Agricultural Journal 3 (5): 429-438, 2008
ISSN: 1816-9155

Evaluation of Macro-and Micro-Volatiles in Hellenic Local Alcoholic Beverage from Opuntia Ficus (Fragosyko)

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Abstract: Opuntia ficus-indica (fragosyko) is a very popular fruit in many Mediterranean countries, in Southwestern United States, Northern Mexico and other areas. This fruit is used for the production of an extraordinary aromatic spirit like tsipouro, after direct steam distillation of fermented Opuntia ficus-indica (fragosyko) pomace. The main objective of the present study was to analyze the unusual local and unique alcoholic beverage from the fermented fruit pomace of Opuntia ficus fragosyko from different areas of Hellas and to study its major and minor volatile compounds. The volatile compounds present in headspace fraction were isolated and identified by using a balance pressure headspace system Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 system (Shimadzu Co, Kyoto, Japan). Totally 66 samples were analysed and 27 volatiles have been isolated.

Key words: Distillation, fermentation, alcoholic beverages, opuntia ficus, spirits, volatile compounds, flavor

INTRODUCTION

About 1500 species of cactus belong to the genus Opuntia and are distributed mainly in many Mediterranean countries, in South-Western United States, Northern.

Mexico, in Africa and other areas (Hegwood *et al*., 1990). The fruit is called by different names, e.g., in Greece fragosyco, in France Indian figs, in United States prickly pears and in Chile tunas etc.

The flavour of this spirit, is made up of a great number of makro and mikro volatiles which be a number of alcohols, esters, acids, carbonyl compounds etc. Their respective concentrations of Opuntia ficus vary considerably between different areas of cultivation (Flath and Takahashi, 1978).

The determination of volatiles involves preparation of sample prior to chromatographic analysis in order to isolate the compounds and to determine their concentration. The most frequently used methods for isolation and concentration of -makro and -mikro constituents involves extractions, distillation and simultaneous distillation-extraction techniques. However, all these techniques are time-consuming and involve excessive manipulation of the sample, which may lead to serious errors. Furthermore, new aromas may arise from the aromatic precursors already present in the samples or from chemical and biochemical reactions promoted by heat, pH and oxidation-reduction conditions

Head-space analysis is such a technique, which makes it possible to analyse the volatile fraction without necessarily attiring it. The dynamic mode involves purging the sample in an inert gas much in the same way as we breathe in the natural fragrance of a fruit.

Recently a dynamic headspace methods has been developed which permits the analysis of the fruit volatile fraction by purging with an inert gas followed by thermal desorption and gas chromatography (Garcia-Jares *et al*., 1995).

The main studies on the Opuntia fruits were the chemical analysis of pulp, skin and seeds (El Kossori *et al*., 1998), analysis of volatile constituents of pulp (Di Cesare and Nani, 1992; Flath and Takahashi, 1978; Ewaidah and Hassan, 1992; Uchoa *et al*.,1998), use of pulp in juice production (Espinosa *et al*., 1973), production of alcoholic beverage (Bustos, 1981), jam production (Sawaya and Khan, 1982; Sawaya *et al*., 1983) and the production of butter equivalents from Opuntia ficus juice fermentation by an unsaturated fatty acid (Hassan *et al*., 1995). An overview of processing technologies concerning the Opuntia ficus (fragosyko 0

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has recently been published by Saenz (2000). Other authors have studied the nutritional significance of *Opuntia* sp. (Stintzing *et al*., 2001).

The extraction and the characterization of the Opuntia ficus (fragosyko) was optimized by several authors (McGarvie and Parolis, 1979; Medina-Torres *et al*., 2000; Trachtenberg and Mayer, 1981, 1982). The macro and micro volatiles of Opuntia Ficus (fragosyko) alcoholic beverage which produced by distillation of fermented pulp from this traditional alcoholic beverage is very attractive to study because the research in this area, as much we know, is very poor. This spirits, it is characterized by a special flavor. Some compounds of this distillate originate from the fruit, but most of them are developed during fruit pulp fermentation. Various factors influence the aromatic profile such as environment, soil and climate, the degree of fruit ripeness, the pulp fermentation conditions and distillation process.

Volatiles of this alcoholic beverage can be classified in different chemical groups such as alcohols, esters, aldehydes, fatty acids etc. These compounds can be characterized by different volatility. Some compounds highly volatile, while others exhibit lower volatility. These volatile compounds exist in a wide concentration range.

