



## Phytochemical Content and Antioxidant Activity of Vinegar Prepared from Four Apple Varieties by Different Methods

Mohammed Kara<sup>1\*</sup>, Amine Assouguem<sup>2</sup>, Abdou R. Zerhouni<sup>1</sup>, Jamila Bahhou<sup>1</sup><sup>1</sup>Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVRN), Department of Biology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences, Dhar El Mahraz, Fez, Atlas, Morocco<sup>2</sup>Laboratory of Functional Ecology and Genie of Environment, Faculty of Sciences and Technology, USMBA, Fez, Morocco

### ARTICLE INFO

#### Article history:

Received 01 May 2021

Revised 04 July 2021

Accepted 23 September 2021

Published online 02 October 2021

**Copyright:** © 2021 Kara *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### ABSTRACT

Apple vinegar (AV) contains several phytochemicals with antioxidant properties which make it finds applications in traditional medicine and food preservation. The phytochemical content of AV has been reported to be influenced by several factors. This study was therefore conducted to investigate the effects of production methods and varietal profile on the phytochemical content and antioxidant activity of AV. Four varieties of apple; *Red Delicious* (V1), *Gala* (V2), *Golden Delicious* (V3), and *Starking Delicious* (V4) were employed for the study. The V3 variety was used to prepare AV using three different methods; cutting apple into small pieces (AP), filtering apple juice (AJ), and crushing apple (CA). Then, the vinegar samples were prepared using the AP method for fermenting the four varieties of apple. Total polyphenol, carotenoid, flavonoid, as well as flavone and flavonol contents of the AV were determined. Free radical scavenging activity and total antioxidant capacity were evaluated. The results obtained showed that the V1 variety is rich in flavonoids, and the V2 and V3 varieties are rich in flavones and flavonols, while the V4 variety contains a significant amount of carotenoids. Also, it was observed that the highest antioxidant activity was obtained in the AV prepared from the V1 variety and by CA method with IC<sub>50</sub> values of 770.333 and 75.507 µg/mL, respectively. The highest total antioxidant capacity value of 822.266 µg EAA/mL was recorded for the V4 variety. Therefore, the findings from this study elucidate that varietal profile and production methods influence polyphenol content and antioxidant activity of AV.

**Keywords:** Antioxidant activity, Apple vinegar, Polyphenols, Production method.

### Introduction

Apple vinegar (AV) is a liquid obtained from a double fermentation of apple fruits, produced either by the slow fermentation process using a traditional method (Orleans method) or rapid procedure used in the industry (Submerged technique).<sup>1</sup> It contains several chemical compounds of nutritional value and bioactive molecules such as carotenoids, phytosterols, phenolic compounds, and vitamins.<sup>1-3</sup> Vinegar can be used for several purposes, such as salad condiments or food preservation, and has found applications in the field of traditional medicine as a disinfectant or cosmetic product.<sup>4,5</sup> It can be used to reduce hyperglycemia, correct dyslipidemia, and help to reduce body weight.<sup>6,7</sup> Research on vinegar has shown that it has antioxidant, antibacterial, and antifungal activities, as well as the capacity to inhibit the action of a set of some pathogens.<sup>8,9</sup> The therapeutic properties of vinegar include anti-diabetic, anti-obesity, and anti-cancer effects.<sup>1</sup> It is also recognized for its ability to decrease blood pressure,<sup>10</sup> prevent lipid peroxidation, nucleic acid damage, inflammation, and improve neurological complications in Alzheimer's disease (AD) via regulation of oxidative stress markers.<sup>11</sup>

\*Corresponding author. E mail: [mohammed.kara@usmba.ac.ma](mailto:mohammed.kara@usmba.ac.ma);  
Tel: +212 658742494

**Citation:** Kara M, Assouguem A, Zerhouni AR, Bahhou J. Phytochemical Content and Antioxidant Activity of Vinegar Prepared from Four Apple Varieties by Different Methods. Trop J Nat Prod Res. 2021; 5(9):1578-1585. [doi.org/10.26538/tjnpr/v5i9.9](https://doi.org/10.26538/tjnpr/v5i9.9)

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Polyphenols are ubiquitous in many foods, fruits, drinks, and fruit vinegar. The content of polyphenols and other phytochemicals in AV differs according to several factors, including the raw materials, climate, harvesting method and its storage,<sup>12,13</sup> production methods,<sup>14</sup> container, temperature variation, and shelf life of vinegar.<sup>15</sup> This generally influences the quality and chemical composition of the final product. This study was aimed at determining the effects of production methods and varietal profile on the phytochemical contents and antioxidant activity of apple vinegar.

### Materials and Methods

#### Sources of plant materials

Four varieties of apple; *Red Delicious* (V1), *Gala* (V2), *Golden Delicious* (V3), and *Starking Delicious* (V4) as shown in Table 1 were randomly harvested from Imouzzer Kander, Sefrou Province, region of Fez-Meknes in the Middle Atlas of Morocco and appropriately transported to the SMBA University, Fez, Morocco. The plants were authenticated by Pr. Amina Bari, a Botanist in the same university. The four varieties were used for the production of AV.

#### Sample preparation

The *Golden Delicious* variety was used to produce the AV using three distinct methods. In the first method, the apples were cut into small pieces (AP) with a volume of 8 cm<sup>3</sup>. The second method was based on filtered apple juice (AJ). As for the third method, all the juice components of the crushed apple (CA) were obtained. Then, the vinegar samples were prepared using the AP method for fermenting the four varieties of apples previously designated as V1, V2, V3, and V4. These preparations were deposited in anaerobic and dark conditions for 40 days for alcoholic fermentation by implementing spontaneous microorganisms existing on the fruit surface.

**Table 1:** Geographical locations, dates of sample collection and voucher number of the studied apple samples.

Variety	Sample ID	Location	Date of sample collection	Voucher Number
<i>Red Delicious</i>	V1		September 2019	DGI0918 191
<i>Gala</i>	V2	Imouzzer Kander 33°44'	August 2019	GI08181 92
<i>Golden Delicious</i>	V3	North, 5°01' West.	September 2019	RDI0918 193
<i>Starking Delicious</i>	V4		September 2019	SDI0918 194

The alcohol obtained from the first fermentation was transformed into acetic acid for ten days under aerobic conditions at room temperature ( $22 \pm 3^\circ\text{C}$ ). The final product was filtered manually and stored in opaque glass bottles for later use.

#### Determination of total polyphenol content

The total polyphenol content of the different samples was estimated using the method described by Singleton and Tsai.<sup>16</sup> This method consists of mixing 0.1 mL of Folin-Ciocalteu reagent (25 %) and 0.1 mL of vinegar. The mixture was stirred vigorously using the vortex, and 2 mL of sodium carbonate solution (2 %) were added. After 30 min of incubation at room temperature ( $22 \pm 3^\circ\text{C}$ ), the mixture was measured by spectrophotometry at 760 nm. The results obtained were expressed in  $\mu\text{g}$  of Gallic acid equivalent per mL of vinegar. A series of Gallic acid dilutions were carried out by successive  $1/2$  X dilution in distilled water ranging from 1000  $\mu\text{g}/\text{mL}$  to 30.6  $\mu\text{g}/\text{mL}$  to draw a standard calibration curve:  $y = 1.2603x + 0.0266$  (x: The concentration of Gallic acid equivalent expressed in  $\mu\text{g}$  of gallic acid per mL of vinegar; y: measured absorbance) with a correlation coefficient:  $R^2 = 0.9237$ .

