ANTIOXIDANT PROPERTIES EVALUATION FOR DIFFERENT TYPES OF APPLE VINEGAR WITH UNALCOHOLIC RED WINE CONCENTRATES ADDITION

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Key words: apple vinegar, total antioxidant capacity, polyphenols content, monomeric anthocyanins, red wine concentrates

Abstracts

In this paper it was obtained the apple vinegar with addition of red wines concentrates in different percents for to improve the antioxidant properties. For processing of red wine concentrates it was used the young red wines Merlot and Cabernet Sauvignon. For resulted vinegar types were analyzed total acidity, extract, total antioxidant capacity (using FRAP method), total polyphenols amount (by Folin-Ciocalteu method) and monomeric anthocyanins (using pH-differential method).

Polyphenols content from vinegar with Cabernet Sauvignon concentrates addition was situated in the range 0.74-3.42 mM gallic acid/L and for vinegar with red wine Merlot concentrates addition between 0.74-2.64 mM gallic acid/L. The antioxidant capacity was presented the values between 0.45-8.18 mM Fe²⁺/L for apple vinegar with Cabernet Sauvignon concentrates addition and between 0.45-6.69 mM Fe²⁺/L for vinegar with Merlot concentrate addition. The polyphenols content and monomeric anthocyanins content of apple vinegars with red wine concentrates increase in rapport with the percent of red wines concentrates added. The values of polyphenols content and total antioxidant capacity were more with approximate 20% in the case of vinegar with Cabernet Sauvignon concentrates addition comparatively with the case of vinegar with Merlot concentrates addition.

INTRODUCTION

Recent scientific investigations clearly demonstrate the antimicrobial properties of vinegar, but mainly in the context of food preparation. The consumption of fruit and vegetables, and all the food and drink derived from these, has been inversely associated with morbidity and mortality from degenerative and coronary heart diseases [Block et al., 1992; Szeto et al., 2002]. The protection that fruit and vegetables provide against diseases has been attributed to the various antioxidants contained in them particularly polyphenols that have received considerable attention for their antioxidant activity and potential health benefits. The wine vinegars are rich in polyphenols being derivative products of wine, and also exhibit potential health benefits, such as the antihypertensive effects observed in rats [Ou et al., 2002; Proteggente et al., 2002].

The chemical and organoleptic properties of vinegars are a function of the starting material and the fermentation method. Acetic acid, the volatile organic acid that identifies the
product as vinegar, is responsible for the tart flavor and pungent, biting odor of vinegars. However, acetic acid should not be considered synonymous with vinegar. Other constituents of vinegar include vitamins, mineral salts, amino acids, polyphenolic compounds (eg, gallic acid, catechin, caffeic acid, ferulic acid), and nonvolatile organic acids (eg, tartaric, citric, malic, lactic). Vinegars are also a dietary source of polyphenols, compounds synthesized by plants to defend against oxidative stress. Ingestion of polyphenols in humans enhances in vivo antioxidant protection and reduces cancer risk [Gey, 1990].

Specialty vinegars are grouped as herbal or fruit vinegars. Herbal vinegars consist of wine vinegars or white distilled vinegars, which may be seasoned with garlic, basil, tarragon, cinnamon, clove, or nutmeg. Fruit vinegars are wine and white vinegars sweetened with fruit or fruit juice to produce a characteristic sweet-sour taste. Epidemiologic data, demonstrated that vinegar ingestion was associated with a decreased risk for esophageal cancer, the vinegar ingestion has the antiglycemic effect in insulin-resistant subjects [Dávalos et al., 2005; Verzelloni et al., 2006].

Currently, much interest surrounds the role of dietary polyphenols, particularly from fruits, vegetables, wine in the prevention of cancers as well as other conditions including cardiovascular disease; perhaps vinegar can be added to this list of foods and its consumption evaluated for disease risk [Simonetti et al. 1997; Landrault et al., 2001; Scalbert et al., 2005].

The young red wines are rich in polyphenolic substances that have the antioxidant properties. The red wine extracts contain many compounds with antioxidant role. By addition of these extracts at different products the antioxidant properties of this is improving.

The purpose of our study is the obtaining of some concentrates from red wine and their addition in apple vinegar. The antioxidant properties of obtained vinegars are correlated with the content in polyphenolic compounds.

MATERIAL AND METHOD

**Apple vinegar processing.** In the present research, for obtaining apple vinegar, apples are washed, damaged parts are taken away and then fruits are cut and are used all parts. Could be used all parts remains from fruits canning. All cut apples are put in a proper recipient; on fruits is pored warm water, which before was boiled (0.5 L water to 0.4 kg apples). For every liter of water is added 100 g honey. To accelerate acetic fermentation, on each L of water is added 120g yeast and 20g black bread. The recipient with this mixture is storage in a room at 20-30°C. Acetic fermentation is favored by a small alcohol content liquid (bellow 20% sugars), a constant temperature of 20°C and a big contact area of mixture with air (aerobe fermentation). The recipient is from glass, wood or ceramics.

