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MICROBIOLOGICAL AND CHEMICAL PROPERTIES OF TRADITIONALLY PRODUCED APPLE, BLACKTHORN, HAWTHORN AND PEAR VINEGAR

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Abstract

The aim of this paper is to determine the quality of vinegars through microbiological and chemical properties using simple chemical and microbiological analysis in different types of vinegar from apple (*Malus sp.*), blackthorn, (*Prunus spinosa* L.), hawthorn (*Crataegus sp.*) and pear (*Pyrus sp.*) obtained in a traditional manner. The amount of acetic acid in vinegar in this investigation varied between 0.72% to 4.5% for the samples.

In apple vinegar, the total number of bacteria amounts to 16 cfu/mL, consists of only acetic bacteria, contains 4.0% acetic acid and has 3.25 pH value. In the samples of blackthorn and hawthorn vinegar, a very small number (6 cfu/mL and 5 cfu/mL respectively) of acetic bacteria was present. Also, a low acidity (pH about 1.0) was found and microscopy revealed Gram (+) rod-shaped asporogenous bacteria (41 cfu/mL in blackthorn and 35 cfu/mL in hawthorn vinegar) and a near absence of acetic bacteria. Hence, samples of blackthorn and hawthorn vinegar were found to undergo no acetic fermentation process at all, meaning the final product is not vinegar. In pear vinegar, the total number of bacteria is 25 cfu/mL and acetic bacteria 16 cfu/mL, it has the highest percentage of acetic acid (4.5%). Yeast cells were observed under the microscope which is characteristic of alcoholic fermentation. Due to it, the process of obtaining the final product goes in an unwanted direction.

A very high negative correlation between pH and acetic acid was determined and between the total number of bacteria and the number of acetic bacteria.

Key words: *Malus sp., Prunus spinosa L., Crataegus sp., Pyrus sp.*

INTRODUCTION

Vinegar, as well as wine, has been known to mankind for many millennia. Ancient winemakers noticed that wine left in an open container becomes sour and turns into a sour liquid with a specific smell. For many years, vinegar was produced without understanding the essence of the process. The first vinegar in history was made from date wine about 7000 years ago in Babylon, Ancient Egypt and Assyria. At that time, vinegar was used as an antiseptic and solvent, it was used as an antidote and general tonic for reducing thirst. There are many myths about the healing properties of vinegar. The vinegar-soaked sponge offered to the crucified Christ was actually a way to ease his suffering. A mixture of apple vinegar and honey was considered an effective remedy for arthritis, although no medical explanation has been found for this.

For centuries, vinegar has been used in food. It was widely used as a spice to improve the taste of hot dishes and to make marinades and spices. Red wine vinegar was used as a salad dressing, and for marinating red meat. White wine vinegar, rice and corn vinegar are combined with chicken and fish dishes and used in baked goods. Malt and cane vinegar are used in various sauces.

In traditional medicine, vinegar is used in its pure form for therapeutic and prophylactic purposes. It is a folk medicine that aids in all kinds of health problems. The most popular is apple vinegar. People used it for therapeutic purposes, such as weight loss, lowering blood sugar levels, treating respiratory diseases, improving cardiovascular circulation, better digestion, against inflammation of throat, bad breath, various inflammatory processes, high temperature, preventing the feeling of fatigue, etc. Vinegar was an ideal natural supplement for skin care (makes it soft and smooth), helps against warts and dandruff, is a perfect balm for smooth and shiny hair, acts as a natural deodorant, completely neutralizes odours.

Some scientific investigations clearly state the beneficial properties of vinegar such as antifungal, antiviral, antibacterial (due to the low pH value) and antioxidants properties. Vinegar decreases triglycerides, helps with weight loss, controls blood sugar levels, improves cardio-vascular health, prevents hypertension, cardiovascular diseases, cancer, diabetes (type 2), neurodegenerative diseases, osteoporosis, relieves arthritis pain and stomach ache, regulates body pH and detoxifies the body, provides numerous benefits related to skin, digestion and immunity health without any side effects (Sakanaka & Ishihara, 2007; Tripathi, 2023). But, like other acids, vinegar can damage tooth enamel.

