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ARTICULO DE INVESTIGACION

Analysis of the volatile compounds in sugarcane juice wine using solid-phase microextraction techniques coupled with gas chromatography-mass spectrometry

Análisis de los compuestos volátiles en el vino de guarapo usando microextracción en fase sólida acoplada con cromatografía de gases-espectrometría de masas

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ABSTRACT

The aim of this work was to characterize the volatile composition of a wine of good quality prepared from sugarcane juice, which may consequently aggregate further values to the sugarcane juice. The volatile constituents of wine obtained from sugarcane wine were analyzed by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry. A fiber of DVB/CAR/PDMS was used for the extraction of volatile compounds. A total of 96 volatile compounds, including 46 esters, 12 alcohols, 10 acids, 4 terpenes, 7 aldehydes, 4 ketones, 4 n-paraffins, 3 acetals, 1 sulfur compound and other 5 of different chemical nature were identified in wine. The major constituents found in wine volatiles were 3-methylbutan-1-ol, ethyl octanoate, ethyl hexanoate, 2-phenylethanol and diethyl succinate. The aroma of the sugarcane wine has a full wine and cane aroma, whereas the flavor was soft, mellow and slightly sweet. According to the sensory evaluation, the panelists considered that the flavor of the sugarcane wine was good. The results of the hedonic scale showed that 56% of the judges preferred the wine as ‘like very much’ and 44% preferred it as ‘like moderately’.

Keywords: wine; sugarcane; volatile compounds; solid-phase microextraction; gas chromatography-mass spectrometry.

RESUMEN

El objetivo de este trabajo fue caracterizar la composición volátil de un vino de calidad buena preparado a partir del guarapo, que pueda agregar mayor valor al guarapo. Los compuestos volátiles del vino de caña de azúcar fueron analizados por microextraction en fase sólida del espacio de cabeza acoplado a la cromatografía de gases-espectrometría de masas. Se usó una fibra de DVB/CAR/PDMS para la extracción de los compuestos volátiles. En el vino fueron identificados 96 compuestos volátiles, incluyendo 46 ésteres, 12 alcoholes, 10 ácidos, 4 terpenos, 7 aldehídos, 4 cetonas, 4 n-parafinas, 3 acetales, 1 compuesto azufrado y otros 5 de naturaleza química diferente fueron identificados. Los constituyentes mayoritarios encontrados en el vino fueron 3-metilbutan-1-ol, octanoato del etilo, hexanoato de etilo, 2-feniletanol y succinato de dietilo. El aroma del vino de caña de azúcar tiene un aroma completo a vino y caña, mientras que el sabor es suave, maduro y ligeramente dulce. Según la evaluación sensorial, los catadores consideraron que el sabor del vino de caña de azúcar es bueno. Los resultados de la escala hedónica mostraron que el 56% de los catadores prefirieron el vino como 'me gusta mucho' y el 44% lo prefirieron como 'me gusta moderadamente'.

Palabras clave: vino; caña de azúcar; compuestos volátiles; microextracción en fase sólida; cromatografía de gases-espectrometría de masas.

INTRODUCCIÓN

The wine commercialization undergoes long and traditional trajectories until it arrives at the table for consumption. However, the product undergoes stabilization treatments and packing that transforms it into a quality product although at many times, turns it to be quite original and personalized. Thus being, the wines should constant improvements in its characteristics and these must be perfectly stabilized as a guarantee to the consumer.

Although the wines better appreciated are made from grapes, yet other fruits could be utilized as raw material for the manufacture of wines. These fruits could be pineapple, mango, guava, papaya, and many other fruits (Pino & Queris, 2010a, 2010b, 2011, 2012). Mostly, the wines made from these fruits result in flavor characteristics of the original fruit used and if proper care is taken, could last for long time storage. Besides this, the production of wine may consequently aggregate further values to the fruits.

Sugarcane juice is the syrup extracted from pressed sugarcane (*Saccharum officinarum* L.). It is consumed as a refreshing drink in many places, particularly where sugarcane is cultivated such as Latin America, Southeast Asia and India. There are some reports about the production of wine from sugarcane juice (Tzeng *et al*., 2009, 2010; Chen *et al.*, 2013).

Wine volatile fraction is extremely complex, because of the great number of compounds present, which may have different polarities, volatilities and a wide range of concentrations (Ebeler, 2001). Therefore, a major task in flavor studies is to separate the odor-active constituents from the odorless nonvolatile compounds present in wines. There are several methods described in the literature that partially fulfill these requisites, one of them is solid-phase microextraction (SPME) (Souza-Silva *et al*., 2015).