Several extraction-concentration methods have been used for spirits, such as liquid-liquid extraction (Ferreira *et al*., 1993, 1999), (Hardy, 1969; Rapp *et al*., 1976; Usseglio, 1971), liquid-liquid extraction with ultrasound (Boidron *et al*., 1988), simultaneous distillation-extraction (Orriols-Fernandez, 1994) and other techniques (Garcia-Jares *et al*., 1995; Salinas *et al*., 1994; Silva *et al*., 1996; Vernin *et al*., 1987).

Above techniques are generally labor-intensive and are characterized by relatively low reproducibility. Sample preparation was mainly concerned to obtain more concentrated samples, but the elimination of interfering substances is also important.

Macro-and micro-volatiles contained in alcoholic beverage from Opuntia ficus (fragosyco) were studied in this work.

MATERIALS AND METHODS

Plant material: Samples of mature Opuntia ficus (fragosyco) fruits, were collected, respectively, in August and September 2003 from many areas of Greece. All samples selected according to FAO/WHO (1986) recommendations. The fruit crushed before fermentation and transferred into wooden barrels of 50 L. The barrels was filled with the pulp up to 35-40 cm below the surface, covered up with plastic wrap, to avoid microbial attack and left to ferment. Then, small quantities of water was added progressively in order to assist the fermentation procedure. According to local producers, the fermentation period was 4 weeks at ambient temperature (20°C). The fermentation was a spontaneous process in which the natural flora of the malt carries out the all procedure.

The distillation of the fermented Opuntiia ficus (fragosyko) pomace was followed.

Physicochemical analyses: Fresh fruit pulp also were measured for their °Brix and pH values and refractive index and analyzed for total acidity according to the relevant EEC Directive (2984/98). °Brix was measured by a portable Brix meter. pH was measured with a digital pH meter (Orion, model 520 A, Boston, MA). Refractive index was determined at 20°C with an Abbè refractometer with temperature adjustment(ATACO, Osaka, Japan). The ash content was determined according to the AOAC method (AOAC, 1990). Acidity was measured by volumetric titration with 0.1 N NaOH using phenolphthalein as indicator. Acidity was expressed as tartaric acid EEC Directive (2984/98).

Distillation: Fermented Opuntia ficus was transferred in a traditional copper alembic of 130 L up to 3/4 of its capacity. Before the beginning of heating, the alambic was hermetically closed in order to prevent any vapour leakage. When the temperature reaches 80-90°C, the liquid distillate of fermented pulp of Opuntia ficus (fragosyco) started to run from the funnel and the freshly distillates were collected and analyzed by extraction and injection in head space GC/MS.

Extraction of distillates: The volatiles constituents of distillate from Opuntia ficus (fragosyco) were extracted and concentrated with combination and modification of the methods described by Moio (1995) and Priser (1997). Distillate (200 mL), dichloromethane (5 mL) and sodium chloride (50 g) were added in a spherical flask. The spherical flask cooled in melting ice was purged with nitrogen for 1 min in order to remove air and the distillate was stirred at 500 rpm for 2 h. The organic extract was dried over anhydrous sodium sulfate and then filtered with special filter (GHP Acrodisc Syringe Filters, GF 0.45) with the help of a gas-tight syringe. The whole process was performed in the absence of air. Filtrates were transferred into small glass-vial and stored at -5°C for analysis.

Analysis of volatile compounds by CG/MS: The headspace volatiles compounds were isolated and detected by a dynamic headspace autosampler Perkin-Elmer HS40 coupled to a GC/MS-Q 5050 system (Shimadzu).

The samples of fresh fruit pulp (5 mL) and of extracted distillates (5 mL) were weighed into 20 mL vials; then the vials were sealed with aluminium-rubber septa. The vials with samples were thermos tatted at 75°C for 15 min, purged and pressurised with 35 mL min^{-1} helium and then the volatile compounds driven through the thermostat via the 90248°C transfer line and injected into the GC/MS.

The volatile compounds were separated on a HP Innowax capillary column (60 m length 0.25 mm i.d., 0.25 mm film thickness) under the following conditions: Injector temperature 200°C; carrier gas helium 0.6 mL min^{-1}; temperature programme: From 35-80°C at a rate of 5°C min^{-1}, held for 3 min and then up to 200°C at a rate of 8°C min^{-1}. The GC column was directly connected without splitting to the ion source of a QP 5050 quadrupole mass spectrometer (interface line 250°C), operating in the scan mode within a mass range of m/z 40-300 at 2 scans/s. Ionization was done by electronic impact at 70 eV and calibration by auto tuning.

