Determination of quality and antioxidant activity of traditional homemade fruit vinegars produced with double spontaneous fermentation

Maja Chochevska[1], Elizabeta Jančovska Seniceva[2], Sanja Kostadinović Veličkovska[3], Galaba Naumova-Leţia[2] and Valentin Mirčeski[4]

[1]Faculty of Medical Science, University "Goce Delčev"-Štip, Krste Misirkov bb, Republic of North Macedonia
[2]Food and Veterinary Agency, III Makedonska brigada No 20, 1000 Skopje, North Macedonia;
[3]Faculty of Agriculture, University "Goce Delčev"-Štip, Krste Misirkov bb, Republic of North Macedonia
[4]Institute of Chemistry, Ss. Cyril and Methodius University, Arhimedova 5, 1000 Skopje, North Macedonia

Introduction

The production of homemade and industrial vinegars is different in the employed procedures.

Generally, fruit vinegars entail two major processing steps: one is the production of ethanol from raw materials, and the second is the conversion of the ethanol into acetic acid

In our study, for the production of homemade fruit vinegars, the fresh fruits were selected, washed, dried and cut with removal of the seeds (whenever necessary). About 1400 g of each fruit were then macerated for 1–3 min to ex-pose the chemical constituents as polyphenols, amino acids, oxidative and degradative enzymes, polyphenol oxidase enzyme, lipids, etc. The puree of each fruit was then dis-tributed into 5–6 L glass vessels and 4.8 L of water and 500 g of sucrose were added. The fermentation process was conducted at room temperature, ranging between 21 and 26 °C, for a time period of 41 days in the absence of starter cultures like yeasts and acetic acid bacteria. Once the acetic fermentation was completed (41 days), the experimental vinegars were filtered, pasteurized (15 min at 80 °C) and transferred, under aseptic conditions, into 100 mL glass airtight containers to reduce the risk of microbial contamination

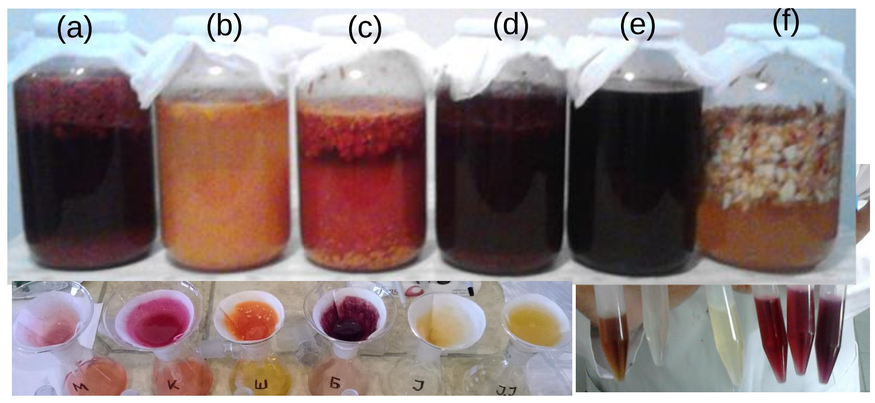

Fig.1 Homemade vinegar (HMV) producing and collecting (a) blueberry (Vaccinium myrtillus); (b) apple (Malus domestica); (c) raspberry (Rubus idaeus); (d) rose hip (Rosa canina); (e) blackberry (Rubus fruticosus); (f) persimmon (Diospyros kaki).

Method and materials

Harvesting and Selection of Plant Feedstocks

The six elected plant feedstocks

- apple (Malus domestica) → apple vinegar (HMV1);
- raspberry (Rubus idaeus) → raspberry vinegar (HMV2);
- blueberry (Vaccinium myrtillus) → blueberry vinegar (HMV3);
- blackberry (Rubus fruticosus) → blackberry vinegar (HMV4);
- rose hip (Rosa canina) → rose hip vinegar (HMV5);
- persimmon (Diospyros kaki) → persimmon vinegar (HMV6)

All the six fruits were obtained in the region of Berovo, Eastern of North Macedonia

Physicochemical characterization and antioxidant activity

- The content of alcohol in homemade and commercial vinegars was determined according to the AOAC standard method
- Acetic acid, as a major organic acid, was determined by titration according to the AOAC standard method
- Soluble dry matter content was determined with a refractometer
- The pH variation of HMV during the fermentation process and of the final products was measured using a pH meter
- Determination of total phenolic content was performed by the Folin–Ciocalteu assay (AOAC SMPR 2015.009: Estimation of total phenolic content using the Folin–Ciocalteu assay, 2015) and expressed as mg/L of gallic acid equivalents

Physicochemical analyses of each sample taken in duplicate for every fermentation time (n = 9 for HMV - at 2, 4, 6, 8, 10, 24, 31 and 41 days of fermentation and at 60 days.

