JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2014, 20(4), 304-310

Journal of Agroalimentary Processes and Technologies

Study concerning the quality of apple vinegar obtained through classical method

Adriana Dabija, Cristian Aurel Hatnean

Faculty of Food Engineering, Stefan cel Mare University of Suceava, 13 University street, 720229 Suceava, Romania

Received: 31 August 2014; Accepted: 12 September 2014

Abstract

Vinegar is the product that is obtained exclusively through biotechnological process of double fermentation, alcoholic and acetic liquids or other substances of agricultural origin.

This paper presents a summary of research concerning the quality of vinegar made from apples, Florina assortment, harvested from Suceava county area.

Apple vinegar is obtained through double fermentation, alcoholic and acetic apple juice through the classic Orleans.

In the different stages of acetic fermentation, the aroma compounds were determined via chromatographic analysis, using a gas chromatograph mass spectrometer equipped with a Shimadzu GCMS. In the apple vinegar - finished product were identified aroma compounds derived from apple - raw material and which are processed during the technological stages, but also new flavour compound resulting from alcoholic and acetic fermentations. All these substances determine the ultimate sensory qualities of the finished product - apple vinegar.

The final product was analyzed in terms of heavy metal content by atomic emission spectrometer using an inductively coupled plasma mass spectrometer equipped with AGILENT 7500.

Keywords: alcoholic fermentation, acetic fermentation, flavor compounds, heavy metals

1. Introduction

Vinegar can be defined as the product obtained exclusively through biotechnological processes by double fermentation, alcoholic and acetic fermentation of liquids or other substances of agricultural origin. Thus, depending on the raw material used, it can be obtained:

- *wine vinegar*, wine - raw material of min. 70 % by volume;
- *fruit and berry vinegar*, vinegar obtained from fruit or berries through biotechnological process of alcoholic fermentation and acetic fermentation;
- *cider vinegar,* vinegar obtained by the biotechnological process of acetic fermentation;
- *alcohol vinegar*, vinegar made from ethyl alcohol, fermentation by biotechnological process of acetic fermentation;
- *grain vinegar*, vinegar made by the biotechnological process of double fermentation, alcohol and acetic, from grains;

Corresponding author: *e-mail:* adriana.dabija@fia.usv.ro

- *malt vinegar*, vinegar made by the biotechnological process of double fermentation, alcohol and acetic, from malt;
- o*ther types of vinegar*, for example, obtained from whey vinegar, vinegar made from beer, vinegar made from honey.

Apple vinegar is part of vinegar fruit apples obtained by biotechnological process of double fermentation, alcoholic and acetic.

Prior to the start of the acetic fermentation apple wine is subjected to alcoholic fermentation which is carried out with yeast, thus obtaining the amount of necessary alcohol to produce acetic acid.

By prolonging the outdoor stay-period of the fermented apple juice it can be seen on its surface a pellicle containing microorganisms with different characteristics belonging to the *Acetobacter* and *Gluconobacter* genus [1,2]

The biochemical mechanism for the conversion of alcohol in acetic acid starts with its oxidation into an acetaldehyde in the presence of alcohol dehydrogenase. Acetaldehyde, by the addition of a molecule of water, is converted into hydrate acetaldehyde, which in the presence of the aldehyde dehydrogenase is transformed into acetic acid - product of the fermentation [3].

To carry out the acetic acid fermentation in optimum conditions it is necessary to achieve and to maintain a certain concentration of dissolved oxygen. The manner in which the oxygen is supplied to acetic bacteria influences the speed of fermentation process and, to some extent, the sensory qualities of the wine vinegar - finished product. Depending on the rate of formation of acetic acid (and the ventilation mode, implicitly) acetic fermentation processes can be divided into two categories: slow and fast processes [4].

For obtaining vinegar was used slow process (type Orleans). This process is one of the oldest manufacturing techniques of vinegar, a principle known since antiquity. At first, the fermented apple juice is placed in a vessel with high diameter/height ratio. After about seven days, during which acetic fermentation is triggered, the liquid is passed to another vessel.

