Safety Evaluation of the Grape Marc Spirit by GC-FID Analysis

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Abstract. There were investigated grape marc spirits regarding their safety on consumers. Major volatile compounds which present risk to human health (such as methanol, furfural, and acetaldehyde) and ethyl alcohol content were compared to European Union Regulation and other values found in previous studies. The aim of our study was to evaluate the safety of grape marc spirits considering their content in major volatile compounds by comparison with the requirements of European Union Regulation. We investigated the ethyl alcohol content by electronic densimetry and 10 major volatile compounds (acetaldehyde, ethyl acetate, methanol, 1-propanol, 1-butanol, 2-butanol, isobutyl alcohol, isoamyl alcohol, amyl active alcohol and furfural) by Gas Chromatography coupled with Flame Ionization Detector. We used reference chemicals to identify these compounds and 3-pentanol as internal standard to quantify the volatiles. Results were compared with the requirements of European Union Regulation. All major volatile compounds registered values in accordance to EU Regulation and the grape pomace spirits samples do not present any kind of risk for consumption.

Keywords: grape marc spirit, food safety, methanol, furfural, acetaldehyde.

INTRODUCTION

The consumption of unrecorded alcohol is a worldwide issue, with impact on consumers' health. Unrecorded alcohol includes any kind of alcohol which is not taxed as alcoholic beverage, such as illegally produced or commercialized alcohols products. The technological process for producing alcohol should be conducted by qualified personnel who have the necessary experience to obtain a safe beverage for consumers.

All alcoholic beverages produced around the world must be considered by the authorities because the resulting product components can present a real risk. In EU countries it is reglemented by law the most harmful components found in alcoholic beverages and many studies evaluated these components (Quesada Granados *et al.*, 1996, Geroyiannaki *et al.*, 2007, Versini *et al.*, 2009, Cortés *et al.*, 2010, Lukić *et al.*, 2011).

Our previous researches were conducted to verify the safety of these drinks on humans regarding their content in organochlorine pesticides, heavy metals, toxic volatile compounds and microbial contamination (Rusu *et al.*, 2010, 2011, Coldea *et al.*, 2012, Rotar *et al.*, 2012).

While not as popular in Romania as fruit brandies, such as plum, pear or apple brandies, grape marc spirit is gaining ground because of the revaluation of waste from the winemaking industry. Being more popular in Mediterranean countries, studies had been developed on eau-de-vie de marc (French grape marc spirit), the Italian grappa, tsipouro (from Greece) or zivania from Cyprus (Danilatos and Harvala, 1981, Apostolopoulou *et al.*, 2005, Geroyiannaki *et al.*, 2007). The Reg. 110/2008 of EU established the general production methodologies and analytical parameters for each type of alcoholic beverage. Grape marc spirit

is produced exclusively from grape marc. Distillation is conducted to an alcoholic strength between 37.5 and 86% vol. Redistillation is permitted at the same range. No added alcohol or flavorings is permitted. Some of the major volatile compounds found in fruit spirits can have harmful effects on our health. Methanol, furfural and acetaldehyde can have toxic effect to the human organism when ingested in large amounts, which is why European Commission established the maximum admissible value for methanol to be 1000 mg% mL pure alcohol (p.a.). Furfural it is regulated by law to a maximum content of 2 mg% mL p.a. Volatile substances must exceed 140 mg% mL p.a. Acetaldehyde represents the main aldehyde present in fruit spirits. Beyond the factors of their appearance in the final spirit drink might be fermentation temperature, the amount of SO₂ in marc or the oxidation of ethanol by acetic acid bacteria in the presence of oxygen. Methanol is formed during the grape processing and fermentation by the hydrolytic demethoxylation of esterified methoxyl groups of the pectin in the presence of pectic enzymes (Lukić *et al.*, 2011). Furfural can be formed during distillation by hydrolysis of acids or by heating the unfermented pentose and Maillard reactions. Other studies presented as a cause of furfural occurrence to be the aging process of the distillates (Quesada Granados *et al.*, 1996).

MATERIALS AND METHODS

Provenience of samples

The grape marc spirits produced in USAMV Cluj-Napoca pilot station were evaluated for the presence of contaminants with risk for consumption. After having pressed the grapes in the winemaking process, the grape pomace is fermented. The volatile components thus formed are then recovered by distillation after in stills made of copper. The distillation process is selected, as two sequential cuts of distillate, a first cut to separate the head products from the heart products, and a second cut to separate the heart products from the tail products. The head fraction, or the first fraction of condensate, is very rich in methanol; the heart fraction, or the second fraction of condensate is a complex mixture of volatiles, most of which contribute to sensorial characteristic (G1); the tail fraction, or the last fraction of condensate is rich in less volatile compounds. The heart fraction is redistilled for separation of toxic volatile compounds (G2). The distillates were collected in two bottles and stored at 4°C before analysis.