Identification of the compounds was carried out by computer-matching of mass spectral data with those in the Shimadzu NIST62 Mass spectral Database and by comparing their retention times and mass spectra to 3-pentanol (50g L^{-1} of ethanol) as internal standards. Quantization was performed by integrating the peak areas of Total Ion Chromatograms (TIC) by the Shimadzu Class 500 software.

Chromatographic runs were carried out in triplicate and their average was used as a single data point in the results section. The average coefficient of variation for the triplicate assays was 1.1%.

The major and minor compounds of Opuntia ficus (fragosyco) are grouped by chemical families Fig. 1 and 2.

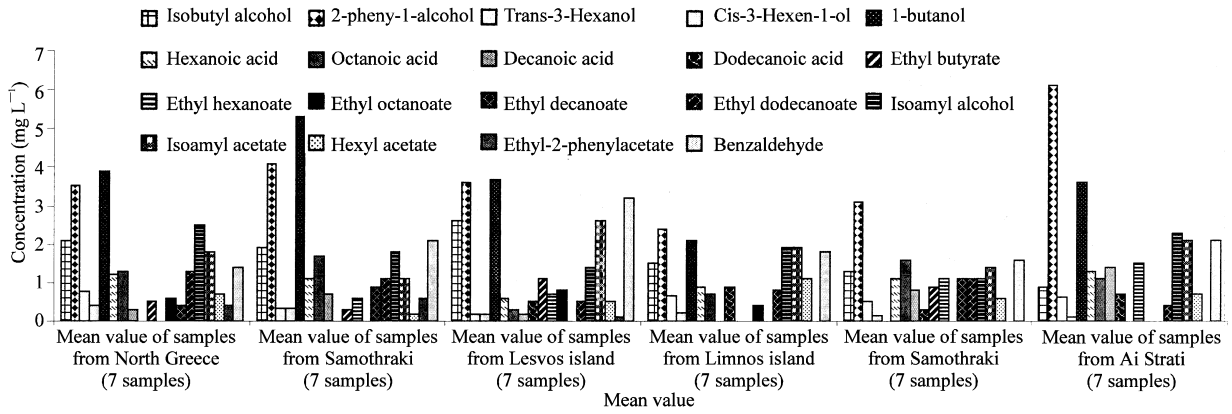

Fig. 1 : Micro-volatiles in distillates from Opuntia ficus (fragosyko)

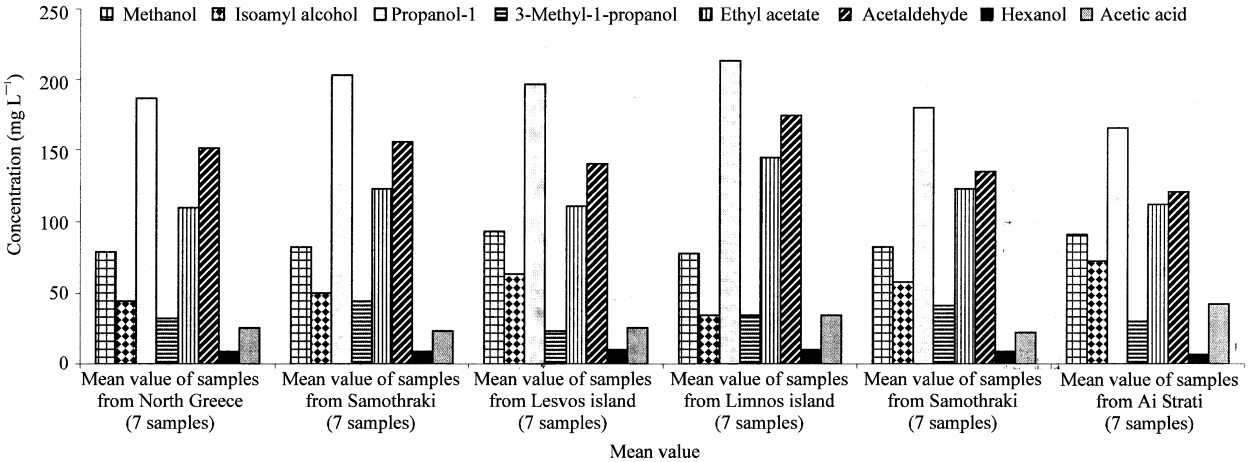

Fig. 2: Macro-volatiles in distillates from Opuntia ficus (fragosyko)