Filling is dispensed so that the liquid occupies 50 ÷ 70% of the total volume of the vessel. Acetic fermentation is slow, taking effect only at the surface of the liquid, where the dissolved oxygen concentration is sufficient to ensure the conversion of alcohol to acetic bacteria. In this way, there is formed a "fermentation veil", where the number of active acetic bacteria is high. The fermentation lasts between 8 to 14 weeks, depending on the initial composition of the alcoholic solution, fermentation temperature, nature of the microorganism, the contact surface liquid / air etc. After the acetic fermentation is considered completed, it is extracted from the vessel an amount of vinegar representing 60 ÷ 70% of the original volume, which is then replaced with raw material and acetic fermentation process may be repeated with the removal of the initial phase [4, 5, 6, 7].

This process leads to vinegar with 4÷7 acetic degrees. When using appropriate materials the sensory qualities of vinegar produced via this slow method are superior than those obtained using rapid methods. For this reason, slow fermentation is preferred to obtain apple vinegar and other types of vinegar derived from different fruit juice, or even balsamic vinegar.

Under the legislation, apple cider vinegar must meet the following conditions:

- total acidity - minimum 50g/1000 mL, expressed in pure acetic acid;
- residual alcohol content - more than 0,5% v / v - volume fraction;
- organoleptic and physico-chemical characteristics must meet the technical specifications of the manufacturers;
- eligibility of contamination level must be in accordance with regulations [8, 9, 10].

The influence of the manufacturing process on the quality of vinegar has an important weight, but the most important factor in obtaining vinegar's aroma is the raw material. The main physico-chemical properties of apple vinegar are shown in Table 1 [4].

2. Materials and methods

To obtain the vinegar were used Florina apples, harvested from Suceava county area. After initial

processing, the crushed fruit was passed into the alcoholic fermentation vessel for 10 days at 18 to 22°C. Finally, the fermented mash had an alcohol content of 5 vol. % and then was subjected to acetic fermentation via slow process, as described in the previous section. The resulting apple vinegar was analyzed after 28 days of acetic fermentation and at the end of the acetic fermentation, respectively after 42 days.

For the analysis of volatile compounds in apple vinegar was used a gas chromatograph equipped with a Shimadzu GCMS mass spectrometer (GCMS - QP2010Plus).

Flavour compounds are separated by chromatography using a capillary column CP IDF 88 with the following characteristics: length - 50 m, outer diameter - 0.33 mm internal diameter - 0,20 ; helium flow - 1 mL / minute; temperature lamp: 65 ÷ 240 °C.

Mass spectrometry is the most sensitive method of structural analysis, a microanalytical technique for measuring the relative molecular weights of compounds per unit, or highlighting certain atomic species and existing functional compound analyzed. The chromatography method is the most effective of all the known methods of separation of different substances of a mixture, used in analytical chemistry and technology.

Chromatography is a separation method of multicomponent mixtures. It is based on distribution of different components of a mixture between a mobile phase and a stationary phase (immiscible), with different speeds due to the movement of the component carried by the mobile phase during the stationary phase. This difference between the rates of migration of the component is depending on the specific chemical nature of their physico-chemical properties.

Quantitative determinations were made using the coupling technique of gas chromatography - mass spectrometry, conducted by means which enhance the sensitivity of up to 1000 times.

Heavy metal analysis was performed using atomic emission spectrometer with inductively coupled plasma mass spectrometer equipped with (ICP-MS) AGILENT 7500. ICP-MS parameters were: nebulizers - 0.9 mL / min, RF power - 1500 W, carrier gas - argon 0.92 mL / min, mass range - 7-205; integration time - 0.1 seconds; acquisition - 22.7 seconds.

3. Results and discussions

Flavour compounds in apple cider vinegar have been identified in two samples at different stages of fermentation, respectively 28 days and 42 days.

After analyzing the obtained spectrum it was observed a variation of flavour compounds during the acetic fermentation process shown in the chromatograms in Fig. 1 and Fig.2.