Because of its variety and rich history, vinegar attracts the attention of collectors and tasters. High-quality, aged vinegar is considered a good investment, and a collection of original vinegars is valued as much as a collection of rare wines. Vinegar lovers experiment with adding this spice to various dishes, create their own mixes, prepare vinegar at home, and even grow new types of acetic acid bacteria.

As a product, vinegar is obtained from various cereals available in nature, sugar cane, grapes and fruits. Traditional vinegar is produced from regional foods according to well established customs. Different types of vinegar are available on the market. The balsamic vinegar of Modena, Italy is made from the local white Trebbiano grapes. Traditional rice wine vinegar is produced in Asia, coconut and cane vinegar is common in India and the Phillipines and date vinegars are popular in the Middle East, persimmon, sour cherry etc. (Sakanaka & Ishihara, 2007; Singh & Mishra, 2017; Wang et al., 2023).

Vinegar is an acidic liquid with a sharp smell that is obtained through the fermentation process (Najdenovska & Čolo, 2013). In French, the word vinegar actually means "sour wine". Fermentation is done by bacteria. The existence of these bacteria was proved by Louis Pasteur in 1864. In the 70's of the 20th century, there was a revolution in the production of vinegar. It was discovered that acetic acid, which is the main component of vinegar, can be obtained not only by fermentation of wine, must, honey, juices and other liquids containing alcohol, but also chemically from natural gas, industrial waste and products of dry distillation of wood. Vinegar prepared through traditional technology has a special taste and aroma. Vinegars prepared according to special recipes are very popular in American, European and Asian cuisine. In Japan, rice, barley and corn vinegars are made using acidobacteria, which makes them particularly useful. Cherry vinegar is made from a mixture of several wines and stored in wooden barrels for a long time.

In Macedonia, vinegar is traditionally produced from apples and pears. Vinegar from these species of fruit is produced in plastic barrels (wooden barrels were used in the past) by fermenting previously ground fruits (Selamovska et al., 2023). The fermentation process lasts several months (Tešić, 1981; Ziberoski, 2006).

MATERIAL AND METHODS

The examination of the quality of vinegar in this paper included laboratory tests of the microbiological and chemical properties of four different types of vinegar samples: apple (*Malus sp.*), blackthorn (*Prunus spinosa* L.), hawthorn (*Crataegus sp.*) and pear (*Pyrus sp.*) (Figure 1), produced in a traditional manner of production. The samples were taken from different producers who produce vinegar using their own technology (Figure 1 and 2).

Figure 1. Samples of vinegar from fruits: 1-apple, 2-blackthorn, 3-hawthorn, 4-pear (Original photo by O. Najdenovska, 2024)

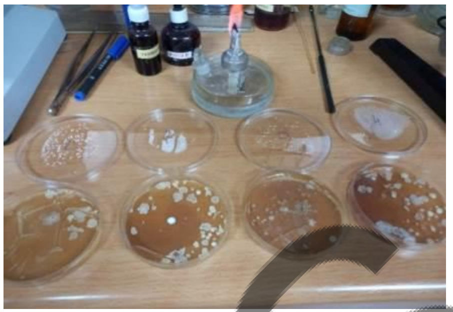


Figure 2. Colonies of bacteria in vinegar in Petri dishes on nutrient medium MPA (Original photo by O. Najdenovska, 2024)

The microbiological analysis of four samples of vinegar was carried out in two iterations. The tested types of microorganisms were cultivated in a thermostat at a temperature of 25 ºC. The microbiological analyses included examinations of the total number of bacteria and presence and total number of acetic bacteria (*Acetobacterium*). The analyses were performed in the microbiological laboratory at the Faculty of Agricultural Sciences and Food in Skopje.

The total number of bacteria, as well as, acetic bacteria, were examined on nutrient medium mesopeptone agar (MPA) according to the method of diluting and seeding of selective nutrient medium (Jarak & Djuric, 2004). In the second iteration, the acetic bacteria were examined by the method of diluting and seeding of selective nutrient medium Henneberg (250 mL distilled water, 7.5 g maltose, 2.5 g peptone, 2.5 g yeast extract, 5.0 g agar and 10 mL of ethyl alcohol).

