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Artículos de investigación

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Análisis de los compuestos volátiles en el vinagre de acerola mediante microextracción en fase sólida acoplada con cromatografía de gases-espectrometría de masas

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Abstract:

Vinegar is derived from the conversion by bacteria of ethanol to acetic acid. It can therefore be produced from any alcoholic material, ranging from alcohol–water mixtures to wines. The aim of this work was to characterize the volatile composition of a vinegar of good and acceptable quality prepared from acerola wine. The volatile constituents of acerola vinegar were isolated by headspace solid-phase microextraction and analyzed by gas chromatography with flame ionization and selective mass detectors. A fiber of Carboxen-polydimethylsiloxane was used for the isolation of volatile compounds with optimized parameters: aliquot (8 mL) of vinegar with 1 g NaCl in a 15-mL vial. The sample was equilibrated at 45°C for 10 min and extracted with the fiber for 30 min under stirring at 500 min^{-1}. A total of 69 volatile compounds, including 20 acids, 19 esters, 6 alcohols, 6 terpenes, 5 aldehydes, 3 n-paraffins, 2 ketones, 1 sulfur-containing compound and other 7 of different chemical nature were identified in acerola vinegar. The major constituents found in vinegar volatiles were acetic acid, 3-methylbutanoic acid and 2-phenylethyl acetate.

Keywords: vinegar, acerola, volatile compounds, solid-phase microextraction, gas chromatography-mass spectrometry.

Resumen:

El vinagre se deriva de la conversión por las bacterias de etanol a ácido acético. Puede producirse por consiguiente de cualquier material alcohólico, desde las mezclas de alcohol-agua a los vinos. El objetivo de este trabajo fue caracterizar la composición volátil de un vinagre de calidad buena y aceptable preparado a partir de vino de acerola. Los compuestos volátiles del vinagre de acerola fueron aislados por microextraction en fase sólida del espacio de cabeza y analizados por cromatografía de gases con detectores de llama de hidrógeno y selectivo de masas. Se usó una fibra de Carboxen-polidimetilsiloxano para el aislamiento de los compuestos volátiles con los parámetros optimizados: alícuota (8 mL) de vinagre con 1 g NaCl en un vial de 15-mL. La muestra fue equilibrada a 45°C por 10 min y extraída con la fibra durante 30 min y bajo agitación a 500 min^{-1}. Un total de 69 compuestos volátiles, que incluyen 20 ácidos, 19 esteres, 6 alcoholes, 6 terpenos, 5 aldehídos, 3 n-parafinas, 2 cetonas, 1 compuesto azufrado y otros 7 de distinta naturaleza química fueron identificados en el vinagre de acerola. Los constituyentes mayoritarios encontrados fueron el ácido acético, ácido 3-metilbutanoico y acetato de 2-feniletilo.

Palabras clave: vino, vinagre, acerola, compuestos volátiles, microextracción en fase sólida, cromatografía de gases-espectrometría de masas.

INTRODUCTION

Vinegar is the result of a two-step fermentation process over almost any fermentable carbohydrate source. First, during alcoholic fermentation, yeasts transform sugars into ethanol, which is then converted into acetic acid during the second fermentation by acetic acid bacteria. Its sensory characteristics depend greatly on the initial substrate (Xia *et al*., 2018). Vinegar production methods range from traditional methods that employ wooden casks and surface culture to submerged fermentation. Nowadays the industrial production is based

on submerged acetification systems, but vinegars produced by slow traditional surface methods generally get higher prices because their better sensory quality (Budak *et al*., 2014).

Because of its worldwide availability and great array of varieties, vinegar is one of the world's most widespread and common products. In USA and Europa, vinegar is commonly made from several cereals, fruits, wines fermented, cider, and malt liquor. At present, innovations in vinegar production falls into two areas: improving production processes and employing different raw materials (Vidra & Németh, 2018).

Acerola (Malpighia emarginata DC.) is a minor non-conventional fruit cultivated in many tropical zones. The fruit is considered as an excellent source of antioxidant and vitamin C and it is consumed fresh and used in food industry to produce juices, jams and beverages (Rondón & Pino, 2015). Since the cultivation of this fruit is improving in Cuba, this makes it an attractive candidate for use as a raw material in producing new types of vinegars.

Aroma is one of the most important determinants in food quality and acceptance. Therefore, when introducing a new food product, the characterization of its aroma is an important aspect to be considered. Aroma is caused by many volatile compounds that are involved in different ways. Although volatile compounds of acerola fruit have been studied to some extent (Rondón & Pino, 2015), there is no information published to date on the volatiles of acerola vinegar.

This work was undertaken to characterize the volatile composition of an acerola vinegar of good and acceptable quality, which may consequently aggregate further values to this plant culture.