#### Determination of total carotenoid content

The method of Mackinney is based on mixing 50 mL of the fresh sample with 10 mL of 80 % (v/v) aqueous acetone. Then, the mixture was centrifuged and filtered. The absorbance was measured at three wavelengths (470, 663, and 647 nm) after which the concentration of the compounds was determined by the formula below.<sup>17</sup>

$$[\text{Carotenoids}] = (5 \times \text{D}0470) + (2.846 \times \text{D}0663) - (14.87 \times \text{D}0647)$$

#### Determination of total flavonoid content

Yang *et al.* described the procedure for the determination of flavonoids.<sup>8</sup> One millilitre of each sample was added to 1 mL of 2 % aluminium chloride methanolic solution. After 15 min of incubation at room temperature, the absorbance of the samples was measured in a UV-visible spectrophotometer at 430 nm. A similar procedure was repeated for Quercetin at different concentrations. The ethanol and  $\text{AlCl}_3$  methanolic solution represented the controls. All the procedures were performed with three repetitions. The concentrations of flavonoid compounds in the test samples were calculated using a Quercetin calibration curve ( $R^2 = 0.9844$ ).

#### Determination of flavone and flavonol contents

Flavones and flavonols were determined by mixing 0.5 mL of AV in a test tube with 1.5 mL of ethanol, 0.1 mL of methanolic solution of  $\text{AlCl}_3$  (10 %; v/v), 0.1 mL of  $\text{CH}_3\text{COONa}$ , and 2.8 mL of distilled water. The solution was incubated at room temperature for 30 min. A UV-visible spectrophotometer was used to measure the absorbance at 415 nm. All the experimental procedures were repeated three times. The samples' concentration of flavones and flavonols was calculated

using the calibration curve obtained using the Quercetin as standard ( $y = 0.0062x + 0.4289$  and a correlation coefficient,  $R^2 = 0.857$ ).<sup>18</sup>

#### Determination of free radical scavenging activity

The radical-scavenging activity of the different samples was determined by the free radical trapping method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a relatively stable oxidizing agent.<sup>19</sup> The method consisted of the addition of 2 mL of each sample to 0.5 mL of DPPH (0.004 %). Simultaneously, a negative control was prepared by mixing 1 mL of methanol with 0.25 mL DPPH.<sup>8</sup> An absorbance reading was recorded against a positive control (ascorbic acid) at 517 nm after 30 min of incubation in the dark and at room temperature. The test was repeated three times, and the % of inhibition was estimated by applying the following equation:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Where (Abs control) is the absorbance of the control, and (Abs sample) is the absorbance of the sample. The  $\text{IC}_{50}$  (which is the concentration inhibiting 50 % of the reaction) of each sample was determined using a linear regression graph ( $y = 5E-05x + 0.0721$ ;  $R^2 = 0.9769$ ).

#### Determination of total antioxidant capacity

In this method, 25  $\mu\text{L}$  of each sample under study were mixed with 1 mL of liquid reactive solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and four mM ammonium molybdate). The solution was then incubated in a water bath at  $95^\circ\text{C}$  for 90 minutes before measuring its absorbance using a spectrophotometer at 695 nm. The antioxidant capacity was expressed in micrograms of ascorbic acid equivalent per millilitre of vinegar ( $\mu\text{g}$  EAA/mL of the sample) from an ascorbic acid curve. Methanol was used as a negative control.<sup>20</sup>

#### Statistical analysis

To investigate the influence of varietal profile and production methods on the physicochemical properties of AV, the production was repeated three times and the data were presented in the form of graphs using means and standard deviations. Mean comparison was performed with the ANOVA test, followed by a post hoc Tukey test at  $p < 0.05$ . The linear correlation between polyphenol content and vinegar antioxidant potency was obtained by the Pearson correlation coefficient at a significance level at  $p < 0.05$ . Principal Component Analyses were accomplished using Minitab19.1 software, LLC, USA.

## Results and Discussion

### Total polyphenol content of vinegar produced from different varieties of apples

Polyphenols are secondary metabolites found in most plant species, often reflecting the bitter and astringent fruit flavor.<sup>21</sup> Its content in the fruit differs according to the species,<sup>22</sup> varieties,<sup>23</sup> and their geographical origins.<sup>24</sup> As scientific literature reports, procyanidins and condensed tannins are the principal substances of most apple varieties.<sup>25</sup> The results shown in Figure 1 indicated a significant difference between the test varieties ( $p = 3.39 \times 10^{-07}$ ). Vinegar prepared by V1 represents a higher concentration (1462.586  $\mu\text{g}$  Gallic acid equivalent/mL) compared to V4, V3, and V2 with concentrations of 1169.523, 1082.772, and 981.751  $\mu\text{g}/\text{mL}$ , respectively. In general, the polyphenol content in the experimental samples varies between 981.751 and 1462.586  $\mu\text{g}/\text{mL}$ . This range is slightly higher than 988 mg GAE/L described by Sengun *et al.*<sup>26</sup> This difference can be explained by the variation in the phenolic content of the initial material from which they were produced.<sup>23</sup> According to this study, the amount of polyphenols in the *Golden Delicious* (V3) is relatively higher than the *Gala* (V2), which was confirmed by research conducted by Minnocci,<sup>23</sup> on these same varieties with concentrations of 1043 and 560  $\mu\text{g}/\text{mL}$ , respectively. Thus, the total polyphenol content for *Golden Delicious* (V3) samples was lower than that recorded for *Starking Delicious* (V4). These results can be explained by the findings from the study carried out by Francini *et al.*, which showed that *Starking Delicious* contains a higher total polyphenol content than the *Golden Delicious*.<sup>27</sup> Wang *et al.*, found a concentration of 1802.1  $\mu\text{g}/\text{mL}$  in *Golden* and 3184.8  $\mu\text{g}/\text{mL}$  for the *Gala gala* (V2) varieties<sup>28</sup> and between 1143.38 and 1229.32 mg/100

g in the *Starkrimson* and *Top Red* varieties, respectively.<sup>29</sup> Similarly, the results obtained by Ozturket *et al.*, indicated that the difference in vinegar samples was due to the types and nature of the raw material and its origin.<sup>30</sup>