For the first phase of fermentation the recipient is stored in a warm environment for 10 days at 20-30°C, mixing twice a day, and then is filtered by pressing. Obtained juice is filtered again, the volume is measured and is stored in a recipient with a big contact area with air. If it’s necessary is added 50-100g honey or sugar for every liter of juice and it’s very good homogenized.

For the second phase of fermentation the recipient is stored in a warm place to continue the fermentation. The fermentation is finished when the liquid is clear. Depending on juice preparation, temperature, etc the vinegar will be done in 40-60 days. After that vinegar is filtered, bottled, waxed and stored in a cool place (4-10°C).

In obtained apple with honey vinegar it was adding the unalcoholic red wine concentrates in followed percents: 0.5; 1; 2; 3; 4; 5 (v/v). For processing of this concentrates the 1 liter for each young red wines from Silagiu (Buzias) vineyard, obtained in 2006 were
concentrated in vacuum condition in the view of alcohol and water removing until to obtain a viscous red dark that was used for improving the antioxidant properties of apple vinegar.

**Reagent and equipment:** All chemicals and reagents were analytical grade or pure quality purchased from Merck, Fluka, Sigma and Chimopar. For reagents preparation and for dilution was used bidistilled water. Absorption determination for FRAP and total polyphenol content was made using Spectrophotometer Specord 205 by Analitik Jena.

**Total antioxidant capacity** will be determined by FRAP assay [Benzie&Strain, 1996]. The total antioxidant capacity in wine samples in mM Fe$^{2+}$/L was calculated. For antioxidant capacity analyse the dilution was 1:50 (v:v). Calibration curve equation was: $Y= -0,02404+3,41362 \cdot X$. Correlation coefficient for calibration curve was $R=0,9991$.

**Total polyphenolic compounds content** was determined by spectroscopic modified method Folin-Ciocalteu and was expressed in (mM gallic acid /L) [Singleton et al., 1999]. For polyphenol analyse the dilution was 1:20 (v:v). Calibration curve equation was: $Y= -0,10164+1,92242 \cdot X$. Correlation coefficient for calibration curve was $R=0,9980$.

**Total monomeric anthocyanins content** will be determined by the pH-differential method [Giusti&Wrolstad, 2000]. Monomeric anthocyanin pigment (mg/L) will be calculated as cyanidin-3-glucoside. For anthocyanins determination the dilution was 1:15 (v:v).

**Analysis of red wine color** is realised in accord with Boulton modified Sommers method [Boulton, 2001]. On the base of this analysis it was quantified the percent of color due to monomeric anthocyanins, copigmented anthocyanins and polymeric pigments.

**RESULTS AND DISCUSSIONS**

From the data showed in the table 1 it was observed that for the Cabernet Sauvignon and Merlot red wine from 2006 harvest year’s the majority color is due to monomeric anthocyanins class (in the range 40-43%). The class of polymeric pigment participate to the total wine color with 24-30% and copigmented anthocyanins with a percent between 29-32%.

From this data is evident that the color of red wine is unstable, because for chromatic stabilized red wines the percent of polymeric pigments is between 75-90%, and the percent of monomeric and copigmented anthocyanins is small. During winemaking and aging, anthocyanins from the skins are thought to react with tannins from the skins and seeds to give rise to polymeric pigments, the stable color compounds in wine. The polymeric pigment can be form on the base of diminishing of amount of monomeric and copigmented anthocyanins. Because they have a high content of anthocyanins and polyphenols (table 2), the antioxidant properties of this is strong. The antioxidant properties of Cabernet Sauvignon wine are more evident than Merlot wine. The unalcoholic concentrates obtained from this young red contain the all these compounds with antioxidant role.

Therefore, these red wines varieties can be successfully used for concentrates obtaining because these wines have the astringent taste, high polyphenolic quantities, are unstable chromatic. The resulted concentrates have the agreeable and strong odor, viscous aspect, red dark color and contain the all constituent that form the wine extract. Owing to this reason, the unalcoholic concentrates are used for improving the antioxidant and organoleptic (especially the color, taste and smell) of some food products: grape juices weakly colored, vinegars, syrups, other beverages categories. In this case, through addition of obtained concentrates, the apple vinegar have the color from weakly pink (the vinegars with 0.5 and 1% concentrates) to red (the vinegar with 5% concentrate addition). The acid taste of obtained vinegars are ameliorated, the odor became agreeable typical of black grapes.
From the data showed in the table 3 and 4 result that the polyphenols content from vinegar with red wine Cabernet Sauvignon concentrates addition is situated in the range 0.74-3.42 mM gallic acid/L and for vinegar with red wine Merlot concentrates addition between 0.74-2.64 mM gallic acid/L.