Table 1. Quality characteristics of apple vinegar

Characteristic	Apple vinegar
Density, kg/m^3	1013-1024
Extract, g/L	19 - 35
Ash, g/L	2-4,5
Total acidity, % acetic acid	3,9 – 9
Nonvolatile acids, % acetic acid	0,1 – 0,55
Glucids (carbohydrates), g/L	1,5 – 7
Malic acid, g/L	0,47 - 0,80
Colour	yellow
Flavour	specifically

Figure 1. Apple vinegar after 28 days of acetic fermentation (sample 1)

Figure 2. Apple vinegar after 42 days of acetic fermentation (sample 2)

Figure 3. Highlighting the presence of pentanol in sample 1

Figure 4. Highlighting the presence of pentanol in sample 2

Table 2. The amount of heavy metals present in apple vinegar obtained

Element	Concentration µg/L
Li	1,59886
Be	0,57182
B	6,25453
Na	37,68977
Mg	19,67193
Al	237,71042
K	0
Ca	32,02768
V	0,06755
Cr	0,65346
Mn	0,91131
Fe	0,09103
Fe	0
Co	0
Ni	13,40967
Cu	6,90455
Zn	0,05752
Ga	0
As	0
Se	0,13169
Rb	0,06642
Sr	14,91454
Ag	0
Cd	0
Cs	0,03184
Ba	0,03734
Tl	0
Pb	0
U	0

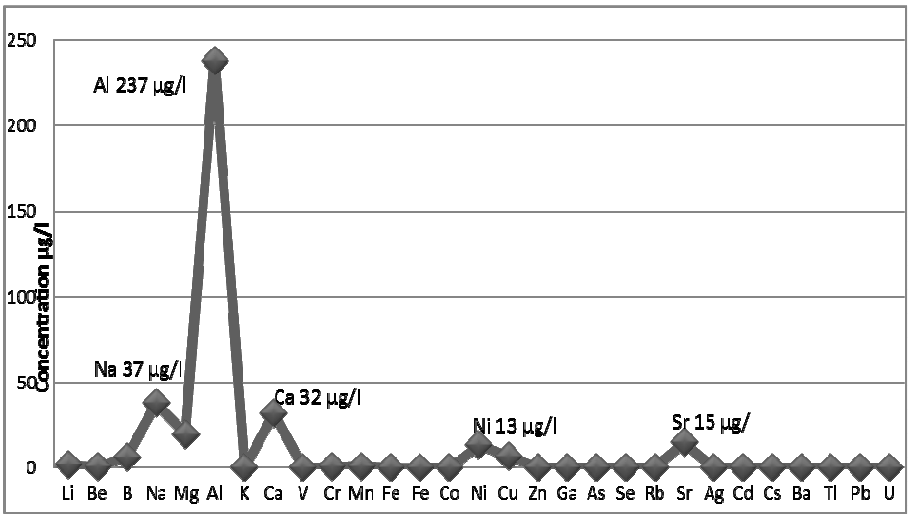

Figure 5. Heavy metals concentrations in apple vinegar – finished product

The main flavour compounds have been identified in both samples, with some differences.

Sample 1 identified flavour compounds, the most important being:

- 1 - pentanol ;
- butanedioic acid ;
- propanoic acid, 2-methyl-, 2-ethyl- 3-hidroxihexil ester ;
- phenylethyl alcohol - (alcohol is a pleasant floral aroma);
- 3,5- di - tert - butyl -4 - hydroxybenzaldehyde .
- In sample 2 the following flavour compounds were identified:
- 1-butanol, 3-methyl acetate - (this compound is converted in the presence of acetic acid in isoamyl acetate with a pleasant banana flavour);
- 1 - pentanol - (this alcohol is responsible for the pungent aroma of vinegar, also the ester formed with acetic acid has banana flavour);
- 1 - hexanol - (compound used in the perfume industry) ;
- butanedioic acid ;
- propanoic acid, 2- methyl- ,2 -ethyl - 3hidroxihexil ester;
- phenylethyl alcohol - (alcohol is a pleasant floral aroma);
- 3,5- di - tert - butyl -4 - hydroxybenzaldehyde .

In Figure 3 and Figure 4 is observed the presence of pentanol, specific to fermentation media in which the acetic fermentation is present.