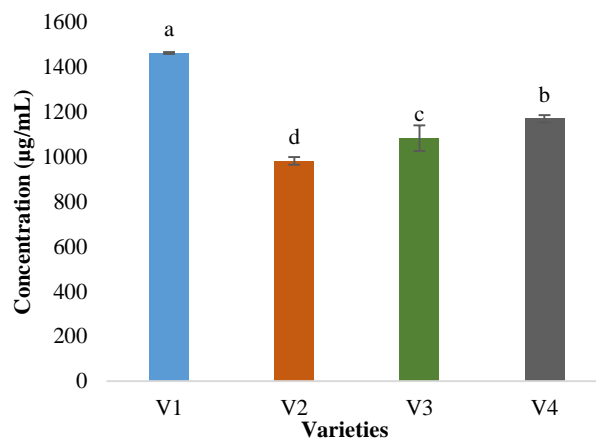
Phenolic compounds can be influenced by various environmental and varietal factors,<sup>31</sup> such as alcoholic fermentation, and acetic fermentation methods.<sup>32</sup> As shown in Figure 2, the vinegar obtained from the CA method represents a high concentration of polyphenols. The observation could be due to the fermentation of the whole fruit composition throughout the production process. This will allow the bioactive compounds to be extracted either through the conditions created by the fermentation or through the effects of natural microorganisms.<sup>33,34</sup> Thus, the AP method is based on the fermentation of apple in its solid-state, which can slow down the extraction of bioactive molecules and makes it difficult to reach all the cells by the enzymes (cellulase and pectinase),<sup>35</sup> secreted spontaneously into the environment by microorganisms. These results can be confirmed by the observation made on the vinegar of apple juice (AJ) which does not exceed 860 µg/mL, which may mean that the elimination of apple pomace, in the beginning, led to a significant loss of Procyanidins and Tannins capable of adsorbing parietal surfaces, polysaccharides, and protein structures by non-covalent bonds.<sup>1,36,37</sup>

It should be noted in this study that the filtration of vinegar samples was carried out by manual pressure filtration during the production process. This procedure indicated that the crushing step allowed polyphenolases (PPO) and polyphenols to directly interact with each other leading to the transformation of polyphenols into other molecules. As a result of this, qualitative and quantitative decrease in the chemical composition of the final vinegar product was achieved.<sup>38</sup> Guyot *et al.* reported that polyphenol concentration decreases during the first alcoholic fermentation from apple to cider.<sup>39,40</sup> During acetification, these phenolic compounds are reduced by 40 % in their initial content in the raw material compared to the red and white vinegar (with a decrease of 13 and 8 %, respectively).<sup>41</sup> Francini and Sebastiani found that polyphenols can reside in apple pomace after juice extraction.<sup>27</sup>

#### Total carotenoid content of vinegar produced from different varieties of apples

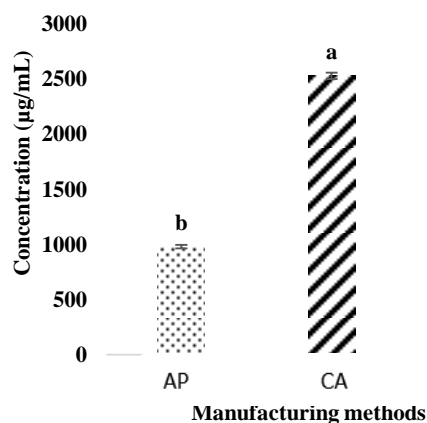
Carotenoids are among the nutrients that the human body cannot synthesize. Their interest in combating severe degenerative diseases such as cancer, coronary heart disease, and Age-related Macular Degeneration (AMD) appears to be very interesting.<sup>42</sup> The contribution of these elements can therefore only be made by plant and animal foods.<sup>43,44</sup> In this study, carotenoids were determined in vinegar made from different varieties of apples. The results obtained revealed that there was a significant difference ( $p=2.51 \times 10^{-09}$ ) between the vinegar of all varieties except for V1 and V2, which appeared identical. Both varieties (V1 and V2) contain a lower concentration of total carotenoids (2.584 and 2.511 µg/mL respectively) than V3 (3.527 µg/mL) and V4 (10.355 µg/mL). Carotenoids are very common pigments in plants, and they appear yellow, red, or orange in color. Delgado-Pelayo *et al.*, showed that *Golden Delicious* has a higher total carotenoid concentration than the *Gala* and *Starking* varieties. Indeed, despite the abundance of carotenoids in the *Starking* variety offered in this study, apples and their by-products remain a resource with low carotenoids compared to other fruits.<sup>45</sup>

To study the effect of manufacturing methods on the carotenoid content in the final vinegar product, three methods mentioned above were used to ferment the variety V1. The results are displayed in Figure 4 and revealed a significant difference ( $p=3.57 \times 10^{-05}$ ) between the AP and CA methods compared to the AJ method. The carotenoid content of AV for the first two methods was higher than for the third method (2.584, 2.741, and 1.415 µg/mL, respectively). This may indicate that a large quantity of these carotenoids was removed during the pressing of the apples to obtain the juice for the AJ method AJ, while for the AP and CA methods, all the compounds of the apple were utilized for the fermentation, which resulted in the extraction of a large number of carotenoids.



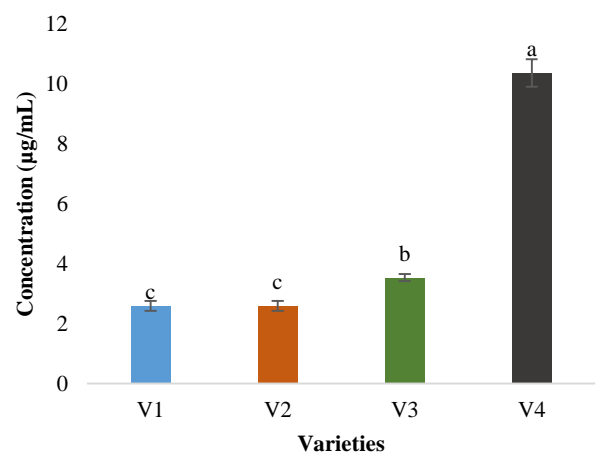
**Figure 1:** Total polyphenol content (µgGAE/mL) of vinegar produced from four varieties of apples.

**V1:** *Red Delicious*; **V2:** *Gala*; **V3:** *Golden Delicious*; **V4:** *Starking Delicious*; a, b, c, and d: Statistically significant results ( $p < 0.05$ ).



**Figure 2:** Total polyphenol content (µgGAE/mL) of vinegar produced from four varieties of apples using different production methods.

**AP:** Pieces of apples; **CA:** Crushed apples; **AJ:** Apple juice; a, b, and c: Statistically significant results ( $p < 0.05$ ).