RESULTS AND DISCUSSION

The mean values of the 66 studied samples of produced distillates from fermented pomace of Opuntia ficus(fragosyco) are given in Table 1 and Fig. 1 and 2. Table 2 reports the chemical and physicochemical parameters of Opuntia ficus (fragosyco) pomace from different geographical areas of Greece. Until now no other studies have been found relating to the Opuntia ficus (fragosyco) distillates except from distillates from grape pomace, wine and other fruit like koumaro(Soufleros *et al*., 2005), mouro, (Soufleros *et al*., 2004), melon Lanikanra and Richard (2002),etc.

In the examined samples, °Brix values which varied between 17 and 18.6, pH values which varied between 3.3 and 4.1 and acidity which varied between 5.4 and 7.4 g L^{-1} tartaric acid (Table 2).

Our results were compared with those from other distilled alcoholic beverages such as plum, Greek grape pomace distillates, bagaceiras, aguardiente, cachacas, whiskey, brandy, rum , cherry, pear and apple, given by (Bertrand, 1975, 1994; Cantagrel *et al*., 1997). Soufleros and Bertrand (1991), Silva *et al*. (1996), Silva and Malcata (1998, 1999), Gerogiannaki *et al*. (2004), Kana *et al*. (1991), Lanikanra and Richard (2002), Danilatos and Harvala (1981), Espinosa, *et al*. (1973) and Salvo *et al*. (2002) show that the different chromatographic profiles of the used for alcoholic beverage production fruits clearly defined different fingerprint which can be used as a basis for varietal differentiation. Characteristic taste of the Opuntia ficus distillates describe that the complex volatiles and some precursors volatiles from the fruit which are difficult to concentrate without special techniques gives the unique profile of this traditional alcoholic beverage.

Table 1: Concentration (mg L^{-1}) of volatile compounds identified in distillates from fermented pulp of Opuntia ficus(fragosyko)

Compounds	Reliability of identification[a]	Mean value of samples Greece (7 samples)	Mean value of samples from Samothraki (6 samples)	Mean value of samples from Lesbos Island (15 samples)	Mean value of samples from Limnos (10 samples)	Mean value of samples from Santorini (7samples)	Mean value of samples from Ai Stratis (7 samples)	Relative Stantrard Daviation
Isobutyl alcohol	a	2.1	1..9	2.6	1.5	1.3	0.9	1.1-2.8
Isoamyl alcohol	a	44.7	49.7	62.9	34.7	57.9	71.4	1.9-3.1
2-phenyl-1-alcohol	a	3..5	4.1	3.6	2.4	3.1	6.1	1.1-2.5
Methanol	a	78.8	81.4	93.1	77.7	90.8	68.2	1.2-2.6
Hexanol	a	9.3	8.4	9.7	9.5	9.2	6..3	0.9-2.5
Trans-3-Hexan-1-ol	b	0.77	0.35	O.18	0.67	0.51	0.62	1.3-3.9
Cis-3-Hexen-1-ol	a	0.41	0.34	0.19	0.23	0.13	0.12	1.5-3.6
Propanol	a	187.3	203.1	197.4	213.6	179.8	166.4	1-2.7
1-butanol	a	3. 9	5.3	3.7	2.1	N.D	3.6	1.4-3.1
3Methyl-1-propanol	b	32.1	44.3	23.7	33,9	41.2	29.9	1.1-1.9
Hexanoic acid	a	1.2	1.1	0.6	0.9	1.1	1.3	1-2.9
Octanoic acid	a	1..3	1.7	0..3	0.7	1. 6	1.1	1.8-2.7
Decanoic acid	a	0. 3	0.7	0.2	N.D.	0.8	1.4	1.3-2.9
Dodecanoic acid	a	N.D	N.D	0. 5	0.9	0. 3	0.7	0.9-1.9
Ethyl butyrate	b	0. 5	0. 3	1.1	N.D	0.9	N.D	1.1-3.2
Ethyl hexanoate	a	N.D.	0. 6	0.7	N.D.	1.1	1.5	1.9-3.6
Ethyl octanoate	a	0. 6	N.D	0. 8	0.4	N.D.	N.D	1.1-3.4
Ethyl decanoate	b	0.4	0. 9	N.D.	N.D	1.1	N.D	1.5-3.1
Ethyl dodecanoate	a	1.3	1.1	0. 5	0. 8	1.1	0.4	1.1-2. 9
Ethyl acetate	a	123	111	145	123	112	169	0.9-2.1
Isoamyl acetate	b	2. 5	1. 8	1.4	1. 9	1.1	2..3	1.6-3.1
Isobutyl acetate	b	1.8	1.1	2.6	1. 9	1.4	2.1	1.1-2.9
Hexyl acetate	b	0.7	0.2	0.5	1.1	0.6	0.7	1.5-2.7
Ethyl -2-phenyl acetate	b	0.4	0.6	0.1	N.D.	N.D.	N.D.	2.7-3.4
Acetaldehyde	a	151	111	178	234	101	89	1.5-2.7
Benzaldehyde	a	1.4	2.1	3.2	1.8	1.6	2.1	0.9-3.2
Acetic acid	a	N.D.	23	N.D	34	N.D	N.D	1.1-2.8[a]