- The presence of pentanol in both samples indicates a high degree of acetic fermentation.
- Apple vinegar - final product was analyzed in terms of heavy metals content, the quantities found are shown in Table 2 and fig.5.

From the analysis of Table 2 data for the analyzed samples, was observed the absence of the following heavy metals: K, Fe, Co, Ga, As, Ag, Cd, Tl, Pb, U. Significant quantities were found for the following : Na, Al, Ca, Ni, Sr, but all within the limits allowed by law.

The chart below shows the variation of heavy metals found in apple vinegar sample.

4. Conclusions

The quality of vinegar is influenced by a number of factors: the raw material used, the technological process adopted, the technological equipment supplied, acetic bacteria species used etc.

To obtain a high quality finished product with a very fine flavour, decisive is the quality of the raw material used in the process. Florina variety of local apples used in the study is a kind of highly flavoured

red apple, some of its flavour compounds are preserved in the final product - apple cider vinegar.

By using modern analytical methods were identified key aroma compounds of apple cider vinegar in different stages of acetic fermentation. Also, the presence of pentanol indicated that both samples had acetic fermentation.

Apple vinegar - finished product was analyzed in terms of heavy metal contamination, their value lying within the range allowed by law.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Dan., V., *Microbiologia alimentelor,* Editura Alma, Galati, **2001,** pp. 337-340
2. Rocío Fernández-Pérez, P; Carmen Torres, C.;Sanz, S.; Ruiz-Larrea, F., Strain typing of acetic acid bacteria responsible for vinegar production by the submerged elaboration method, *Food Microbiology*, **2010**, *27*(8), 973-978
3. J.L. Ordóñez, J.L.; Callejón, R.M.; Morales, M.L.; García-Parrilla, M.C., A survey of biogenic amines in vinegars, *Food Chemistry*, **2013,** *141*(3), 2713-2719, doi: 10.1016/j.foodchem.2013.05.087
4. Banu, C., coord., *Biotehnologii in industria alimentara,* Editura Tehnica, Bucuresti, **2000,** pp.307-318
5. Úbeda, C.; Callejón, R.; Troncoso, A.M.;Rojas, J.M.; Peña, F.;Lourdes Morales, M., *Impact Odorants in Strawberry Vinegars*, In: Flavour Science, Proceedings from XIII Weurman Flavour Research Symposium, **2014**, pp. 177-181
6. Antonelli, A.; Zeppa, G.; Gerbi, V.; Carnacini, A., Polyalcohols in vinegar as an origin discriminator, *Food Chemistry*, **1997**, *60*(3), 403-407, doi: 10.1016/S0308-8146(96)00360-3
7. Ji-Cheng Chen; Qi-He Chen;Qin Guo; Sue Ruan; Hui Ruan; Guo-Qing He; Qing Gu, Simultaneous determination of acetoin and tetramethylpyrazine in traditional vinegars by HPLC method, *Food Chemistry*, **2010**, *122*(4), 1247-1252, doi: 10.1016/j.foodchem.2010.03.072
8. Giudici, P.; Gullo, M.; Solieri, L.; Falcone, P.M., Technological and Microbiological Aspects of Traditional Balsamic Vinegar and Their Influence on Quality and Sensorial Properties, In: *Advances in Food and Nutrition Research*, Edited by Jeyakumar Henry, **2009,** *58*, 137-182
9. Tesfaye, W.; Morales, M.L.; García-Parrilla, M.C.; A.M Troncoso, A.M., Wine vinegar: technology, authenticity and quality evaluation, *Trends in Food Science & Technology*, **2002**, *13*(1), 12-21, doi: 10.1016/S0924-2244(02)00023-7
10. Ju-Hye Lee; Hyun-Dong Cho; Ji-Hye Jeong; Mi-Kyung Lee; Yong-Ki Jeong;Ki-Hwan Shim; Kwon-Il Seo, New vinegar produced by tomato suppresses adipocyte differentiation and fat accumulation in 3T3-L1 cells and obese rat model, *Food Chemistry*, **2013**, *141*(3), 3241-3249, doi:10.1016/j.foodchem.2013.05.126