**Figure 3:** Total carotenoids (µg/mL) of vinegar produced from four varieties of apples.

Thus, the cubic shape of the apples for the AP method may require the presence of enzymes such as cellulase and pectinase that could facilitate its extraction.<sup>35</sup> Pigment content is often higher in the peel (58.72–1510.77 µg/g DW) than in the apple pulp (14.80–71.57 µg/g DW).<sup>45</sup>

#### Total flavonoid content of vinegar produced from different varieties of apples

In addition to the phenolic acids, the flavonoid group is considered among the most abundant bioactive compounds in fruits. The concentration of flavonoids in the samples of this study was determined using a calibration curve ( $R^2 = 0.984$ ). As highlighted in Figure 5, the results displayed a significant difference in the concentration values ( $p=1.12 \times 10^{-09}$ ). Vinegar prepared with V1 variety contains a higher amount of total flavonoids (122.37 µg QE/mL) compared to that recorded for V3, V2, and V4 (95.927, 76.72, and 24.977 µg QE/mL, respectively). Several pieces of research have shown that the concentration of flavonoids in vinegar is often lower than in apples. Sengun *et al.* reported a decreasing order of flavonoid content in AV (174.79 µg QE/mL),<sup>26</sup> Gale gala (132.94 µg QE/100mL), and Golden Delicious (121.24 µg QE/100mL).<sup>28</sup>

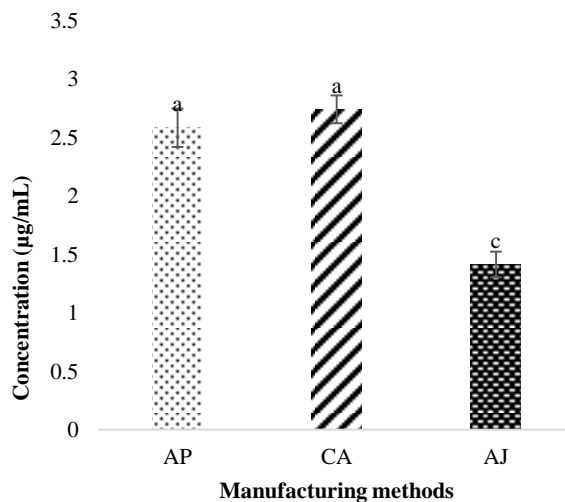
The results presented in Figure 6 indicated an abundance of flavonoids in the vinegar prepared by the AP method compared to those obtained from the CA and AJ methods with values of 122.377, 62.605, and 14.543 µg QE/mL, respectively. In this study, the production methods affected the composition of flavonoid content. This observation indicated that bioactive chemicals in vinegar vary depending on several factors such as the varietal profile and the production procedures stated above.

#### Flavone and flavonol content of vinegar produced from different varieties of apples

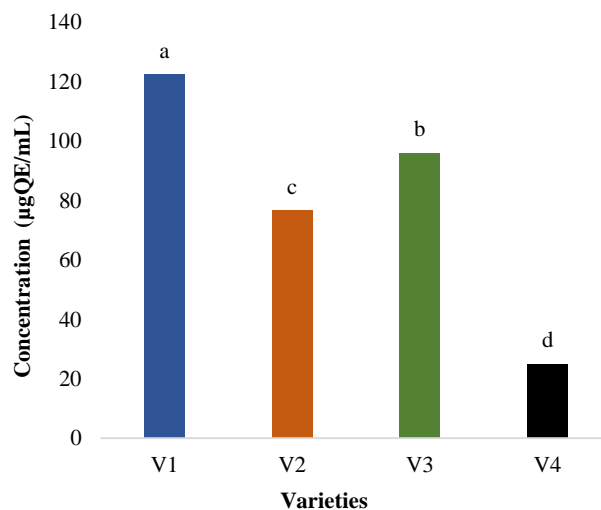
Flavonols (3-hydroxyflavones) and flavones (3-deoxyflavonols) are part of the polyphenol family and are highly responsive in the plant kingdom,<sup>46</sup> at different concentrations depending on several factors. This part of the study consists of measuring flavonols/flavones in vinegar samples produced from different varieties of apples. The results obtained (Figure 7) showed that the vinegar prepared from V2 and V3 varieties showed a significant difference in the amounts of flavonols/flavones (45.96 and 46.02 µg/mL respectively) compared to the concentrations obtained in the V1 and V4 varieties (36.822 and 27.797 µg/mL, respectively;  $p=4.62 \times 10^{-05}$ ). These results showed the effect of varieties on flavone/flavonol content, which is in agreement with the previous study.<sup>23</sup> Based on the results presented in Figure 8, a maximum concentration of flavones/flavonols (72.871 µg/mL) was obtained by the crushed apple (CA) method compared to the AP and AJ methods (36.822 and 25.724 µg/mL, respectively). This observation explains the efficiency of the CA method to obtain a better extract of these flavones/flavonols compounds from apples during the production of vinegar. This method may allow the creation of the conditions for the emergence of these compounds stored in the vacuole of the cells of the plant material.

#### Free radical scavenging activities of vinegar produced from different varieties of apples

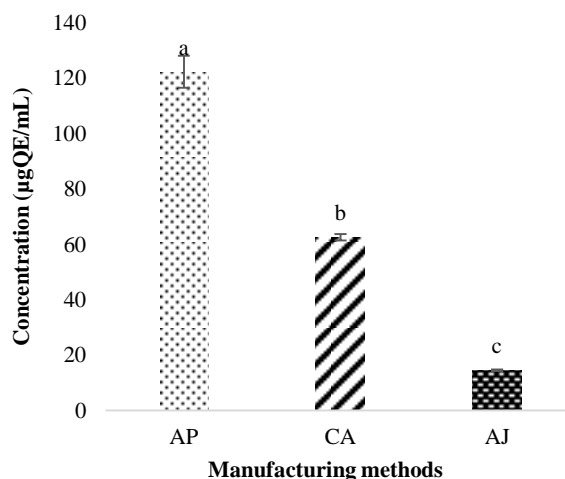
Fruit vinegar is a product rich in bioactive compounds such as polyphenols, carotenoids, and flavonoids, making it one of the most preferred food ingredients compared to synthetic products. The antioxidant power of these compounds is manifested by direct trapping of free radicals by chelation of traces of metal ions involved in its formation by proton atom exchange.<sup>47</sup> After 30 minutes of incubation in the dark, the values obtained from the antioxidant activity test using DPPH indicate the concentration of vinegar required to inhibit 50 % of the antioxidant agent (DPPH).<sup>8</sup> As shown in Figures 9 and 10, there was a significant difference ( $p<0.05$ ) between samples according to the production method and the initial raw material used. Figure 9 shows the DPPH scavenging activity values of vinegar prepared by apple pieces (AP) of different varieties (V1 to V4). The results obtained revealed that the V1 and V3 samples had an inhibition concentrations of 770.333 and 824.051 µg/mL, respectively which were identical to that of the positive control



**Figure 4:** Total carotenoids (µg/mL) of vinegar produced from four varieties of apples using different production methods.



**Figure 5:** Total flavonoids content (µgQE/mL) of vinegar produced from four varieties of apples.



produced from four varieties of apples.

**Figure 6:** Total flavonoids content (µgQE/mL) of vinegar produced from four varieties of apples using different production methods.