MATERIALS AND METHODS

Vinegar making by surface culture

Fresh acerola juice (18.5 % m/m soluble solids and 0.37 % m/m anhydrous citric acid) was added at 10% m/m to a wort containing brown sugar (190 g/L), dibasic ammonium phosphate (1 g/L), and anhydrous citric acid (2 g/L). The wort was transferred to a stainless-steel fermenter and it was inoculated with dried *Saccharomyces cerevisiae* yeast (1 g/L, Fermipan Lefersa, Havana). Fermentation was performed at 26 oC until the soluble solids content was constant (5.1 % m/m) and alcohol content reached 10 % v/v. The wine was inoculated with 10% m/m of an industrial mother preparation and placed into an open stainless-steel fermenter at 26 oC until the volatile acidity did not change. The vinegar was filtered through four layers of fine cheesecloth and placed at -17 oC for subsequent analyses.

Standard chemical analyses

Soluble solids (method 932.12) and pH (method 981.12) were measured in the juice and alcohol content (method 969.12), soluble solids (method 932.12), total acidity (method 962.12) and pH (method 960.19) were determined in the wine according to standard methods (AOAC, 2012).

SPME procedure

In previous researches silica fiber coated with carboxen-polydimethylsiloxane (CAR-PDMS) was found to be more efficient at extracting the volatile compounds of vinegar than other fibers (Xiao et al., 2011; Cirlini et al., 2011). The SPME manual device equipped with a 75 μm CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) was used for the extraction of volatile compounds. The fiber was conditioned in the GC injector port at 250 °C for 1 h prior to use. An aliquot (8 mL) of vinegar with 1 g NaCl was placed into a 15-mL vial

containing a stirring bar. The sample was equilibrated at 45 °C for 10 min and extracted with the fiber for 30 min at the same temperature under stirring (500 min-1). Each analytical sample was measured in triplicate.

Gas chromatography analyses

The analytical systems were gas chromatography with flame ionization detector (GC-FID) and mass selective detector (GC-MS). SPME injections were splitless (straight glass liner, 0.75 mm I.D.) at 250 oC for 4 min during which time thermal desorption of analytes from the fiber occurred. Following SPME desorption, the inlet was switched to purge-on for the remainder of the GC run and the SPME fiber was conditioned for 5 min more before it was removed from the injector.

GC-FID analysis was accomplished with a Konik 4000A instrument (Konik, Barcelona) equipped with a DB-5 ms (30 m x 0.25 mm x 0.25 μm; J & W Scientific) column, working with the following temperature program and conditions: 50 oC for 2 min, ramp of 4 oC/min up to 250 oC; injector and detector temperatures 250 oC; carrier gas hydrogen (1 mL/min).

GC-MS analysis was performed with a QP-2010 Ultra instrument (Shimadzu, Japan) equipped with a BP-5 (30 m x 0.25 mm i.d. x 0.25 μm; SGE Analytical Science Pty. Ltd., Victoria, Australia) column. Analytical conditions were the same as GC-FID analysis. Injector and transfer line temperatures 250 and 230 oC, respectively; carrier gas helium at 1 mL/min. Detection by MS was performed in the electron impact mode (70 eV ionization energy). Acquisition was performed in scanning mode (mass range 35-400 m/z).

The volatile compounds were identified by comparing their retention index and their mass spectra to those of commercial spectra databases (Wiley 6, NBS 75k and NIST05) and the in-house Flavorlib library created from previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC-FID system. Linear retention indices (LRI) of the compounds were calculated using an n-alkane series.

RESULTS AND DISCUSSION

According to standard methods, the volatile acidity expressed as acetic and pH value were 4.9 % m/m and 2.90, respectively. These values are in accordance with other vinegars (Aceña *et al*., 2011; Cejudo-Bastante *et al*., 2017; Xia et al., 2018).

The volatile compounds identified in acerola vinegar are reported in Table 1. Although the used isolation method is not suitable to determine major volatile compounds (acetaldehyde, ethanol, methanol, acetic acid and ethyl acetate), a total of 69 volatile compounds were identified.

Acids were found to be the most abundant volatile constituents (20 compounds), as they accounted for the largest proportion of the total aroma. Also, 19 esters, 6 alcohols, 6 terpenes, 5 aldehydes, 3 .-paraffins, 2 ketones, 1 sulfur-containing compound and other 7 of different chemical nature were identified in acerola vinegar. This qualitative profile is like those found in other vinegars (Chinnici *et al*., 2009; Min-Sheng & Po-Jung, 2010; Aceña *et al*., 2011).

Acids mainly come from alcohol oxidation acted by acetic bacteria. Their presence, hence, is expected to be somehow related to the amount of alcohols in the raw material. Besides acetic acid, 3-methylbutanoic acid represents by far the second major acid in the acerola vinegar. This result agrees with other reports (Chinnici *et al*., 2009; Min-Sheng & Po-Jung, 2010; Aceña *et al*., 2011; Liang *et al*., 2016).

The esters usually are considered most important to flavor of vinegars. They can be formed by the reaction of alcohols with organic acids under the effect of the esterase, yeast or mold (Liang *et al*., 2016). Among them, acetates and ethylic esters were the major components in acerola vinegar. Acetates are formed by esterification between acetic acid and alcohols, while ethylic esters are related with the ethanol content of the raw material.

