The Evolution Of The Volatile Compounds By Aging, In Apple Vinegar Flavored With Rosmarinus Officinalis L., Using A HS/GC-MS Technique

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Abstract

Apple vinegar, made from apple juice or concentrated apple juice through a double fermentation, it’s widely used as a beverage and food preserver in traditional medicine and healthy diets.

The apple vinegar used in this study was flavored using 2 methods of extraction for the active principles from Rosmarinus officinalis L.: maceration and ultrasounds. This paper aims for the analysis and identification of the biological active compounds from the flavored vinegar, using a Headspace /Gas Chromatographic – Mass Spectrometry analysis, at the bottling of the process and after a year of aging the vinegar in glass bottles.

A various number of volatile compounds, specific to rosemary, were identified in the vinegar also from the beginning of bottling. The volatile compounds ranged during the aging process, but the main ones, that were specific to rosemary, and to the apple vinegar, were the same.

Keywords: Apple vinegar, Rosmarinus officinalis L., HS/GC-MS, volatile compounds.

1. Introduction

Vinegar by definition is „a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars, or starch and sugars by the process of double fermentation, alcoholic and acetous, and contains a specified amount of acetic acid” [4].

Apple vinegar is well known for it’s containing in aromatic compounds, some naturally present in the apples and some formed during the process of obtaining. The main ingredient of apple vinegar, or any vinegar, is acetic acid, but it also have other acids, vitamins, mineral salts, and amino acids that have a great impact on the overall aroma of all vinegars.

The flavor is one of the most important factors in the determination on vinegar character and quality. The volatile fraction of the vinegar and its aromatic composition is weighty influenced by many factors, the most important being the raw materials and especially the aromatic plant used for flavoring. Therefore, the volatile profile of the vinegar is a fingerprint that analysis the volatile compounds and is considered an efficient way of assessing its authenticity [5].

In vinegars, these volatile compounds are normally present at low concentrations (µg/L or less) this is way they are determined using a sensitive analytical technique, a headspace coupled with a gas chromatography (GC) and a mass spectrometry (MS) method [1,3,6].

The aim of this work is the identification of the volatile compounds from rosemary flavored apple vinegar, employing a headspace gas chromatography-
mass spectrometry and the analysis of its evolution during a year of storage in glass bottles with a green leaf of rosemary [2,7].

2. Materials and methods

Gas chromatography analysis were performed with a Shimadzu GC-MS QP-2010 model equipped with an AOC-5000 autosampler (CombiPAL) and a AT-5 capillary column, of 30m length, 0.25mm inner diameter and 0.25µm film thickness (Alltech, USA). The carrier gas was helium, at a constant flow rate of 1.5 ml/min and the oven program temperature was from 60ºC, with a initial time hold of 5 min, then rised with 4ºC/min to 160ºC, and then at 240ºC with 15ºC/min and held for 1 min at this final temperature.

The injector temperature was 250.0ºC; the pressure 37.1 kPa; the linear velocity 32.4 cm/s; the split ratio 1:200., the detector: MS, the ion source temperature 250.0oC and the interface temperature 250.0oC. All mass spectra were acquired in electron impact mode (EI) Mass spectra were recorded over 40-400 amu range with electron impact ionization (EI) energy of 70 eV and a scan speed of 769u/s.

The analytes were partitioned into the headspace by heating the sample at 95ºC for 15min in order to extract all the volatile constituents that were still present in our sample and inject them in the GC-MS, for the analysis of volatile compounds. The compounds were identified by comparison of the obtained mass spectra with the ones from the mass spectra libraries, NIST 27 and NIST 147.

3. Results and discussion

The flavored apple vinegar volatile compounds studied, in this paper, were: 1-Butanol-3-methyl-acetate; 1-Butanol-2-methyl-acetate; 3-Octanona; Eucalyptol; Camphor; Borneol; Terpining-4-ol; alfa-Terpineol and I-Verbenona, identified in a previous study in 2009, by Truta D. & all., after the product was obtained.

The chromatograms of three flavoured apple vinegar, obtained using different flavouring technique are shown in figure 1 as an example. Three major volatile compounds, 3-Octanone, Eucalyptol and Camphor, were found in all samples chromatographic fingerprints. Using a similar technique, Socaci S. & all., in 2008 and 2009, has identified all these compounds in different rosemary plants of different age and variety.

![Figure 1. Fingerprint signals obtained from the HS/GC-MS analysis of three samples corresponding to the same apple vinegar flavoured with: a) rosemary dry leaves; b) rosemary green leaves and c) rosemary dry leaves by ultrasounds](image-url)
The distinct aroma and flavor characteristics were given to the apple vinegar by the apples used as row material and especially by the rosemary plants used for flavouring. Both apples and rosemary plants contribute trace elements of many volatile substances which gave to the final product its distinctive aroma characteristics. As shown in figure 1, Camphor was found to have the highest concentration (33.48%) in the flavoured vinegar obtained by maceration extraction from green rosemary in contrast with the other extraction methods.

Eucalyptol was also found in high amounts in apple vinegar, but it was two times smaller in the sample flavoured by ultrasound technique (17.07%) comparing with that flavoured by maceration techniques (35.72%), although the ultrasound technique offered the highest concentration of 1-Butanol-methyl-acetate (33.67%).

Analysing after one year the evolution of the volatile compounds from the above mentioned samples, it can be noticed that the main aroma compounds found initially in the vinegars were also present in vinegar after a year. It can be seen in table 1., that, six different aroma compounds were identified in all the samples.

As it can be seen in table 1, the concentration of the main volatile compounds was modified by time, some of them were found in higher concentration (Eucalyptol, 3-Octanone, 1-Butanol-3-methyl-acetate and 1-Butanol-2-methyl-acetate) or smaller concentrations (Camphor, Borneol) and others were lost during time (Terpinen-4-ol, Alfa-Terpineol, I-Verbenona).

Also by analyzing the three flavoring methods regarding the best method of keeping the volatile compounds in time in the liquid substrate, we noticed that the ultrasound technique managed to keep a higher content of volatiles compounds and moreover to intensify its concentration by time.

The maceration technique has brought some new compounds (Borneol, 1-Butanol-3-methyl-acetat, 1-Butanol-2-methyl-acetate) in the flavored vinegar but also has lost some of them.

4. Conclusion
The HS/GC-MS method used for the determination of the volatile constituents from apple vinegar flavored with Rosmarinus Officinalis L., allowed us to compare the different flavoring methods and the evolution of volatile composition of vinegar samples after one year.

Because of the high concentration of these three compounds specific to the rosemary plants and found in the vinegars samples, the analysis method needs further optimization in order separate and identify other volatile compounds specific to the apple vinegar used as extraction substrate.

By comparing the three methods of flavouring the apple vinegar we found that the maceration extraction of green rosemary plant brought more aroma compound to the apple vinegar compare to the maceration extraction of dry rosemary plant.

As it was expected, after a year, we noticed that in all flavoured vinegar samples (independent of the extraction method) some of the volatile compounds extracted from rosemary were lost or oxidized.
References


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