The same analyses for ethanol content, total acids, pH and dry matter during 41 days of fermentation were performed after the time period of two months (60 d)—i.e., the final fruit homemade vinegars.

Result and discusion

- During alcoholic fermentation the ethanol level in all HMV samples steadily increased, which happened up to day 24 (Fig 1a). As a consequence of the loss of metabolic activity by yeasts as ethanol concentration in the fermentative medium increases, the subsequent time period (from 24th to 41st day) was characterized by the consumption (decrease) of ethanol and concomitant production (increase) of acetic acid by the autochthonous AAB present therein.
Such behavior was observed in all samples with the highest slope for the blueberry (HMV3) and black-berry (HMV4) vinegars (Fig 1a). The levels of ethanol were significantly ($p < 0.05$) found to be the lowest in HMV6 and the highest in HMV5.

- As well known, the decrease of ethanol content usually indicates the completion of the alcoholic fermentation by yeasts and the concomitant initiation of acetic fermentation by AAB, causing the total acidity to start increasing (Fig 1b). In the time period from 24th till 41st day, the total acidity rapidly increased, reaching a significant maxi-mum level of 45.6 g/L ($p = 0.001$) for the sample of persimmon vinegar (HMV6). Indeed, the significant ($p < 0.05$) lowest and highest levels of TA were obtained in samples of HMV6 and HMV1, respectively.

- pH values of the apple vinegar (HMV1) and blueberry vinegar (HMV3) showed the largest significant decrease ($p < 0.05$), i.e., the highest negative slope of pH values within time (Fig 1c). Furthermore, such a pH decrease was substantially observed as acetic acid fermentation became dominant (approximately from 24th fermentation day onwards).

-The content of dry matter in all samples constantly decreased during fermentation. The sharpest decrease was observed for raspberry vinegar (HMV2), i.e., from 11.4 to 3.0% (Fig.1d). In addition, when both consecutive spontaneous fermentations are compared, the decrease of dry matter was more visible during the alcoholic fermentation. In fact, during the acetic acid fermentation, the amounts of dry matter were not significantly different ($p = 0.07$). The significant ($p < 0.05$) lowest and highest levels of dry matter were obtained in samples of HMV2 and HMV6, respectively