SPME is now widely used for analysis of aroma volatiles in many food and beverage matrices. This is a solventless sampling technique that can be faster and easier than solvent extractions and distillations, as well as being highly reproducible and sensitive.

This work was undertaken to characterize the volatile composition of a wine of good and acceptable quality prepared from sugarcane juice, which may consequently aggregate further values to this juice.

MATERIALES Y MÉTODOS

Wine making

Fresh sugarcane juice (20 L), with 26 °Brix and pH 5.5, were prepared from Cuban peeled sugarcanes, cut in pieces and passed through a mill. The characteristics of the sugarcane juice were pH 5.1, soluble solids 19.7 ^{o}Brix and total acidity 1.2 g/L as anhydrous citric acid. The juice was added at 10% (w/w) to a wort containing brown sugar (190 g/L), dibasic ammonium phosphate (1 g/L), and anhydrous citric acid (2 g/L). Then, the wort was transferred into one stainless steel tank for the fermentation using dried bakery yeast (1 g/L, Fermipan Lefersa, La Habana). Fermentation was performed in duplicate at controlled temperatures (26 ± 2 ^{o}C). After fermentation, the wine was racked by adding 0.4 g/L sodium bisulphite and clarified by adding 0.1 g/L agar. After 5 days, the wine was decanted and it was stored at 25 ^{o}C for 1 month.

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

The SPME manual device equipped with a 50/30 μm DVB/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. Aliquots (8 mL) of wine with 1 g NaCl was placed into a 15-mL vial containing a stirring bar. The sample was equilibrated at 30°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 duplicate.

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 ^{o}C 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 ^{o}C for 2 min, ramp of 4 ^{o}C/min up to 250 ^{o}C; injector and detector temperatures 250 ^{o}C; carrier gas hydrogen (1 mL/min).

GC-MS analysis was performed with a QP-2010 Ultra instrument (Shimadzu, Japan) equipped with a 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 ^{o}C, 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 m/z 35-400 u).

The volatile compounds were identified by comparing their retention index and their mass spectra to those of commercial spectra databases (Wiley 6, NBS 75k, 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. Retention indices of the compounds were calculated using an *n*-alkane series.

Sensory evaluation

The wine was evaluated by 50 panelists, males and females, 20-55 years of age. The panelists were selected for participation based on their preference for wines and availability. Refrigerated (15 ^{o}C) samples of 20 mL were served in clear glasses with a volume of 100 mL and covered with plastic Petri dishes. Evaluations were conducted at room temperature (25 ^{o}C) under white light. The wine was evaluated for its general acceptability according to the five points (dislike very much to like very much) hedonic scale (Espinosa, 2007).

RESULTADOS Y DISCUSIÓN

The general composition of the wine was alcohol 13.3% v/v, soluble solids 7.5 ^{o}Brix, total acidity 0.23 g/L as anhydrous citric acid and pH 3.4. These results are in the range reported in other works (Tzeng *et al*., 2009, 2010).

The compounds formed during alcoholic fermentation have a decisive influence on the volatiles of wine and therefore they are the responsible of its flavor. Esters, higher alcohols, volatile fatty acids and, to a lesser extent, aldehydes are the main volatile compounds formed during alcoholic fermentation (Ebeler, 2001).

A total of 96 volatile constituents were identified in sugarcane wine (Table 1), in which esters were found to be the most abundant volatile constituents (46 compounds), as they accounted for the largest proportion of the total aroma. Also, 12 alcohols, 10 acids, 4 terpenes, 7 aldehydes, 4 ketones, 4 *n*-paraffins, 3 acetals, 1 sulfur compound and other 5 of different chemical nature were identified in the wine. This qualitative profile is like those found in other tropical fruit wines (Pino & Queris, 2010a, 2010b, 2011, 2012). However, it is something different to those reported in a sugarcane wine produced in Taiwan, where only 15 constituents were identified (Tzeng *et al*., 2010). These differences could be attributed to the use of a different yeast strain or volatile isolation procedure because in the previous report a Carboxen-polydimethylsiloxane fiber was used.

Alcohols were the major quantitative components of sugarcane wine (46.3%). Among them, 3-methylbutan-1-ol and 2-phenylethanol were markedly the most abundant alcohols. However, it is well-known that it is not the higher contents of volatile compounds occurring in a food that contribute to its aroma; only those with concentrations higher than their odor thresholds can contribute to the aroma (Ebeler, 2001). The odor thresholds of 3-methylbutan-1-ol (alcohol note) and 2-phenylethanol (floral note) are 60 and 200 mg/L in 10% ethanol-water solution, adjusted to pH 3.5 with tartaric acid (Peinado *et al*., 2006), respectively. Thus, this means that 3-methylbutan-1-ol and 2-phenylethanol could contribute to the aroma of sugarcane wine.

