SJIF Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 9.
Issue: 3
Page N. 69-71
Year: 2025

Original Article www.wjahr.com

DETERMINATION OF APPLE CIDER VINEGAR'S PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY

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Received date: 07 January 2025 **Revised date:** 27 January 2025 **Published date:** 17 February 2025

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ABSTRACT
Phenolic compounds, which are formed during the normal development of plants, are one of the most widespread groups of compounds in plants. Phenolic compounds include a variety of molecules classified as secondary metabolites in food foods and their products, which have antioxidant capacity that protects the body from the occurrence of many diseases. Apple cider vinegar is one of the foods rich in phenolic compounds, as its content was measured by the Folin-Ciocalteu method, and the antioxidant capacity was measured by the restorative capacity method.

KEYWORDS: Apple, Cider Vinegar, Total Phenolic Content, Folin-Ciocalteu, Reducing power.

INTRODUCTION

In the past few decades, interest in the therapeutic application of natural ingredients and food products has increased, leading to the use of food by-products in industry as a good source of bioactive compounds with high nutritional value.[1] Apples are good suppliers of natural antioxidants in the diet, because of both their high antioxidant content but of its widespread consumption. Vinson *et al* reported that 22% of ingested phenolic compounds in the United States come from apples[2], and Eberhardt *et al* stated that 100g of fresh apples have the antioxidant capacity equivalent to 1500mg of Vitamin C.[3]

Vinegar is used since the eighteenth century to treat many diseases.[4] Nowadays is known for pickling vegetables and fruits and in manufacturing mayonnaise and ketchup.[5] It is obtained from an alcoholic fermentation process followed by a pickling process of glucose found in apples, grapes, honey and many foods rich in carbohydrates.[6] Apple cider vinegar is the most marketed type of vinegar among Western and European countries as a fresh, nutritious, acidic vital product with a distinctive flavor, and without any additives.[7]

In addition to the sensory properties, including the color, smell, and bitter and astringent taste[8], phenolic compounds may be responsible for many other different vital rules that characterize apple cider vinegar.

This research aims to determine the total phenolic compounds found in apple cider vinegar as well as determine its antioxidant capacity.

MATERIALS AND METHODS

Chemicals

The following Chemicals were supplied from the indicated sources: Folin-Denis (Sigma Aldrich, Switzerland), sodium carbonate (BDH, England), Gallic acid (Biotech LTD, India), Trichloroacetic acid (TCA) (Riedel-De Haen AGm Germany), Potassium ferricyanide (May & Baker LTD, England), Ferric Chloride Anhydrous (Qualikems, India).

Apple Cider Vinegar products

Six different types of locally manufactured and marketed liquid apple cider vinegar products were collected, three of them were commercial (V1, V2, V3) and the other three were home-made (M1, M2, M3).

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using Folin-Ciocalteu assay.[8] A standard curve with different concentrations of gallic acid was prepared, then 7 ml of distilled water and 1.5 ml of 20% anhydrous sodium

carbonate solution were added to 0.1 ml of each solution of the standard series, as well as to 0.1 ml of the vinegar sample after diluting it by adding 2 ml of vinegar samples to 20 ml of water. After that, the solutions were mixed well, incubated for one minute, and then 0.5 ml of Folin-Denis reagent was added. After 60 min incubation at room temperature in the dark, the absorbance was measured at 765 nm and the results were expressed as gramm Gallic acid equivalent (GAE) in one liter apple cider vinegar.

Reducing power Musearment

The antioxidant activity of apple cider vinegar was determined by the Reducing power method[9], using the potassium ferricyanide reagent. Briefly, 0.5 ml of the sample solution was mixed with 0.5 ml phosphate buffer (PH = 6.6, 0.2 M), and 0.5 ml of 1% potassium ferricyanide solution was added, then the previous mixture was incubated in a water bath at 50 °C for 20 minutes. Subsequently, 0.5 ml of 10% TCA solution was added and mixed well. 1 ml of the previous yellow-colored mixture was taken and 1 ml of distilled water and 0.2 ml of 0.1% anhydrous iron chloride solution were added, and the absorbance of the resulting green solution was measured at 700 nm using a spectrophotometer. Three replicates were made for each sample. An increase in absorbance of the reaction mixture indicated an increase in reducing power of the sample.