The chemical analyses were performed in two iterations, on 10.12.2023 and 26.02.2024. The measurement of pH in vinegar was performed with a pH meter, while the percentage presence of acetic acid in vinegar was determined volumetrically by 0.1 M NaOH, in the presence of the indicator phenolphthalein. The consumed volume of the base was used to calculate the percentage of acetic acid (OIV-MA-VI-01- Compendium of international methods of analysis for vinegar- Methods of analysis for vinegar). The analyses were conducted in the Oenological laboratory of Institute of Agriculture, Skopje.

Correlation analysis (r.) between determined variables was applied using XLSTAT 2014 software. Data matrix has been introduced using descriptive statistical analysis: minimum, maximum, mean value and standard deviation.

Correlation coefficient (r.) description (LaMorte, 2021) is as follows:

- +1.0 Perfect positive
- +0.8 to 1.0 Very strong positive
- +0.6 to 0.8 Strong positive
- +0.4 to 0.6 Moderate positive
- +0.2 to 0.4 Weak positive
- 0.0 to +0.2 Very weak positive or no association
- 0.0 to -0.2 Very weak negative or no association
- -0.2 to – 0.4 Weak negative
- -0.4 to -0.6 Moderate negative
- -0.6 to -0.8 Strong negative
- -0.8 to -1.0 Very strong negative
- -1.0 Perfect negative

RESULTS AND DISCUSSION

According to the results obtained from the microbiological analysis of the total number of microorganisms and acetic bacteria in all four samples of apple, blackthorn, hawthorn and pear vinegar in both iterations, no presence of other types of microorganisms was found except acetic bacteria, yeasts and rod-shaped asporogenous bacteria. In apple vinegar, no other types of bacteria were observed except for acetic bacteria (16 cfu/mL) of the genus *Acetobacter* (Table 1). The determined acidity (pH) of the environment in all samples ranges from 3.12 to 3.96 (Table 2).

Table 1. Total number of bacteria and acetic bacteria (cfu/mL) in apple, thorn, hawthorn and pear vinegar samples.

Samples	Apple vinegar	Blackthorn vinegar	Hawthorn vinegar	Pear vinegar
Total number of bacteria/cfu/mL	16	41	35	25
Total number of acetic bacteria (*Acetobacter)* /cfu/mL	16	6	5	16

Table 2. Chemical analyses for vinegars obtained in December, 2023.

Samples	Acetic acid /%	pH
Apple (*Malus sp.*)	4.00	3.35
Blackthorn (*Prunus spinosa* L.)	0.96	3.68
Hawthorn (*Crataegus sp.*)	0.72	3.96
Pear (*Pyrus sp.*)	4.50	3.12

These tests showed that the samples of blackthorn and hawthorn vinegar contained a low number of acetobacteria (6 cfu/mL in blackthorn and 5 cfu/mL in hawthorn vinegar). Also, in these two vinegar samples, a high quantity of other types of bacteria was determined (41 cfu/mL in blackthorn and 35 cfu/mL in hawthorn vinegar). The obtained pH values were 3.96 in hawthorn, 3.68 in blackthorn vinegar, while 3.35 in apple and 3.12 in pear vinegar. During the first chemical analysis, pear vinegar had the highest percentage (4.50%) of acetic acid (Table 2).

During the determination of the organoleptic properties of the examined vinegar samples, it was determined that the apple vinegar sample had a pleasant, apple smell and taste of vinegar. The smell of the blackthorn vinegar sample did not have a recognizable smell of vinegar, while the smell of hawthorn vinegar had an unpleasant smell. Pear vinegar didn't have a smell recognizable as vinegar but it was not unpleasant, probably due to the presence of yeasts and acetic bacteria. Pear vinegar contains 4.50% acetic acid and had pH value 3.12 (Table 2). The assumption is that the pH value affects the development of yeasts that's why they are present in large quantities (25 cfu/mL) (Table 1).

Acetic bacteria that are obligately aerobic, rod-shaped, Gram negative, asporogenous and motile with polar cilia usually produce 5% to 14% acetic acid (Najdenovska et al., 2013; Najdenovska & Čolo, 2012). Acetic bacteria of the genus *Acetomonas xsylina* produce a small amount of acetic acid, about 4.5%, but they produce a thicker coating - cuticle (Leifson, 1954). In our research, only the apple vinegar sample was observed to form a coating - cuticle.

