

PHYSICO-CHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF ARTISANAL APPLE CIDER VINEGAR WITH HONEY AND SOME NATURAL ADDITIVES

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RESEARCH ARTICLE

Abstract

Artisanal apple cider vinegar, obtained through traditional slow fermentation methods, is appreciated not only for its complex flavor, but also for its potential health benefits, highlighted both in the specialized literature and in traditional medicine. Fruit-infused variants, such as quince or *Prunus laurocerasus* fruits, add additional nutritional value, through their high content of antioxidants, vitamins and bioactive compounds. This product diversification responds to the increasing demand for functional food products, which combine taste with biological functionality. The aim of this study was to obtain artisanal apple cider vinegar with honey in the classic version, as well as infused with quince, respectively *Prunus laurocerasus* extract, using traditional fermentation methods, characterizing the samples obtained from a physico-chemical and biochemical point of view, and finally evaluating the impact of natural additives on the final quality of the product.

Keywords: vinegar, apple, honey, quince, *Prunus laurocerasus*

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INTRODUCTION

Artisanal apple cider vinegar, obtained through traditional slow fermentation methods, is appreciated not only for its complex flavor, but also for its potential health benefits, highlighted both in the specialized literature and in traditional medicine. Fruit-infused variants, such as quince or *Prunus laurocerasus* fruits, add additional nutritional value, through their high content of antioxidants, vitamins and bioactive compounds. This product diversification responds to the increasing demand for functional food products that combine taste with biological functionality.

Depending on the raw material used, different types of vinegar can be obtained: wine vinegar, cider vinegar, berry and other fruit vinegar obtained, alcohol vinegar, grain vinegar (Dabija and Hatnean, 2014).

Depending on the geographical location, the assortments vary, being strongly influenced by culture, traditions and available raw materials. Thus, we can find assortments such as: whey vinegar, in Europe where the processed raw material is deproteinized whey and, if necessary, enriched with lactose to stimulate alcoholic fermentation (Parrondo et al., 2009), honey vinegar in Europe, America and Africa, kombucha (tea and sugar vinegar) in Russia, Japan and China, rice vinegar in East and Southeast China, sugarcane vinegar in France and the USA, banana vinegar in Southeast Asia, and various other assortments (Boonsupa, 2021).

Fermented foods are recognized for its beneficial properties on the human body (Mukherjee et al., 2024). Recent studies have highlighted the beneficial effect of vinegar consumption. Apple cider vinegar is rich in bioactive substances, such as phenols, flavonoids, acetic acid, having positive effects on the body, including antioxidant, antimicrobial, antidiabetic, antitumor and antiinflammatory properties (Kandyliis et al., 2021). It supports the digestive process, relieves fatigue and balances appetite (Guine et al., 2021; Ling et al., 2019).

Studies conducted by researchers have confirmed the following benefits of vinegar consumption:

- alpha-amylase involved in the transformation of starch into glucose is temporarily inactivated by the acetic acid in vinegar, thus flattening the glucose curve (Santos et al., 2019);

- pre-prandial vinegar consumption for three months led to a reduction in visceral fat as well as a decrease in triglycerides (Kondo et al., 2009);

- regular consumption of apple cider vinegar influences glycosylated hemoglobin values in patients suffering from type 2 diabetes (Johnston et al., 2009);

- in the case of a strict diet applied identically in 2 groups, respondents who consumed vinegar lost 5 kg, compared to those who only followed the diet and lost 3 kg (Khezri et al., 2018);

- stimulates the acceleration of glycogen production at the muscle level, thus better assimilating glucose, and at the DNA level

stimulates fat burning in mitochondria (Santos et al., 2019);

-supplementation with vinegar increases satiety after consuming bakery products and decreases glycemic peaks and insulin response in healthy individuals (Ostman et al., 2005);

-apple cider vinegar is considered superior to other supplements with a thermogenic effect, being more effective in burning fat (Santos et al., 2019);

- vinegar consumption is associated with the recovery of ovulation function in women suffering from polycystic ovary syndrome (Wu et al., 2013).

The main objective of this study was to obtain artisanal apple cider vinegar with honey in the classic version, as well as infused with *Prunus laurocerasus* extract respectively with quince, using traditional fermentation methods, characterizing the obtained samples from a physico-chemical and biochemical point of view.

MATERIALS AND METHODS

The research methodology included the preparation of raw materials, fermentation processes, as well as physico-chemical and biochemical analysis of products.