Alcohol concentration and the relative density

The alcohol content and relative density analyses were made by the electronic densitometer type DDM2911, with digital display and measuring cell connected to an incorporated temperature regulator, made by Rudolf Research Analytical, series: 2045, measuring domain: 0-3 g/cm³. The measurement result was an average of five values obtained for alcohol concentration and respectively, for the relative density. The density was displayed with 5 decimals and alcoholic concentration with 2 decimals.

Volatile compounds analysis

Analysis of major volatile compounds of the two fractions of distillate was adapted after the EU reference method for volatile compounds found in alcoholic beverages. For the determinations of the major volatile compounds, the samples were injected directly into the gas chromatograph column, from a GC-FID Agilent Technologies gas chromatograph, 6850A, without preliminary treatment. Each sample was injected twice in the GC-FID. One micro liter from each sample was introduced in the capillary chromatography column ZB-WAX plus (characteristics: 60 m length, 0.25 mm diameter, 0.25 µm film thickness, stationary phase:

cross linked polyethylene glycol) produced by Zebron Company. Inside the oven, the initial temperature was 35⁰C. The injector temperature was 240⁰C, automatic injection. The carrier gas was Helium. Detector (FID) temperature was 250 ⁰C (Rusu *et al.*, 2011). The temperature program is represented in Tab.1. The total analysis time of each sample was 30.63 min.

Tab.1

Temperature program used for the GC-FID analysis of grape spirits

Ramps	Rate	Final temperature	Final time
1	12 ⁰ C/min	35-58 ⁰ C	4 min
2	3 ⁰ C/min	58-85 ⁰ C	0 min
3	30 ⁰ C/min	155 ⁰ C	3 min
4	200 ⁰ C/min	230 ⁰ C	5 min

The main components (methanol, acetaldehyde, ethyl acetate, 1-propanol, 2-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-butanol, furfural), were identified by comparing their retention times with those of authentic compounds (see *Chemicals and reagents*). For quantitative evaluation it was applied the internal standard method, with a known amount of 3-pentanol, as internal standard (IS). As such, a solution containing 0.1 mL 3-pentanol was added to 10 mL of every each sample. For all volatiles, the quantitative evaluation was based on automatic calculation, based on peak area integration, while for furfural the integration was done, manually.

Chemicals and reagents

All used chemicals (ethanol, acetaldehyde, methanol, propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl acetate, 3-pentanol, furfural) with purity over 99% were purchased from Merck and Sigma Aldrich Company.

Statistical analysis

The results obtained from the individual experiments were used to calculate the mean values and standard deviation values for the two apple brandy fractions samples.

RESULTS AND DISCUSSIONS

Alcohol concentration based on relative density determination

The values obtained for alcohol concentration based on relative density measurement were for G1 and G2, 36.16 %v/v, and 39.85 %v/v respectively, which are in accordance with previous studies (Apostolopoulou *et al.*, 2005).

GC-FID analysis

There were identified the main volatile compounds by their retention times and by comparison with pure standards. The internal standard used in all cases was 3-pentanol. Based on the peak areas, for each sample it was calculated the concentration of each component, expressed as mg% mL p.a. The individual and mean values for each volatile found in the samples resulted from the distillation process were compared with those reglemented by the EU legislation and by comparison with other references (Tab. 2).

Tab. 2

Major volatile compounds identified in the samples collected from the two distillation stages (G1, G2) in comparison with other references