According to the results of the research of microbiological properties of the vinegar samples in the first iteration (10.12.2023), it was found that the blackthorn and hawthorn vinegar samples contain the lowest quantity of acetic bacteria and a high quantity of total number of bacteria. This condition corresponds to the low percentage presence of acetic acid in vinegar (Table 2). Specifically, the percentage of acetic acid in blackthorn vinegar is 0.96% (0.96 g/100 mL acetic acid), while in hawthorn vinegar it is 0.72% (0.72 g/100 mL acetic acid). Therefore, it was established that the samples of blackthorn and hawthorn vinegar, according to the parameter of amount of acetic acid, the uncharacteristic smell for vinegar, the presence of other Gram-positive asporogenous bacteria and the absence of acetic bacteria (Tešiċ, 1981), do not represent vinegar.

During the second iteration of the microbiological and chemical analysis (Table 3) of the vinegar samples (26.02.2024), the following properties and parameters were determined:

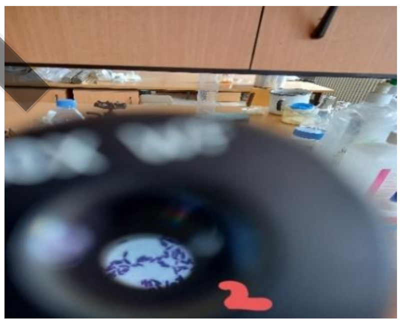
Table 3. Chemical analyses for vinegars obtained in February, 2024.

Samples	Acetic acid /%	pH
Apple (*Malus sp.*)	4.00	3.25
Blackthorn (*Prunus spinosa* L.)	1.10	3.62
Hawthorn (*Crataegus sp.*)	1.00	3.96
Pear (*Pyrus sp.*)	4.50	3.13

The apple vinegar sample had a completely pleasant taste and smell of apple vinegar, and during microscopy only acetic bacteria were observed in the field of view (Figure 3a,b). In the same apple vinegar, a visible slimy coating from the so-called "mother of vinegar", starter acetic bacteria were observed. Acetic bacteria are able to oxidize sugars and ethanol, producing acetic acid in the fermentation process (Raspor & Goranovic, 2008; Mamlouc & Gullo, 2013; Salieri & Giudici, 2008).

Figure 3. Acetic bacteria in apple vinegar (under microscope).

During the second analysis of the samples, a quantity of 4.0 g/100 mL of acetic acid was determined in the sample of apple vinegar and only the presence of acetic bacteria (16 cfu/mL) (Figure 3) was determined under the microscope. Also, the results obtained for blackthorn and hawthorn vinegar, show that the samples have not a recognizable smell and taste characteristic for vinegar, which was also confirmed through chemical analysis (Table 3), very low content of acetic acid (around 1.0%). Under the microscope, it was found that only Gram (+) rod-shaped asporogenous bacteria (Figure 4,5) dominated, while acetic bacteria were not found, that's why the blackthorn and hawthorn vinegar samples do not represent vinegar according to the established microbiological-chemical parameters.



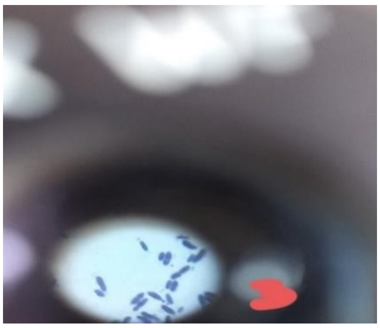


Figure 4. Microorganisms in blackthorn vinegar. Figure 5. Microorganisms in hawthorn vinegar.

During the second microscopic analysis, the sample of pear vinegar, in addition to acetic bacteria, showed yeasts (Figure 6) characteristic of alcoholic fermentation. Another characteristic of the sample of pear vinegar is that it doesn't smell like vinegar, but like pomace, probably due to the presence of yeasts.