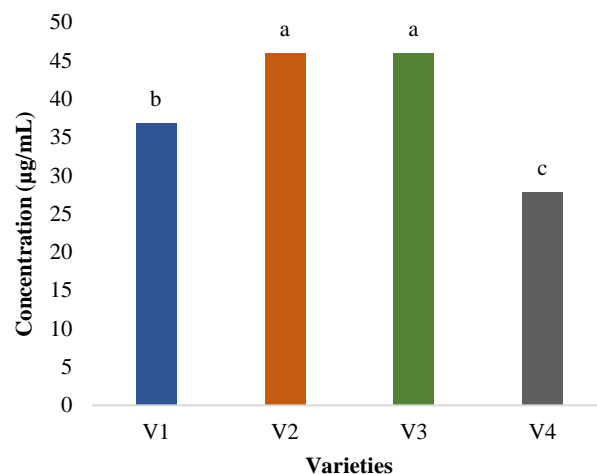
(Vitamin C with  $IC_{50}$  = 748.108  $\mu\text{g/mL}$ ) and slightly lower than the V4 and V2 varieties (with  $IC_{50}$  values of 921.972 and 1599.336  $\mu\text{g/mL}$ , respectively). This indicates that the vinegar of variety V2 had lower antioxidant activity than the other varieties and reference antioxidant. These results are consistent with the finding reported by Francini *et al.*<sup>27</sup> The *Gala* variety possesses a large number of antioxidants found in apple pomace.<sup>27</sup> Thus, according to Bakir *et al.*, AV remains the most defective product in antioxidant compounds than vinegar from other fruits.<sup>48</sup> The positive control (vitamin C) used in this study showed a lower antioxidant activity ( $IC_{50}$  = 748.108  $\mu\text{g/mL}$ ) than that recorded for the four varieties. The results obtained from the vinegar samples prepared by the different methods studied indicated a higher  $IC_{50}$  (951.015  $\mu\text{g/mL}$ ) for the AJ method and a lower  $IC_{50}$  for the CA procedure with a value of 54.648  $\mu\text{g/mL}$  (Figure 10). Therefore, the CA method represents a higher antioxidant capacity. These results can be explained by the phenolic content of these samples involved in neutralizing free radicals of the oxidizing agents used.<sup>49</sup> The contents of polyphenols, flavonoids, and carotenoids may be influenced by the method applied since it appears that the CA method can maintain the antioxidant power of vinegar while the other two procedures, AP and AJ, decrease this power. This observation shows that crushed apple facilitates the extraction of bioactive compounds that react as an antioxidant which gives vinegar a very high antioxidant power. According to Bakir *et al.*, the antioxidant activity of AV prepared by the traditional method was higher than that prepared by the submerged method.<sup>48</sup> Several pieces of research have shown that antioxidant activity is highly correlated with the total polyphenol content of samples,<sup>27</sup> and each polyphenol has a different antioxidant potential depending on its chemical structure.<sup>50</sup> In addition to the enzymes involved in protecting the body against oxidizing agents, certain amino acids exist in vinegar that can act as antioxidant agents such as Histidine, Glycine, Alanine, Tyrosine, and Lysine.<sup>51-53</sup> Catechins, epicatechin, and chlorogenic acid,<sup>27</sup> procyanidins,<sup>50</sup> and carotenoids are considered to be among the most potent antioxidants in plants compared to vitamin C.<sup>54</sup>

#### Total antioxidant capacity of vinegar produced from different varieties of apples

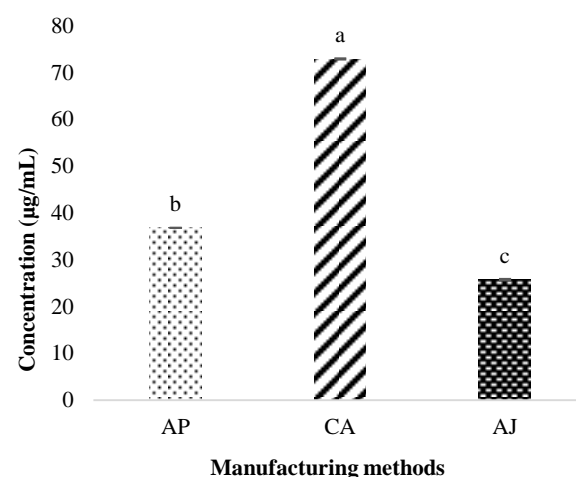
The total antioxidant capacity (TAC) shown by the molybdate-based method indicated that the capacity of the V4 variety (822.622  $\mu\text{g EAA/mL}$ ) had higher activity than that recorded for the other varieties; V3, V2, and V1 (700.681, 536.468, and 136.409  $\mu\text{g EAA/mL}$ , respectively) as presented in Figure 11. From Figure 12, it was observed that the vinegar prepared by the CA method had a TAC (674.460  $\mu\text{g EAA/mL}$ ) higher than vinegar obtained by the AP and AJ methods (536.468 and 113.118  $\mu\text{g EAA/mL}$ , respectively).

#### Relationships between different parameters of the apple vinegar samples

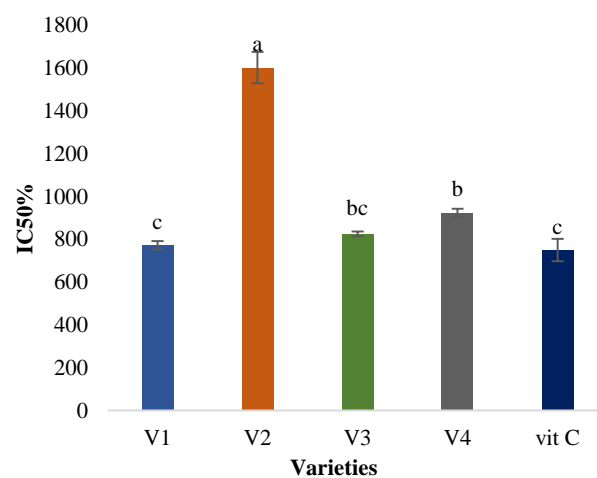
The coefficients of Pearson's correlation between the different parameters of samples used in this research showed a strong positive correlation between flavonoids and polyphenols ( $r = 0.838$ ) and a negative correlation between the two latter parameters and DPPH ( $r = -0.771$ ). Simultaneously, carotenoids and TAC were positively correlated with a coefficient  $r = 0.631$  (Table 2). The results of the PCA obtained in Figure 13 highlighted that the eigenvalues of the first two principal components represent 72.4 % of the variation in the data. The first principal component shows a strong positive association with polyphenols and flavonols/flavones. The second component shows a strong negative association with flavonoids and a positive association with flavonoids and TAC. The projection of the scoring diagram and the contribution diagram visually shows a strong positive contribution of CA on the first main axis in positive correlation with flavonoids and polyphenols and negatively with DPPH. Therefore, it reflects an important content of bioactive compounds that contribute to the strong antioxidant activity indicated by low values of  $IC_{50}$  (DPPH).<sup>55</sup> The second component is positively correlated with the carotenoids and TAC and strongly contributing to the V4. According to research conducted by Young *et al.*,<sup>44</sup> this correlation can indicate that the richness of V4 in carotenoids reflects a very high total antioxidant capacity indicated by TAC values.