Among the esters (45.1% of total volatiles), ethyl octanoate, ethyl hexanoate and diethyl succinate were the major components in sugarcane wine. Ethyl octanoate, ethyl hexanoate and diethyl succinate, with their fruity notes, could be important aroma compounds because their odor thresholds are 0.58, 0.08 and 1200 mg/L in 10% ethanol-water solution, adjusted to pH 3.5 with tartaric acid (Peinado *et al*., 2006), respectively.

Carbonyl compounds including aldehydes and ketones were detected in the sugarcane wine. Among them, 2-furfural, benzaldehyde and nonanal were the most abundant, but in concentrations lower than the major alcohols and esters detected. With regard to acids, acetic, hexadecanoic and tetradecanoic acids were found as the major ones, but their contribution to the aroma of wine cannot be considered important because they have relatively high odor thresholds (Ebeler, 2001).

The aroma of the sugarcane wine has a full wine and cane aroma, whereas the flavor was soft, mild and mellow, slightly sweet. According to the sensory evaluation, the panelists considered that the flavor of the sugarcane wine was good. The sensory analysis of the wine showed a high acceptance (73%) by the tasters. The results of the hedonic scale showed that 56% of the judges preferred the wine as 'like very much' and 44% preferred it as 'like moderately'. However, the panelists proposed that the addition of some fruit flavor increased even more the acceptance of the product.

Table 1. Chemical composition of the volatile compounds in sugarcane wine.

Compound	RI	Area%
Acetals		
1,1-Diethoxyethane	726	1.4
1,1-Diethoxy-2-methylpropane	861	0.1
1,1-Diethoxy-3-methylbutane	955	tr
Acids		
Acetic acid	645	2.0
Propanoic acid	721	tr
Butanoic acid	821	tr
2-Methylbutanoic acid	870	tr
Decanoic acid	1386	tr
Dodecanoic acid	1568	0.3
Tridecanoic acid	1662	tr
Tetradecanoic acid	1779	0.5
Pentadecanoic acid	1868	0.2
Hexadecanoic acid	1960	0.6
Alcohols		
3-Methylbutan-1-ol	741	32.2
2-Methylbutan-1-ol	742	3.1
Butane-2,3-diol	806	1.6
Hexan-1-ol	873	0.1
Heptan-1-ol	969	0.3
1-Octen-3-ol	982	tr
5-Methyltetrahydro-2-furanmethanol	993	0.2
2-Ethylhexan-1-ol	1032	1.0
Octan-1-ol	1068	0.1
Nonan-2-ol	1095	0.1
2-Phenylethanol	1107	7.6
Dodecan-1-ol	1473	tr
Aldehydes		
2-Furfural	836	0.2
Benzaldehyde	961	0.2
Octanal	999	tr
Phenylacetaldehyde	1041	tr

Nonanal	1101	0.2
Decanal	1202	0.1
Dodecanal	1407	tr
Esters		
Ethyl acetate	615	0.4
Ethyl acrylate	702	0.3
Propyl acetate	707	tr
Ethyl propanoate	717	0.3
Ethyl 2-methylpropanoate	751	1.4
2-Methylpropyl acetate	768	0.2
Ethyl butanoate	805	1.7
Ethyl lactate	826	0.5
Ethyl 2-methylbutanoate	851	0.5
Ethyl 3-methylbutanoate	859	0.6
3-Methylbutyl acetate	881	2.4
2-Methylbutyl acetate	884	0.4
Ethyl pentanoate	901	0.1
Ethyl 2,3-dimethylbutanoate	926	0.1
Ethyl 3-hydroxybutanoate	935	tr
Ethyl 3-oxobutanoate	944	tr
Ethyl hexanoate	998	8.7
Ethyl (*E*)-3-hexenoate	1006	tr
3-Methylbutyl 2-methylpropanoate	1017	0.1
Ethyl (*E*)-2-hexenoate	1044	tr
Ethyl 2-hydroxy-4-methylpentanoate	1062	0.2
3-Methylbutyl lactate	1065	tr
Allyl hexanoate	1083	0.1
Ethyl heptanoate	1098	0.1
3-Methylbutyl 3-methylpentanoate	1104	tr
2-Methylpropyl hexanoate	1149	0.1
Ethyl benzoate	1175	0.3
Diethyl succinate	1179	4.7
Ethyl (*Z*)-4-octenoate	1187	0.1
Ethyl octanoate	1196	18.3
(*Z*)-3-Hexenyl 2-methylbutanoate	1232	tr
Hexyl 3-methylbutanoate	1244	tr
Ethyl 2-phenylacetate	1248	0.2
3-Methylbutyl hexanoate	1254	tr
2-Phenylethyl acetate	1258	0.1
Ethyl salicylate	1270	tr
Ethyl nonanoate	1320	0.1
Heptyl 3-methylbutanoate	1338	tr
2-Methylpropyl octanoate	1345	tr
Ethyl 9-decenoate	1389	0.9
Ethyl decanoate	1396	2.0
3-Methylbutyl octanoate	1451	tr
Ethyl undecanoate	1494	tr