Raw materials

-Golden delicious apples were purchased in November 2024, from a local producer from Oradea, Bihor County, Romania. Fresh apple juice was obtained from apples

-*Prunus laurocerasus* concentrate was obtained from *Prunus laurocerasus* fruits macerated in a 1:1 solution of 96% ethanol and drinking water, after which it was stored for 6 months in a room protected from sunlight and with a constant temperature of 17°C. After this maceration period, the fruits were pressed and filtered. The obtained solution was concentrated using a Heidolph Laborota 4010 digital rotavapor, at a temperature of 45°C, for 45 minutes.

-Quince from the local market. The quince fruits were grated.

-Polyfloral honey from a local producer.

From these raw materials, 3 mixtures were prepared according to the following recipes:

900 ml of apple juice was mixed with 3.6% local polyfloral honey and distributed in 3 Erlenmayer flasks.

After dosing, 3 samples were obtained:

-sample 1 (AV): classic apple vinegar with honey, without other fruit additions.

-sample 2 (AQV): apple vinegar with honey and addition of 10% quince.

-sample 3 (APLV): apple vinegar with honey and addition of 10% non-alcoholic concentrated extract of *Prunus laurocerasus*.

After preparing the samples, they were dosed according to the proposed recipe and mixed by shaking, in Erlenmayer flasks.

Fermentation processes



Fig.1. Fermentation processes

The *alcoholic fermentation* process lasted 3 weeks, during which the concentration of soluble dry matter was checked to monitor the progress and completion of the process (Fig.1.I-III)

For the *acetic fermentation*, the cider samples obtained from the alcoholic fermentation were filtered on Watman No. 1 paper and then with 0.22 μm membrane filters. The pH was between 4.42 and 4.52.

The acetic bacteria strains used came from the commercial apple cider vinegar that we used as a starter culture. To stimulate the acetic fermentation, a carrier consisting of sterilized wood chips was used.

The equipment used for the acetic fermentation is shown in Fig.1.IV and consisted of 3 bioreactor units provided at the top with a closing cap.

Each cap was equipped with an air inlet crossed by a glass rod provided at the top with a 0.22 μm filter and at the bottom with a 100 μm air diffuser. This diffuser allows the dispersion of air in the form of small bubbles and avoids the coalescence of the bubbles. The reactor incorporated a top exhaust of the gas flow through a 0.45 μm filter. O_2 saturation conditions were ensured by a constant flow air pump, which provided for each bioreactor an air flow of 1.2 l/min. Agitation was achieved by placing the reactors on an orbital shaker at a stirring rate of 120 rpm.

The following physico-chemical and biochemical methods were performed:

- *Total soluble substances (TSS)*, refractometric method, using a digital refractometer (KRUS, AR 2008 model, Germany).

- *pH* potentiometric determination using Crison pH meter.

-*Electrical conductivity (EC)* potentiometric method, using HACH Sensilon378 multiparameter meter.

-*Total acidity (TA)* was determined by titration with 0.1 N NaOH (STAS 157-86).

-*Total polyphenols (TP)* – Folin Ciocâlteu spectrophotometric method (Thaipong et al 2006)

-*Total flavonoid (TF)* – spectrophotometric method (Atanassova et al. 2011).

-*Total antioxidant activity (AA)* using FRAP – spectrophotometric method, Benzie and Strain 1996).

Spectrophotometric determinations were made with a Shimadzu UV Mini 1240 spectrophotometer.

RESULTS AND DISCUSSIONS

The results obtained after performing analysis for studying the vinegar samples were inserted in Table 1 and 2 and Fig.2, 3 and 4.

Table 1

Mean values for Physico-chemical parameters

sample	TSS (°Brix)	pH	TA (g acetic acid%)	E.C. mS
AV	4.82	3.67	3.89	3.81
AQV	4.02	3.69	3.67	3.78
APLV	4.32	3.68	3.89	4.27

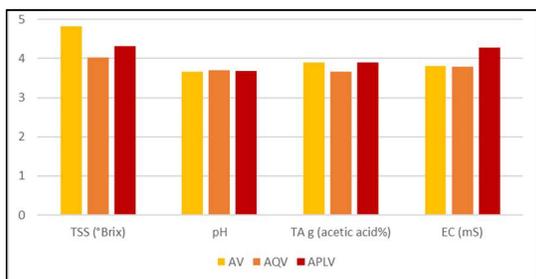


Fig 2. Graphic representation of mean values for physico-chemical parameters

The artisanal vinegar samples have a SUS value between 4.02–4.82°Brix, which suggests a higher residual sugar content in these products.