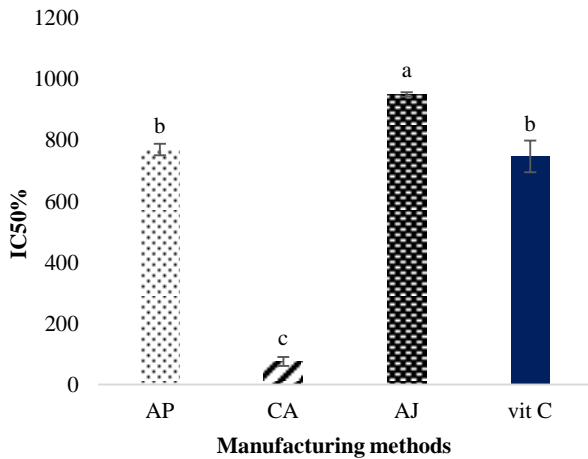
**Figure 7:** Content of flavonols and flavones ( $\mu\text{gQE/mL}$ ) of vinegar produced from four varieties of apples.



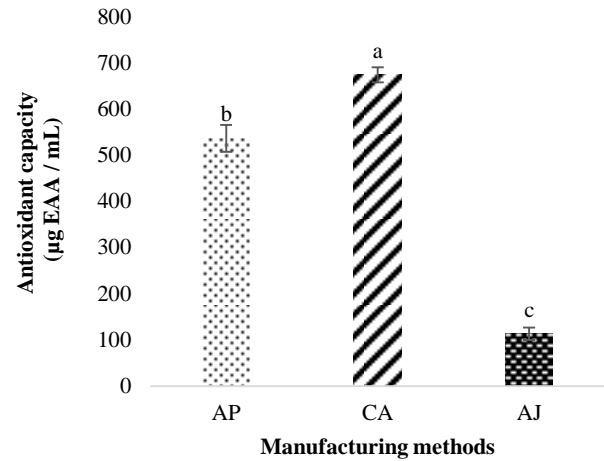
**Figure 8:** Flavonols and flavones contents ( $\mu\text{gQE/mL}$ ) of vinegar produced from four varieties of apples using different production methods



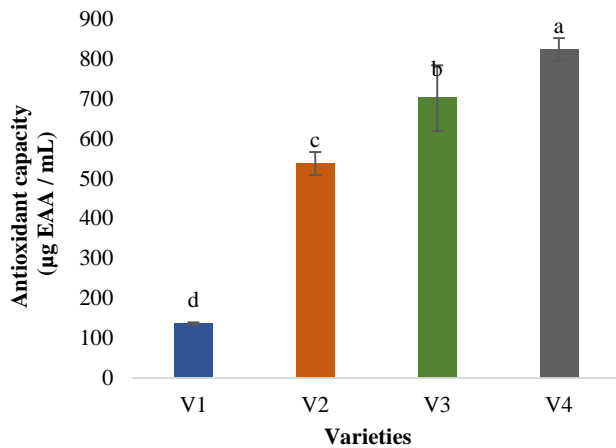
**Figure 9:** Antioxidant activity ( $IC_{50}$ ) of vinegar produced from four varieties of apples.



**Figure 10:** Antioxidant activity (IC<sub>50</sub>) of vinegar produced from four varieties of apples using different production methods.



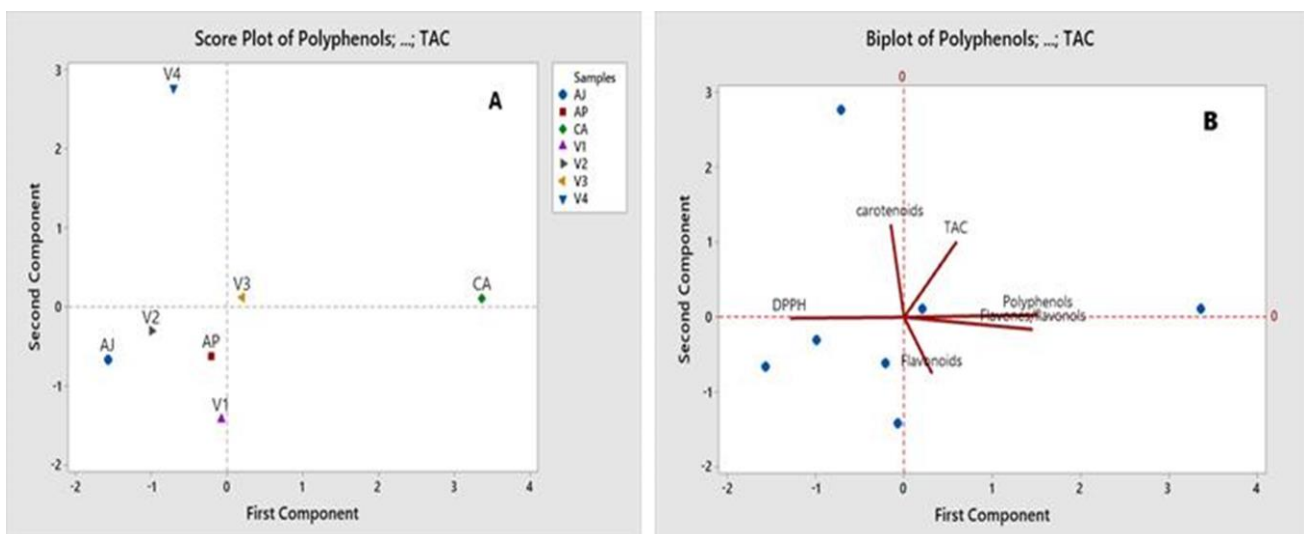
**Figure 12:** Total antioxidant activity (µg EAA/ml) of vinegar produced from four varieties of apples using production methods.



**Figure 11:** Total antioxidant activity (µg EAA/ml) of vinegar produced from four varieties of apples.

### Conclusion

Bioactive compounds have an important role as antioxidants. The content of these compounds in AV varies according to several factors. Indeed, in this research, it has been shown that flavonoid content is higher in the V1 variety. For carotenoids, their abundance was recorded in the V4 variety sample. Regarding the effect of production methods, it was discovered that the AP method produced the maximum extraction of flavonoids, flavones/flavonols, and polyphenols. It was shown through the results obtained that the V4 variety and the CA method revealed a higher antioxidant activity than that recorded for the other samples. The choice of the method to produce AV can contribute to obtaining vinegar with a high nutritional value which is rich in bioactive molecules. The future direction to this study is the structure identification of chemical components and the investigation of the relationship between the chemical structures and their biological activities.



**Figure 12:** Principal component analysis of different studied parameters.

A: Contribution Score Chart for the First 2 Components; B: Double projection diagram for the first two components; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAC: Total antioxidant activity.