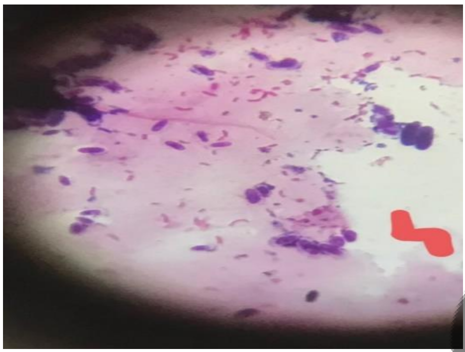


Figure 6. Microorganisms in a sample of pear vinegar

According to legal regulations, vinegar that is commercially available must contain 4-8% acetic acid and low pH value. Vinegar containing 10% acetic acid is corrosive and can cause chemical burns. Spirit vinegar is a stronger form of vinegar that contains about 20% acetic acid. The other chemicals in vinegar depend on its source. Many types of vinegar include added flavourings (sugar, malt or caramel, various spices, etc.) that are added after the fermentation process.

According to the results from the examination, the blackthorn and hawthorn vinegar samples do not comply with the legal regulations (Rules for the quality of vinegar and diluted acetic acid No. 24/1989) for vinegar due to the very low amount of acetic acid (about 1%).

Table 4. Correlation between pH and acetic acid.

Variables	Acetic acid /%	pH
Acetic acid /%	**1**	**-0.9516**
pH	**-0.9516**	**1**

Values in bold are different from 0 with a significance level alpha=0.05.

p-values

Variables	Acetic acid /%	pH
Acetic acid /%	**1**	0.0484
pH	0.0484	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Coefficients of determination (R^2)

Variables	Acetic acid /%	pH
Acetic acid /%	**1**	0.9055
pH	0.9055	**1**

The obtained results presented in Table 4. show a very high negative correlation for pH and acetic acid, when the concentration of acetic acid increases, the pH value decreases and vice versa. In this case, the value of p is 0.0484, which means that there is a statistical significance of the aforementioned correlation dependence between the parameters.

The coefficient of determination R^2 is a statistical value that shows how much the variation of one parameter depends on and it is explained by the variation of another parameter. The higher the coefficient, the higher the dependence. In this case there is a very high coefficient of determination between the studied parameters: acetic acid and pH, when 91% of the variation in the concentration of acetic acid is explained by the pH value and vice versa.

Table 5. Correlation between acetic acid and pH, correlation matrix (Pearson).

Variables	Acetic acid /%	pH
Acetic acid /%	**1**	-0.9370
pH	-0.9370	**1**

Values in bold are different from 0 with a significance level alpha=0.05

p-values

Variables	Acetic acid /%	pH
Acetic acid /%	**0**	0.0630
pH	0.0630	**0**

Values in bold are different from 0 with a significance level alpha=0.05

Coefficients of determination (R^2)

Variables	Acetic acid /%	pH
Acetic acid /%	**1**	0.8779
pH	0.8779	**1**

The results in Table 5 show that there is a very high negative correlation between pH and acetic acid, but somewhat lower compared to the results from the year 2023. When the content of acetic acid increases, the pH value decreases and vice versa. The table shows that there is a very high negative correlation between pH and acetic acid, but somewhat lower compared to the results from the year 2023. When the content of acetic acid increases, the pH value decreases and vice versa. In this case, the value of p (0.063) in year 2024 does not have statistical significance of the aforementioned correlation dependence between the parameters. In this case there is a very high (but somewhat lower in year 2024) coefficient of determination between the studied properties: acetic acid and pH, when 88% of the variation in the concentration of acetic acid is explained by the pH value and vice versa.

Table 6. Correlation between total and acetic bacteria number.
Correlation matrix (Pearson)

Variables	Total number of bacteria/cfu/mL	Total number of acetic bacteria (Acetobacter) /cfu/mL
Total number of bacteria/cfu/mL	**1**	-0.8993
Total number of acetic bacteria (Acetobacter) /cfu/mL	-0.8993	**1**

Values in bold are different from 0 with a significance level alpha=0.05

p-values

Variables	Total number of bacteria/cfu/mL	Total number of acetic bacteria (Acetobacter) /cfu/mL
Total number of bacteria/cfu/mL	**0**	0.1007
Total number of acetic bacteria (Acetobacter) /cfu/mL	0.1007	**0**

Values in bold are different from 0 with a significance level alpha=0.05

Coefficients of determination (R^2)