Table 1. The chromatic structure for red wine Cabernet Sauvignon and Merlot

<table>
<thead>
<tr>
<th>No. crt.</th>
<th>Wine variety</th>
<th>Polymeric pigments (%)</th>
<th>Monomeric anthocyanins (%)</th>
<th>Copigmented anthocyanins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cabernet Sauvignon (CS)</td>
<td>24.18</td>
<td>43.65</td>
<td>32.17</td>
</tr>
<tr>
<td>2</td>
<td>Merlot (M)</td>
<td>30.55</td>
<td>40.21</td>
<td>29.24</td>
</tr>
</tbody>
</table>

Table 2. Antioxidant properties for red wine Cabernet Sauvignon and Merlot

<table>
<thead>
<tr>
<th>No. crt.</th>
<th>Wine variety</th>
<th>Polyphenols (mM acid gallic/L)</th>
<th>Total antioxidant capacity (mM Fe²⁺/L)</th>
<th>Monomeric anthocyanins (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cabernet Sauvignon (CS)</td>
<td>27.87</td>
<td>31.15</td>
<td>231.01</td>
</tr>
<tr>
<td>2</td>
<td>Merlot (M)</td>
<td>21.21</td>
<td>25.09</td>
<td>173.05</td>
</tr>
</tbody>
</table>

Table 3. The values of analyzed parameters for apple vinegar with Cabernet Sauvignon concentrate addition

<table>
<thead>
<tr>
<th>Investigated parameters</th>
<th>Vinegar type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AV</td>
</tr>
<tr>
<td>Total acidity (g acetic acid/100 mL)</td>
<td>5</td>
</tr>
<tr>
<td>Extract (g/100 mL)</td>
<td>0.21</td>
</tr>
<tr>
<td>Total antioxidant capacity (mM Fe²⁺/L)</td>
<td>0.45</td>
</tr>
<tr>
<td>Polyphenols (P) (mM acid gallic/L)</td>
<td>0.74</td>
</tr>
<tr>
<td>Monomeric anthocyanins (mg/L)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table 4. The values of analyzed parameters for apple vinegar with Merlot concentrate addition

<table>
<thead>
<tr>
<th>Investigated parameters</th>
<th>Vinegar type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AV</td>
</tr>
<tr>
<td>Total acidity (g acetic acid/100 mL)</td>
<td>5</td>
</tr>
<tr>
<td>Extract (g/100 mL)</td>
<td>0.21</td>
</tr>
<tr>
<td>Total antioxidant capacity (mM Fe²⁺/L)</td>
<td>0.45</td>
</tr>
<tr>
<td>Polyphenols (P) (mM acid gallic/L)</td>
<td>0.74</td>
</tr>
<tr>
<td>Monomeric anthocyanins (mg/L)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Total antioxidant capacity for vinegar with Cabernet Sauvignon concentrate addition was superior given the Merlot concentrate addition. The values for polyphenols amount and antioxidant capacity are more with approximate 20% in the case of vinegar with Cabernet
Sauvignon concentrates addition comparatively with the case of vinegars with Merlot concentrates addition. The biggest values it was observed in the case of vinegar with 5% Cabernet Sauvignon concentrate addition. From these values results that the antioxidant properties of raw red wines have the strong influence about antioxidant properties of vinegar types obtained.

The polyphenols content has the same evolution direction of with total antioxidant capacity. After processing the obtained data with Origin Program between these parameters it was observed a linear correlation \( R=0.9958 \) for the case of Cabernet Sauvignon concentrate addition, fig. 1, and \( R=0.9907 \) for the case of Merlot concentrate addition, fig. 2.

\[
Y = A + B \\
A = -1.7260 \\
B = 3.0379 \\
R = 0.9958 \\
SD = 0.2796 \\
N = 7
\]

\[
Y = A + B \cdot X \\
A = -1.2670 \\
B = 3.0379 \\
R = 0.9907 \\
SD = 0.3281 \\
N = 7
\]

**Fig. 1.** The dependence established between total antioxidant capacity and polyphenols for vinegars with Cabernet Sauvignon concentrate addition

**Fig. 2.** The dependence established between total antioxidant capacity and polyphenols for vinegars with Merlot concentrate addition

**CONCLUSIONS**

For the red wine used for concentrates obtaining it was observed that the majority color is due to monomeric anthocyanins class (in the range 40-43%). The class of polymeric
pigment participates to the total wine color with 24-30% and copigmented anthocyanins with a percent between 29-32%.

From this data is evident that chromatic structure of these red wine is unstable (for stabilized wine the percent of polymeric pigments is between 75-90%, and the percent of monomeric and copigmented anthocyanins is small). The young red wines have a high content of anthocyanins and polyphenols and the strong antioxidant properties. The antioxidant properties of Cabernet Sauvignon wine are more evident than Merlot red wine.

The vinegar resulted by red wine concentrates addition have the color from weakly pink to red. The acid taste of obtained vinegars are ameliorated, the odor became agreeable typical of black grapes.

The polyphenols content, monomeric anthocyanins amount and total antioxidant capacity from vinegars with red wine Cabernet Sauvignon concentrates addition was superior given the vinegar with red wine Merlot concentrates.

The polyphenols content has the same evolution direction of with total antioxidant capacity (between these parameters it was observed a linear correlation).

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