**Table 2:** Pearson correlation coefficients between various phytochemical parameters of different studied vinegar samples

	Polyphenols	Carotenoids	Flavones/flavonols	Flavonoids	DPPH*	TAC*
Polyphenols	1.000					
Carotenoids	-0.039	1.000				
Flavones/flavonols	<b>0.838</b>	-0.279	1.000			
Flavonoids	0.043	-0.382	0.245	1.000		
DPPH *	<b>-0.771</b>	0.021	<b>-0.521</b>	-0.046	1.000	
TAC *	0.228	<b>0.631</b>	0.369	-0.072	-0.132	1.000

\* DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAC: Total antioxidant activity.

### Conflict of interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

### Acknowledgements

The authors acknowledge Covenant University, Ota; Ogun State, Nigeria, and the Enzyme Division of the Federal Institute of Industrial Research, Oshodi (FIRO) Lagos State, Nigeria, for the use of their facilities. The authors also thank Folake Fashola, Olubunmi I. Ibidapo, Ifeoma C. James, Vera O. Ofoegbu for helping out in media preparation, and enzyme assay.

### References

- Guiné RPF, Barroca MJ, Coldea TE, Bartkiene E, Anjos O. Apple Fermented Products: An Overview of Technology, Properties and Health Effects. *Proc.* 2021; 9(2):223-248.
- Charoenkiatkul S, Thiyajai P, Judprasong K. Nutrients and bioactive compounds in popular and indigenous durian (*Durio zibethinus murr.*). [online]. 2016 [cited 2019 Aug 26]; Available from: <http://www.sciencedirect.com/science/article/pii/S0308814615002988>
- El Barnossi A, Moussaid F, Iraqi Housseini A. Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review. *Biotechnol Rep* [online]. 2021 [cited 2021 Aug 16]. Available from: <https://www.sciencedirect.com/science/article/pii/S2215017X20309863>
- Pianta L, Vinciguerra A, Bertazzoni G, Morello R, Mangiardi F, Lund VJ, Trimarchi M. Acetic acid disinfection as a potential adjunctive therapy for non-severe COVID-19. *Eur Arch Otorhinolaryngol.* 2020; 277(10):1-4.
- Tripathi S, Mazumder PM. Apple Cider Vinegar (ACV) and their Pharmacological Approach towards Alzheimer's Disease (AD): A Review. *Indian J Pharm Educ Res.* 2020; 54(2):67-74.
- Morgan J and Shibebe S. The Potential of Apple Cider Vinegar in the Management of Type 2 Diabetes. *J Diabet Res.* 2016; 5(6): 129-134.
- Ho CW, Lazim AM, Fazry S, Zaki UKHH, Lim SJ. Varieties, production, composition and health benefits of vinegars: A review. *Food Chem.* 2017; 221(60): 1621-1630.
- 8Yang J-F, Yang C-H, Liang M-T, Gao Z-J, Wu Y-W, Chuang L-Y. Chemical Composition, Antioxidant, and Antibacterial Activity of Wood Vinegar from Litchi chinensis. [online]. 2016 [cited 2019 Sep 2]. Available from: <https://www.mdpi.com/1420-3049/21/9/1150>
- Watson RR, Preedy VR, Zibadi S. *Polyphenols: Mechanisms of Action in Human Health and Disease.* (2nd Ed.). USA: Academic Press; 2018. 470 p.
- Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, Sato Y. Acetic Acid Feeding Enhances Glycogen Repletion in Liver and Skeletal Muscle of Rats. [online]. 2001 [cited 2019 Aug 31]. Available from: <https://academic.oup.com/jn/article/131/7/1973/4686778>
- Tripathi S, Kumari U, Mazumder PM. Ameliorative effects of apple cider vinegar on neurological complications via regulation of oxidative stress markers. *J Food Biochem* [online]. 2020 [cited 2021 Aug 16]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/jfbc.13504>
- Kschonsek J, Wolfram T, Stöckl A, Böhm V. Polyphenolic Compounds Analysis of Old and New Apple Cultivars and Contribution of Polyphenolic Profile to the In Vitro Antioxidant Capacity. *Antioxidants* [online]. 2018 [cited 2021 Aug 16]. Available from: <https://www.mdpi.com/2076-3921/7/1/20>
- Geană E-I, Ciucure CT, Ionete RE, Ciocârlan A, Aricu A, Fica A, Andronescu E. Profiling of Phenolic Compounds and Triterpene Acids of Twelve Apple (*Malus domestica* Borkh.) Cultivars. *Foods* [online]. 2021 [cited 2021 Aug 16]. Available from: <https://www.mdpi.com/2304-8158/10/2/267>
- Kokkinomagoulos E, Nikolaou A, Kourkoutas Y, Kandyli P. Evaluation of Yeast Strains for Pomegranate Alcoholic Beverage Production: Effect on Physicochemical Characteristics, Antioxidant Activity, and Aroma Compounds. *Microorganisms* [online]. 2020 [cited 2021 Aug 16]. Available from: <https://www.mdpi.com/2076-2607/8/10/1583>
- Zhang H, He P, Kang H, Li X. Antioxidant and antimicrobial effects of edible coating based on chitosan and bamboo vinegar in ready to cook pork chops. *LWT - Food Sci Tech.* 2018; 93(26):470-476.
- Bisognin DA, Luz LVD, Lencina KH, Santos COD, Sautter CK. Contents of total phenolics and flavonoids in and antioxidant activity of *Ilex paraguariensis* leaves. [online]. 2019 Available from: <https://dx.doi.org/10.1590/s1678-3921.pab2019.v54.00856>.
- Mackinney G. Absorption of light by chlorophyll solutions. *J Biol Chem.* 1941; 140(2):315-322.
- Ivan K, Marina B, Stjepan P, Sanda V-K. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta pharmaceutica (Zagreb, Croatia).* *Acta Pharm.* 2004; 54(1):65-72.
- Amarowicz R, Pegg RB. Chapter One - Natural antioxidants of plant origin. In: Ferreira ICFR, Barros L, editors. *Advances in Food and Nutrition Research.* USA: Academic Press. 2019; 1-81p.
- Agour A, Mssillou I, Saghrouchni H, Bari A, Badiia L, Derwich E. Chemical Composition, Antioxidant Potential and Antimicrobial Properties of the Essential Oils of *Haplophyllum tuberculatum* (Forsskal) A. Juss from Morocco. *Trop J Nat Prod Res.* 2021; 4(12): 1108-1115.