Ethyl dodecanoate	1594	0.1
Ethyl tetradecanoate	1798	tr
Ethyl hexadecanoate	1995	tr
Ketones		
Pentan-2-one	688	0.1
Heptan-4-one	878	tr
Nonan-2-one	1090	0.1
Undecan-2-one	1293	tr
n-Paraffins		
n-Tetradecane	1400	0.1
n-Pentadecane	1500	tr
n-Hexadecane	1600	tr
n-Heptadecane	1700	tr
Sulfur compounds		
3-(Methylthio)-1-propanol	987	tr
Terpenes		
Limonene	1029	tr
Borneol	1167	tr
Menthol	1172	tr
Geranyl acetone	1455	tr
Others		
2,4,5-Trimethyl-1,3-dioxolane	724	2.7
γ-Butyrolactone	918	0.3
m-Cymenene	1086	tr
Dehydroionene	1348	0.1
2,7-Dimethylnaphthalene	1410	tr

tr: traces (< 0.1 %).

CONCLUSIONES

It can be concluded that it is feasible the elaboration of wine with acceptable characteristics, using sugarcane juice as a substrate. This study revealed 96 volatile compounds that are responsible for the overall flavor of the wine produced from sugarcane juice using solid-phase microextraction coupled to gas chromatography. The major chemical group of compounds were predominantly alcohols and esters. Within these, 3-methylbutan-1-ol, ethyl octanoate, ethyl hexanoate, 2-phenylethanol and diethyl succinate were the most abundant

REFERENCIAS BIBLIOGRÁFICAS

AOAC. (2012). *Official Methods of Analysis*, 19th edition, Association of Official Analytical Chemist. Washington DC.: AOAC International.

Chen, G.-L., Zheng, F.-J., Lin, B., Wang, T.-S., Li, Y. R. (2013). Preparation and characteristics of sugarcane low alcoholic drink by submerged alcoholic fermentation. *Sugar Tech.*, *15* (4), 412-416.

Ebeler, S. E. (2001). Analytical chemistry: Unlocking the secrets of wine flavour. *Food Rev. Int.*, *17*, 45-64.

Espinosa J. (2007). *Evaluación Sensorial de los Alimentos*. La Habana: Editorial Universitaria.

Peinado, R. A., Mauricio, J. C., & Moreno, J. (2006). Aromatic series in sherry wines with gluconic acid subjected to different biological aging conditions by *Saccharomyces cerevisia*e var. *Capensis*. *Food Chem., 94*, 232-239.

Pino, J., & Queris, O. (2010a). Analysis of volatile compounds of pineapple wine using solid-phase microextraction techniques. *Food Chem., 122*, 1241-1246.

Pino, J., & Queris, O. (2010b). Analysis of volatile compounds of mango wine. *Food Chem., 125*, 1141-1146.

Pino, J., & Queris, O. (2011). Characterization of impact odorants of guava wine. *J. Agric. Food Chem., 59*, 4885-4890.

Pino, J., & Queris, O. (2012). Characterization of odour-active compounds in papaya wine. *Int. J. Food Sci. Technol., 47*, 262-268.

Souza-Silva, É. A., Gionfriddo, E., Pawliszyn, J. (2015). A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis. *Trends Anal. Chem., 71*, 236-248.

Tzeng, D. I., Tai, C. Y., Chia, Y. C., Lin, Y. P., & Ou, A. S. M. (2009). Investigation of fermenting conditions for sugarcane (*Saccharum officinarum* L.) wine using response surface methodology. *J. Food Process. Preserv., 33*, 330-346.

Tzeng, D. I., Tai, C. Y., Chia, Y. C., & Ou, A. S. M. (2010). Investigation of chemical quality of sugarcane (*Saccharum officinarum* L.) wine during fermentation by *Saccharomyces cerevisiae*. *J. Food Qual., 33*, 248-267.