Of them, 2-phenylethyl acetate was the most abundant. Overall, the alcohols found in acerola vinegar were substantially in accordance with other works on vinegars (Chinnici *et al*., 2009; Min-Sheng & Po-Jung, 2010; Aceña *et al*., 2011; Liang *et al*., 2016). Lower amount of these compounds could be seen due to the almost absent alcoholic fermentation during the production phases of the vinegar. This is particularly evident for 3-methylbutan-1-ol and to a lesser extent, for 2-phenylethanol, which typically derive from amino acid metabolism of yeast cells during alcoholic fermentation (Swiegers *et al*., 2005). With regards to aldehydes, they may derive from alcohol oxidation and the few found were at trace level.

Two ketones were identified, 3-hydroxy-2-butanone and acetophenone. The first one usually is present in fermented foods and beverages such as wine, which can be produced in alcoholic fermentation by the action of microorganisms (Liang *et al*., 2016). Acetophenone should come from the plant-based raw materials used in the fermentation process.

The sulfur-containing compounds can be formed during the thermal processing via Maillard reaction. The Strecker degradation of sulfur amino acids such as cysteine and methionine in Maillard reaction can produce the sulfur compounds. Only dimethyl trisulfide was found, which it is an important odorant in Shanxi vinegar due to its low odor threshold (Liang *et al*., 2016).

HS-SPME showed that is an adequate isolation method that could be used to determine the absolute concentration of the compounds identified. Also, sensory studies need to be done to determine the definite contribution of these volatiles to the acerola vinegar.

TABLE 1
Chemical composition of the volatile compounds in acerola vinegar

Compound	LRI	%
Methyl acetate	559	tr
Acetic acid	645	50.7
Propanoic acid	711	tr
3-Hydroxy-2-butanone	718	0.1
Butane-2,3-diol	789	1.2
Ethyl 2-methylbutanoate	851	tr
Ethyl 3-methylbutanoate	859	tr
3-Methylbutyl acetate	881	0.8
2-Methylbutyl acetate	883	0.2
2-Methylbutanoic acid	886	2.0
3-Methylbutanoic acid	889	5.9
Methyl 3-hydroxybutanoate	890	tr
g-Butyrolactone	918	tr
Benzaldehyde	960	tr
2,2,6-Trimethyl-6-vinyltetrahydropyran	972	tr
Dimethyl trisulfide	976	tr
1-Octen-3-ol	982	tr
Hexanoic acid	998	0.2
Ethyl hexanoate	1000	tr
(Z)-3-Hexenyl acetate	1007	tr
2-Ethylbutanoic acid	1011	tr
1,4-Cineole	1015	tr
p-Cymene	1025	tr
Limonene	1029	tr
2-Ethylhexan-1-ol	1032	0.1
Phenylacetaldehyde	1042	tr
2,2-Dimethyl-5-[(1E)-1-methyl-1-propenyl] tetrahydrofuran	1045	tr
Ethyl 2-furoate	1047	0.1
Acetophenone	1064	tr
Octan-1-ol	1068	tr
p-Cymenene	1091	tr
Ethyl heptanoate	1098	tr
Nonanal	1101	tr
2-Phenylethanol	1107	1.8
endo-Fenchol	1116	tr
Methyl octanoate	1127	tr
2-Ethylhexyl acetate	1153	tr
Nerol oxide	1158	tr
Benzyl acetate	1162	tr
Benzoic acid	1185	tr
α-Terpineol	1189	tr
Ethyl octanoate	1195	tr
Octanoic acid	1198	3.1
Decanal	1204	tr
Hexyl 2-methylbutanoate	1236	tr
2-Phenylethyl acetate	1258	4.1
Nonanoic acid	1297	2.8
3-Methylbenzoic acid	1325	tr
Eugenol	1359	tr
Decanoic acid	1386	3.5
Ethyl decanoate	1396	tr
Dodecanal	1409	0.1
Ethyl (E)-cinnamate	1467	0.1
Dodecan-1-ol	1471	0.1
Undecanoic acid	1490	2.4
(E)-Megastigma-5,8-dien-4-one	1495	1.5
n-Pentadecane	1500	0.1
Dodecanoic acid	1568	3.8
n-Hexadecane	1600	0.1
Benzophenone	1628	tr
Tridecanoic acid	1664	3.2
Tetradecanoic acid	1779	3.7
Ethyl tetradecanoate	1796	tr
Pentadecanoic acid	1868	1.5
(Z)-11-Hexadecenoic acid	1951	1.8
Hexadecanoic acid	1960	2.1
n-Eicosane	2000	0.0
Oleic acid	2141	1.5
Octadecanoic acid	2200	1.1

tr: traces (< 0.1 %).

CONCLUSIONS

This study revealed 69 volatile compounds that are responsible for the overall flavor of the acerola vinegar using solid-phase microextraction coupled to gas chromatography. The major chemical classes of compounds were predominantly acids and esters. Within these, acetic acid, 3-methylbutanoic acid and 2-phenylethyl acetate were the most abundant.

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