Variables	Total number of bacteria/cfu/mL	Total number of acetic bacteria (Acetobacter) /cfu/mL
Total number of bacteria/cfu/mL	**1**	0.8088

Total number of acetic bacteria (Acetobacter) /cfu/mL	0.8088	**1**

The results in Table 6 show that there is a very high negative correlation between the total number of bacteria and number of acetic bacteria. When the number of total bacteria increases, the number of acetic bacteria decreases. According to the p value (0.1007), there is no statistical significance of the aforementioned correlation dependence. In this case there is a very high coefficient of determination between the investigated parameters: total number of bacteria and total number of acetic bacteria, when 81% of the variation in total number of bacteria is explained by total number of acetic bacteria and vice versa.

CONCLUDING REMARKS

According to the results obtained from the research, it has been established that the different types of fruits have an influence on the quality and course of vinegar production in a traditional manner.

According to the parameters of the chemical and microbiological analysis, apple vinegar has the best quality, which contains 4.0% acetic acid, pH 3.35 and 16 cfu/mL acetic bacteria.

In the blackthorn and hawthorn vinegar samples, the presence of Gram (+) rod-shaped asporogenous bacteria was determined, that's why the fermentation process does not unfold according to the scientifically established postulates for the production of vinegar. The percentage of acetic acid in blackthorn and hawthorn vinegar is about 1.0%.

Pear vinegar had the presence of acetic bacteria (16 cfu/mL), pH value 3.12, and 4.50% acetic acid. In addition to acetic bacteria, the presence of yeasts has also been determined. Due to the fermentation process unfolded in the direction of alcoholic fermentation, this sample smells of pomace. The process of acetic fermentation should continue to be monitored.

Blackthorn and hawthorn samples do not comply with the legal regulations for vinegar due to the very low amount of acetic acid.

According to statistical analysis, a very high negative correlation between pH and acetic acid was determined. When the content of acetic acid increases, the pH value decreases and vice versa.

A very high negative correlation was found between the total number of bacteria and the number of acetic bacteria. When the number of total bacteria increases, the number of acetic bacteria decreases.

The number of acetic bacteria is negatively correlated with pH and the total number of bacteria.

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МИКРОБИОЛОШКИ И ХЕМИСКИ КАРАКТЕРИСТИКИ НА ТРАДИЦИОНАЛНО ПРОИЗВЕДЕН ОЦЕТ ОД ЈАБОЛКО, ТРНИНКА, ГЛОГ И КРУША

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Резиме

Целта на овој труд е со хемиски и микробиолошки анализи да се утврди квалитетот на примероците од оцет добиен од некои овошни видови: јаболко (*Malus sp*.), трнинка (*Prunus Spinosa* L.), глог (*Crataegus sp*.) и круша (*Pyrus sp*.) добиени на традиционален начин. Количеството на оцетна киселина во мострите варира од 0,72 % до 4,5 %. Во јаболковиот оцет вкупниот број на бактерии изнесува 16 cfu/mL и истиот се состои само од оцетни бактерии, содржи 4,0 % оцетна киселина и има pH вредност 3,25.

Во примероците оцет од трнинка и глог присутен е мал број на оцетни бактерии (6 cfu/mL, односно 5 cfu/mL). Исто така, утврдена е ниска киселост (pH околу 1,0) и микроскопијата утврди грам (+) неспорогени стапчести бактерии (41 cfu/mL кај трнинка и 35 cfu/mL во оцет од глог) и речиси целосно отсуство на оцетни бактерии. Утврдено е дека во примероците оцет од трнинка и глог воопшто не се одвивал процес на оцетна ферментација, што значи дека финалниот производ не е оцет.

Во оцетот од круша присутни се 25 cfu/mL вкупен број на бактерии, 16 cfu/mL оцетни бактерии и во него има најголем процент на оцетна киселина (4,5 %). Во примероците се забележани и клетки од квасци, карактеристични за алкохолна

ферментација, поради што процесот за добивање на краен производ оди во несакан правец.

Утврдена е многу висока негативна корелација помеѓу pH и оцетна киселина и помеѓу вкупниот број на бактерии и бројот на оцетни бактерии.

Клучни зборови: *Malus sp., Prunus spinosa L., Crataegus sp., Pyrus sp.*