21. Cheyner V. Polyphenols in foods are more complex than often thought. *Am J Clin Nutr.* 2005; 81(1):223-229.
22. Sakanaka S and Ishihara Y. Comparison of antioxidant properties of persimmon vinegar and some other commercial vinegars in radical-scavenging assays and on lipid oxidation in tuna homogenates. *Food Chem.* 2008; 107(2):739-744.
23. Minnocci A, Iacopini P, Martinelli F, Sebastiani L. Micromorphological, biochemical, and genetic characterization of two ancient, late-bearing apple varieties. *Eur J Horticult Sci.* 2010; 75(1):1-7.
24. McGhie TK, Hunt M, Barnett LE. Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. *J Agric Food Chem.* 2005; 53(8):3065-3070.
25. Guyot S, Le Bourvellec C, Marnet N, Drilleau JF. Procyanidins are the most Abundant Polyphenols in Dessert Apples at Maturity. *LWT - Food Sci Technol.* 2002; 35(3):289-291.
26. Sengun IY, Kilic G, Ozturk B. Screening physicochemical, microbiological and bioactive properties of fruit vinegars produced from various raw materials. *Food Sci Biotechnol.* 2020; 29(3):401-408.
27. Francini A and Sebastiani L. Phenolic Compounds in Apple (*Malus x domestica* Borkh.): Compounds Characterization and Stability during Postharvest and after Processing. *Antioxidants.* 2013; 2(3):181-193.
28. Wang X, Li C, Liang D, Zou Y, Li P, Ma F. Phenolic compounds and antioxidant activity in red-fleshed apples. *J Funct Foods.* 2015; 18(Part B):1086-1094.
29. Sethi S, Joshi A, Arora B, Bhowmik A, Sharma RR, Kumar P. Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. *Eur Food Res Technol.* 2020; 246(3):591-598.
30. Ozturk I, Caliskan O, Tornuk F, Ozcan N, Yalcin H, Baslar M, et al. Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. *LWT - Food Sci Technol.* 2015; 63(1):144-151.
31. Deng G-F, Lin X, Xu X-R, Gao L-L, Xie J-F, Li H-B. Antioxidant capacities and total phenolic contents of 56 vegetables. *J Funct Foods.* 2013; 5(1):260-266.
32. Raspor P and Goranovič D. Biotechnological Applications of Acetic Acid Bacteria. *Crit Rev Biotechnol.* 2008; 28(2):101-124.
33. Karabiyikli S and Sengun I. Beneficial Effects of Acetic Acid Bacteria and Their Food Products. In: *Acetic Acid Bacteria.* États-Unis: CRC Press; 2017:22 p.
34. Lynch KM, Zannini E, Wilkinson S, Daenen L, Arendt EK. Physiology of Acetic Acid Bacteria and Their Role in Vinegar and Fermented Beverages. *Comp Rev Food Sci Food Saf.* 2019; 18(3):587-625.
35. Saini RK and Keum Y-S. Carotenoid extraction methods: A review of recent developments. *Food Chem.* 2018; 240(60):90-103.
36. Renard CMGC, Baron A, Guyot S, Drilleau J-F. Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int J Biol Macromol.* 2001; 29(2):115-125.
37. Le Bourvellec C, Guyot S, Renard CMGC. Non-covalent interaction between procyanidins and apple cell wall material: Part I. Effect of some environmental parameters. *Biochim Biophys Acta BBA - Gen Subj.* 2004; 1672(3):192-202.
38. Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot M-J, Aubert SY. Enzymatic browning reactions in apple and apple products. *Crit Rev Food Sci Nutr.* 1994; 34(2):109-157.
39. Wicklund T, Skottheim ER, Remberg SF. Various factors affect product properties in apple cider production. *Int J Food Stud.* 2020; 9(17):84-96.
40. Symoneaux R, Le Quére J-M, Baudin R. Les Polyphénols de la pomme aux cidres : diversité variétale et procédés, facteurs clé de la modulation des saveurs et des couleurs. *Innov Agron.* 2014; 42(8):105-123.
41. Andlauer W, Stumpf C, Fürst P. Influence of the Acetification Process on Phenolic Compounds. *J Agric Food Chem.* 2000; 48(8):3533-3536.
42. Pennington KL and DeAngelis MM. Epidemiology of age-related macular degeneration (AMD): associations with cardiovascular disease phenotypes and lipid factors. *Eye Vis.* 2016; 3(1):1-20.
43. Choubert G. Les pigments caroténoïdes: nature, origine et importance métabolique. I. Caroténoïdes et pigmentation. *Caroténoïdes Pigment.* Paris: Actes du Colloque CNERNA. 1979. 376 p.
44. Young AJ and Lowe GL. Carotenoids—Antioxidant Properties. *Antioxidants* [online]. 2018 [cited 2021 Sep 1]. Available from: <https://www.mdpi.com/2076-3921/7/2/28>
45. Delgado-Pelayo R, Gallardo-Guerrero L, Hornero-Méndez D. Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Res Int.* 2014; 65(23):272–281.
46. Kaurinovic B and Vastag D. Flavonoids and Phenolic Acids as Potential Natural Antioxidants. [online]. 2019 [cited 2021 jul 16]. Available from: <https://www.intechopen.com/chapters/65331>
47. Belščak-Cvitanović A, Durgo K, Huđek A, Bačun-Družina V, Komes D. 1 - Overview of polyphenols and their properties. In: Galanakis CM, editor. *Polyphenols: Properties, Recovery, and Applications.* Woodhead Publishing; 2018; 3-44 p.
48. Bakir S, Devcioglu D, Kayacan S, Toydemir G, Karbancioglu-Guler F, Capanoglu E. Investigating the antioxidant and antimicrobial activities of different vinegars. *Eur Food Res Technol.* 2017; 243(12): 2083–2094.
49. Alonso ÁM, Castro R, Rodríguez MC, Guillén DA, Barroso CG. Study of the antioxidant power of brandies and vinegars derived from Sherry wines and correlation with their content in polyphenols. [online]. 2004 [cited 2019 Aug 31]. Available from: <http://www.sciencedirect.com/science/article/pii/S0963996904000730>
50. Plumb GW, De Pascual-Teresa S, Santos-Buelga C, Cheyner V, Williamson G. Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. *Free Radic Res.* 1998; 29(4):351-358.
51. Shahidi F. *Natural Antioxidants: Chemistry, Health Effects, and Applications.* (1<sup>st</sup> ed). Urbana: The American Oil Chemists Society; 1997; 436p.
52. Wu H-C, Shiau C-Y, Chen H-M, Chiou T-K. Antioxidant Activities of Carnosine, Anserine, Some Free Amino Acids and Their Combination. *J Food Drug Anal.* 2003; 11(2):148-153.
53. Tamang JP. *Health Benefits of Fermented Foods and Beverages.* (1<sup>st</sup> ed). United States: CRC Press; 2015; 624 p.
54. Lee KW, Kim YJ, Kim D-O, Lee HJ, Lee CY. Major Phenolics in Apple and Their Contribution to the Total Antioxidant Capacity. *J Agric Food Chem.* 2003; 51(22):6516-6520.
55. Kiani R, Arzani A, Maibody MSAM. Polyphenols, Flavonoids, and Antioxidant Activity Involved in Salt Tolerance in Wheat, *Aegilops cylindrica* and Their Amphidiploids. [online]. 2021 [cited 2021 Jul 18]. Available from: <https://www.frontiersin.org/articles/10.3389/fpls.2021.646221/full>.