremental microorganisms were eliminated, and at the same time, the number of yeast and lactic acid bacteria

decreased. It has been suggested that the factors determining the vinegar microbiota are environmental factors such as temperature and humidity, as well as the composition of the vinegar **[34].** In addition, it has been stated that ethanol obtained in the first stages of fermentation with lactic acid bacteria and yeast and then acid prevents the development of harmful microorganisms and increases the shelf life of vinegar **[35].** It has also been suggested that vinegars produced by spontaneous natural fermentation carry a great risk of spoilage **[36].**

## CONCLUSION

Kiwi is a fruit rich in phenolic content. Although a slight decrease was observed in the fermentation process, it was found that the phenolic content of the kiwi vinegars produced in this study was high. It was determined that the total acidity increased during the fermentation period in all vinegars. With this increase in total acidity, a significant decrease in the total coliform count was observed. It was observed that the phenolic content was lower but the number of lactic acid bacteria and yeast was higher when vinegar was produced in an anaerobic environment instead of an aerobic environment. In this study, it has been reported that molds can be observed from time to time on the surfaces of vinegars produced under aerobic conditions during the fermentation process. However, kiwi fruits have the potential to be used in vinegar production.

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