RESULT AND DISCUSSION

The phenolic compounds content of the vinegar samples is shown and compared to the antioxidant capacity measured by the reducing power method in Table 1. The results of our study showed a variable content of phenolic compounds, ranging between 0.026 ± 0.01 gGAE/l and 2.90 ± 0.5 gGAE/l. The highest content of phenolic compounds found in M3 sample (2.9 ± 0.5 gGAE/l), while V2 showed the lowest content of phenolic compounds (0.026 ± 0.01 gGAE/l). Comparing the results to other studies, Liu *et al* observed that the total content of phenolic compounds ranged between (29.64 ± 0.16 - 469.10 ± 8.79 mgGAE/l)[10] in several types of apple cider vinegar collected from different countries. The values of phenolic compounds ranged between 689.1 ± 4.5 and 1475.4 ± 12.1 mgGAE/l in the Ousaaid *et al* study.[11] While in a study conducted in Morocco by Kara *et al*, the values of phenolic compounds, estimated in mgGAE/l, ranged between 6.22 ± 4.81 and 655.00 ± 22.2 [12]. Many factors may explain the variable content of phenolic compounds, such as the type of apple used in vinegar production.[13] Budak *et al* reported that the total content of phenolic compounds increases during maceration of apple juice with pulp during the alcoholic fermentation process thus obtaining the highest content of phenolic compounds.[14] Du *et al* also reported that the content of phenolics in peel pomace is much higher than that of apple pulp by about 10 times, indicating that peels and seeds are valuable sources of phenolic compounds.[8] The technology used in vinegar industry could be one of the factors affecting the phenolic content of vinegar. Budak *et al* indicated that the amount of phenolic compounds in vinegar samples prepared by the traditional method (the surface method) is higher than those prepared by the submerged method (the modern method).[15] Additionally, it was found that the total phenolics in apple juice made by cold pressing are lower than those made by traditional process.[8] Moreover, the amount of phenolic compounds varied depending on the type of the used yeast, as the highest amount of phenolic compounds (470.30 ± 2.40 mgGAE/100 ml) was observed when using homemade organic vinegar as yeast. While using commercial vinegar as yeast, the value of phenolic compounds decreased (44.45 ± 8.69 mgGAE/100 ml), while the amount of Phenolic compounds was (349.10 ± 9.75) when using 0.3% Saccharomyces cerevisiae.[16]

Table 1: The content of TPC and the Reducing power of apple cider vinegar.

Apple Vinegar sample	TPC (g/l GAE)	Reducing Power value expressed as absorbance
V1	2.58 ± 0.09	0.383 ± 0.080
V2	0.026 ± 0.01	0.011 ± 0.001
V3	0.173 ± 0.02	0.174 ± 0.010
M1	0.599 ± 0.1	0.525 ± 0.094
M2	1.49 ± 0.05	0.170 ± 0.074
M3	2.90 ± 0.5	0.883 ± 0.019

Our study showed the variation in antioxidant capacity among study samples, as M3 showed the highest antioxidant capacity (0.883 ± 0.019) as it had the highest amount of phenolic compounds (2.90 ± 0.5 gGAE/l), while V2 was with the lowest antioxidant capacity (0.011 ± 0.001) due to its low content of phenolic compounds (0.026 ± 0.01 gGAE/l). The reduction capacity is directly proportional to the sample's content of phenolic compounds.[8] Similar to our study, the antioxidant capacity of various types of apple cider vinegar was determined using the DPPH method by Ozturk *et al* in Turkey.[17] It was noted that the highest value of antioxidant capacity was due to a sample manufactured using the traditional method, DPPH = 65.12 ± 0.73, and containing the highest amount of phenolic compounds (434.88 mgGAE/l). likewise, it was noted that the sample containing the largest amount of phenolic compounds had the highest antioxidant capacity as measured by the ABTs method (13.27 mmol TEAC/l) according to Budak *et al*.[14] Bakir *et al* in Turkey reported the amount of

phenolic compounds as well as the antioxidant capacity by FRAP, DPPH and ABTs method and compared them for two types of apple cider vinegar. The first type contained an amount of phenolic substances (31 ± 4 mgGAE/100 ml) more than that found in the second type (17 ± 1 mgGAE/100 ml) due to the difference in the raw material, and therefore the antioxidant capacity measured by the DPPH method was for the first type (21 ± 2 mg Trolox/100 ml) higher than that for type 2 (13 ± 1 mg Trolox/100 ml). Antioxidant capacity values varied depending on the measurement method used (FRAP, ABTs, and DPPH).[18]

CONCLUSION

An increase in the amount of phenolic compounds was correlated with an increase in antioxidant capacity. Both of phenolic compounds content and the antioxidant activity varied among the studied samples with the highest amount of phenolic compounds and therefore the highest antioxidant capacity in M3, whereas V2 had the least antioxidant capacity due to the smallest amount of phenolic compounds.

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