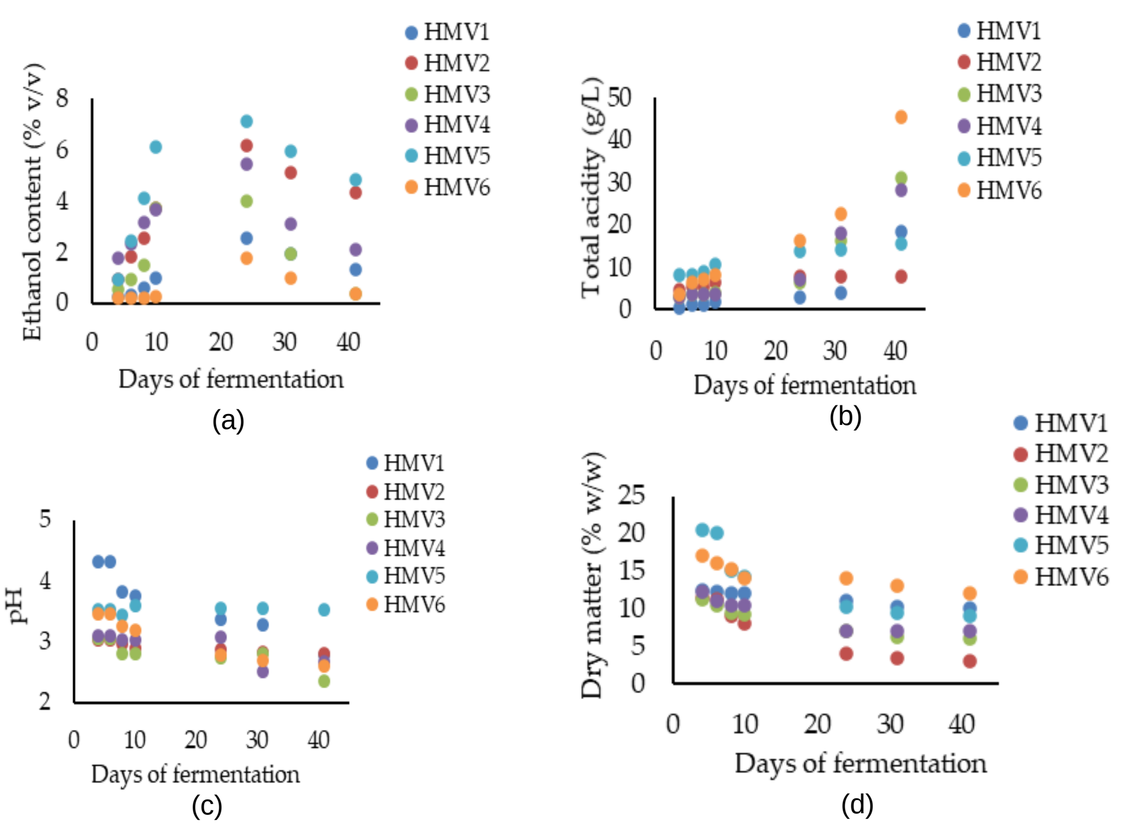

Fig.2 Variation of (a) ethanol content (%, v/v), (b) total acidity (g/L), (c) pH {−log[H+]} and (d) dry matter (%, w/w) in HMV throughout fermentation time (days): apple vinegar (HMV1); raspberry vinegar (HMV2); blueberry vinegar (HMV3); blackberry vinegar (HMV4); rose hip vinegar (HMV5); persimmon vinegar (HMV6).

The results were expressed as mean values and standard deviations (mean ± STDV) and calculated from two replicates of each fermentation and three analytical measurements.

Table 1. Chemical composition of final homemade fruit vinegars (HMV) after two months (60 d) of fermentation, for ethanol content (% v/v), total titratable acids (g/L), pH and dry matter (%, w/w): apple vinegar (HMV1); raspberry vinegar (HMV2); blueberry vinegar (HMV3); blackberry vinegar (HMV4); rose hip vinegar (HMV5); persimmon vinegar (HMV6)..

Final HMV	Fruit	Ethanol (%, *v/v*)	Total Acid (g/L)	pH	Dry Matter (%, *w/w*)	Total Phenolic Content (mg of Gallic Acid/mL)
HMV1	Apple	0.13 ± 0.01 ^{d}	20.4 ± 2.1 ^{b}	2.7 ± 0.0 ^{b}	11.1 ± 1.4 ^{a}	0.29± 0.1 ^{d}
HMV2	Raspberry	4.24 ± 0.08 ^{a}	9.0 ± 0.8 ^{c}	2.9 ± 0.1 ^{b}	3.0 ± 0.7 ^{d}	0.99 ± 0.0 ^{b,c}
HMV3	Blueberry	0.47 ± 0.00 ^{c}	43.6 ± 4.7 ^{a}	2.5 ± 0.0 ^{b}	6.2 ± 0.9 ^{c}	1.24 ± 0.5 ^{b}
HMV4	Blackberry	0.54 ± 0.01 ^{c}	42.6 ± 5.1 ^{a}	2.3 ± 0.2 ^{b}	8.1 ± 1.1 ^{b}	1.70 ± 0.8 ^{b}
HMV5	Rose hip	3.7 ± 0.07 ^{b}	17.4 ± 1.1 ^{b}	3.4 ± 0.1 ^{a}	9.3 ± 2.3 ^{b}	20.2 ± 2.4 ^{a}
HMV6	Persimmon	0.34 ± 0.01 ^{c}	46.8 ± 6.0 ^{a}	2.4 ± 0.0 ^{b}	12.1 ± 2.2 ^{a}	0.44 ± 0.0 ^{d}

The results were expressed as mean values and standard deviations (mean ± STDV) and calculated from one/two replicates of each fermentation and three analytical measurements

In the final vinegar products:

- the pH values were lower than 2.9 but not for the rose hip vinegar HMV5 - According to the Codex Alimentarius Commission (CL 2000/18-EURO, 2000)
- total phenolic content showed that rose hip vinegar had the highest total phenolic content in comparison to all other vinegars
- the samples of apple vinegar (HMV1) and persimmon vinegar (HMV6) showed a significantly higher percentage of dry matter in comparison to the other HMV samples because of large amounts of non-fermentable sugars
- the content of ethanol in vinegars should be lower than 0.5% (v/v)

Conclusions

The highest total phenolic compounds were measured by vinegar produced from rose hip (20.2 mg of gallic acid/mL) while the lowest concentration was determined for apple vinegar (0.29 mg of gallic acid/mL). The results from total phenolic compounds were in strong correlation with the antioxidant capacity. In this way, the use of traditional processes for the production of fruit vinegars proved to be very promising in terms of producing differentiated vinegars and, concomitantly, reaching high levels of health-promoting antioxidant capacities.