-*pH* of the artisanal samples is relatively similar (3.67-3.60)

-*Total acidity*, expressed in grams of acetic acid per 100 ml of vinegar, in the artisanal vinegar samples had an acidity between 3.67 and 3.89 g acetic acid%, a lower value than that of commercial vinegar where the standard value is 5g acetic acid%. This may be due to incomplete acetic fermentation under laboratory conditions, where the fermentation conditions are much more difficult to control.

-The highest *electrical conductivity* was recorded in the apple cider vinegar sample with honey and *Prunus laurocerasus* extract (4.27 mS), closely followed by the other 2 artisanal vinegar samples (3.78-3.81mS),

In general, in the case of food, electrical conductivity is directly proportional to the content of total minerals. This was highlighted by (Ousaaïd et al., 2022) following the comparative analysis between artisanal and industrial apple cider vinegar. They found that artisanal vinegar had a higher content of mineral salts and higher electrical conductivity, compared to the industrial one.

Thus, we can say that:

-AV from artisanal production had the highest sugar content with moderate acidity;

-AQV has a balanced profile, rich in sugars and minerals with a medium pH and acidity;

-APLV has a good combination of sugar and salt content.

Analyzing the data in table 2 and fig. 3, the following is observed:

- APLV has the highest content of polyphenolic compounds (8.02 mg GAE/100ml) followed by AQV and AV (6.99 and 6.34mg GAE/100ml).

- Flavonoids are in higher quantity in AQV (40.16 mg Que/100ml), followed by APLV (35.16 mg Que/100ml) and AV (29.69mg Que/100ml).

Comparing the results obtained from the determination of AA (Tab 2 and fig 4), with the FRAP method, the highest AA was recorded in the APLV concentrate (0.96 mM FeSO₄), followed by AQV (0.95 mM FeSO₄), confirming previous observations suggesting a superior antioxidant activity, associated with a rich content in bioactive compounds.

Table 2

Average values for absorbances and concentrations of biochemical parameters

Sample	TP		TF		AA	
	Abs	GAE mg/100 ml	Abs.	Que mg/100 ml	Abs.	mM FeSO ₄
AV	0.63	6.34	0.28	29.69	2.16	0.94
AQV	0.69	6.99	0.38	40.16	2.19	0.95
APLV	0.79	8.02	0.34	35.16	2.2	0.96

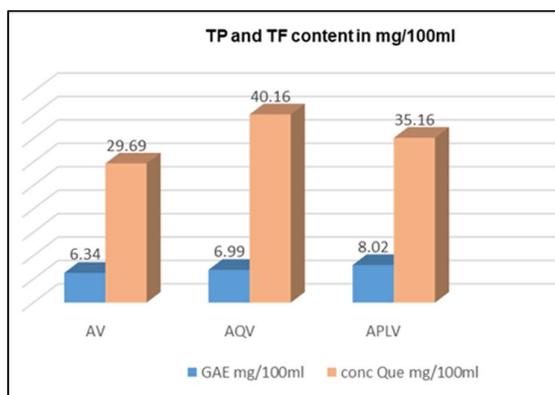


Fig.3. Graphic representation of mean values for Total polyphenol and flavonoid content

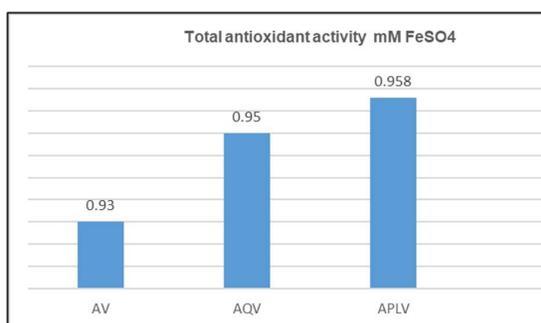


Fig.4. Graphic representation of mean values for Total antioxidant activity FRAP method

CONCLUSIONS

In conclusion, the results obtained from this study prove that following the application of controlled laboratory conditions, quality products with superior nutritional and functional values were obtained.

The advantages of artisanal vinegar with the addition of apple, quince or *Prunus Laurocerasus* concentrate consist of: high content of polyphenols, flavonoids and mineral salts, with antioxidant benefits and potential positive effects on health.

The integration of quinces and *Prunus laurocerasus* concentrate in apple vinegar with honey supports the current trend of producing functional foods, which contribute to the health of consumers.

For artisanal production, more rigorous control of acetic fermentation conditions is recommended to increase acidity while preserving nutritional benefits. It is recommended to continue acetic fermentation until an acetic concentration of at least 5g pure acetic acid/100ml is obtained, in order to comply with the legislative requirements.

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