Volatile compound, mg% mL p.a.	G1 ($\pm$ SD [*])	G2 ($\pm$ SD)	References	
			Different fruit brandies	Grape marc spirit
Acetaldehyde	45.57 ($\pm$ 0.06)	30.96 ($\pm$ 0.03)	1.3-59.7 ⁽¹⁾ ; 7.4-31.0 ⁽²⁾ ; 14.49-19.92 ⁽³⁾ ;	20.3-53.7 ⁽⁵⁾ ; 51.7-76.71 ⁽⁶⁾ ; 1.57-51.1 ⁽⁷⁾ ; 21.4-29.9 ⁽⁸⁾
Ethyl acetate	1625.15 ($\pm$ 0.93)	1037.79 ($\pm$ 4.02)	7.6-692.1 ⁽¹⁾ ; 150.5-376.8 ⁽²⁾ ; 17-128 ⁽⁴⁾ ; 246.04-312.62 ⁽³⁾	73.8-166.91 ⁽⁶⁾ ; 21.5-69.2 ⁽⁷⁾ ; 26.3-51.7 ⁽⁸⁾
Methanol	739.98 ($\pm$ 5.79)	658.28 ($\pm$ 1.09)	93.2-1205.3 ⁽¹⁾ ; 346.4-895.1 ⁽²⁾ ; 540.0-1140.0 ⁽⁴⁾ ;	165.7-955.8 ⁽⁵⁾ ; 576.9-791.7 ⁽⁶⁾ ; 136.0-340.6 ⁽⁷⁾ ; 85.0-454.3 ⁽⁸⁾
Furfural	0.00	0.00	0.93-6.23 ⁽⁴⁾ ; 2.68-3.66 ⁽³⁾	0.0-1.67 ⁽⁷⁾ ; 0.04-0.37 ⁽⁸⁾
2-Butanol	0.69 ($\pm$ 0.00)	0.78 ($\pm$ 0.01)	0.6-171.5 ⁽¹⁾	0.36-6.9 ⁽⁶⁾ ; 0.0-40.9 ⁽⁷⁾ ; 0.0-0.2 ⁽⁸⁾
1-Propanol	31.03 ($\pm$ 0.17)	32.93 ($\pm$ 0.02)	12.1-706.8 ⁽¹⁾ ; 65.6-186.8 ⁽²⁾ ; 15.0-73.0 ⁽⁴⁾	43.91-69.71 ⁽⁶⁾ ; 12.3-32.3 ⁽⁷⁾ ; 9.15-40.6 ⁽⁸⁾
2-methyl-1-propanol	79.78 ($\pm$ 0.23)	88.95 ($\pm$ 0.15)	11.3-195.5 ⁽¹⁾ ; 40.6-110.0 ⁽²⁾ ; 30.0-135.0 ⁽⁴⁾	62.61-87.79 ⁽⁶⁾ ; 13.0-35.2 ⁽⁷⁾
1-Butanol	2.41 ($\pm$ 0.02)	2.68 ($\pm$ 0.01)	0.5-51.6 ⁽¹⁾ ; 3.0-33.5 ⁽²⁾ ; 6.3-116.0 ⁽⁴⁾	1.55-3.13 ⁽⁶⁾ ; 0.25-1.5 ⁽⁷⁾ ; 0.5-0.8 ⁽⁸⁾
2-methyl-1-butanol	34.02 ($\pm$ 0.17)	39.43 ($\pm$ 0.07)	11.0-81.8 ⁽¹⁾ ; 42.0-96.5 ⁽⁴⁾	42.7-57.9 ⁽⁶⁾ ; 20-28.1 ⁽⁷⁾
3-methyl-1-butanol	129.57 ($\pm$ 0.43)	148.67 ($\pm$ 0.03)	58.9-422.5 ⁽¹⁾ ; 129.0-359.0 ⁽⁴⁾	118.5-200.7 ⁽⁶⁾ ; 25.4-104.9 ⁽⁷⁾ ; 79.6-159.4 ⁽⁸⁾

*standard deviation; (1 - Winterová *et al.*, 2008; (2 - Satora and Tuszynski, 2008; (3 - Rusu *et al.*, 2011; (4 - Versini *et al.*, 2009; (5 - Geroyiannaki *et al.*, 2007; (6 - López-Vázquez *et al.*, 2010; (7 - Cortés *et al.*, 2010; (8 - Lukić *et al.*, 2011

Acetaldehyde and higher alcohols registered values in accordance with fruit brandies and other grape marc spirits evaluated in presented references. With higher boiling points than ethanol, 3-methyl-1-butanol and 1-propanol are found in tails fraction (Claus and Berglund, 2005). Among higher alcohols only 2-methyl-1-propanol had similar values with fruit brandies but higher compared to other grape marc spirits (Satora and Tuszynski, 2008, Winterová *et al.*, 2008, Versini *et al.*, 2009, Cortés *et al.*, 2010, López-Vázquez *et al.*, 2010). Ethyl acetate and methanol registered substantially higher values than fruit and grape marc spirits, maybe due to the not sufficient separation of the fractions in the distillation process. Still, no compound was found to be out of the values admitted by legislation (Reg. 110/2008).

CONCLUSION

The major volatile compounds found in two distillate fractions collected from the double distillation process of grape marc spirit were evaluated from the safety to consumers' point of view. Ethanol content evaluated by electronic densimetry and volatile compounds determined by GC-FID analysis, presented similar values to other references and no volatile compound was found to be out of the regulation value.

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