Table 2:Chemical and physicochemical parameters of Opuntia Ficus (fragosyko) fresh fruit pulp

Physicochemical parametres	Mean value of 7 samples from North Greece	Mean value of 6 samples from Samothraki	Mean value of 15 samples from Lesvos Island	Mean value of 10 samples from Limnos	Mean value of 7 samples from Santorini	Mean value of 7 samples from Ai Stratis
pH	4.7	4.1	5.1	4.4	4.5	3.9
Volatile Acidity (g/L of HA_C)	0.57	1.21	0.98	0.54	0.59	0.97
°Brix	11.4	10.9	11.3	11.6	10.4	10
Density[c]	1.475±0.002	1.455±0.001	1.451±0.001	1.455±0.003	1.448±0.001	1.459±0.002
Ash (%)	1.11±0.10	1.19±0.14	1.21±0.13	1.21±0.19	1.11±0.16	1.55±0.15

[c]at 20°C

Methanol, in this alcoholic beverage, witch is formed by pectinolytic enzymes that split the methoxyl group from pectin present in crushed fruits is very low, compared with distillates from other fruits. This means that the manipulation of the fruit pulp was the proper one and was managed with great sensitivity and also very good distillation procedures were performed. According to the European legislation (EEC no. 1576/89), the distillate must have a methanol concentration lower than 1000 g hL^{-1} AA.

The levels for our samples were found to be much lower than the European limit, varying from 68.2-93.1 g hL^{-1} AA (Table 1). This means that the manipulation of the raw material was fermented with great sensitivity and also very good distillation procedures were performed (Silva *et al*., 1996). Soufleros and Bertrand (1991), Soufleros (1987) and Da Porto (1998) demonstrated lower values of methanol in grape pomace distillates ranging from 50.4-84 g hL^{-1} AA. Silva *et al*. (1996) and Silva and Malcata (1998) presented for bagaceiras a much more higher concentration of methanol with a mean value equal to 755 g hL^{-1} AA or higher than the European limit, ranging from 1021-1031 g hL^{-1} AA and from 346.8-3828 g hL^{-1} AA, respectively. These values are dependent mainly on the applied technique of the fruit treatment and the distillation and secondly from the fruit variety. In other reports, the concentrations of methanol in grape pomace distillates range between 530 and 1590 g hL^{-1} AA, Cordonnier (1987), from 39-2860 g hL^{-1} AA (Amerine, 1980), from 205-1157 g hL^{-1} AA (Bertrand, 1975).

The last authors also gave mean values of methanol for distillate from apple 359 g hL^{-1} AA, from cherry 457 g hL^{-1} AA, from pear 796 g hL^{-1} AA and from plum 866 g hL^{-1} AA. Lehtonen *et al*. (1999) for whiskey introduced significantly low mean concentrations of 0.077 g hL^{-1} AA and for rum even lower of 0.023 g hL^{-1} AA. After the above comparison, it can be said that Opuntia ficus(fragosyco) distillate has a very low levels of methanol in relation to other fruit pomace distillates. The relative similarity between the other fruit distillates and Opuntia ficus (fragosyco) distillate, even though they are derived from different raw materials, indicates the high effect that the use of the same technique of distillation and the type of alambic has on the chemical composition of the distillate.

Higher alcohols constitute the group with the highest concentration in distillates, which gives to them a flavouring aroma and an essential character (Ferreira *et al*., 1999; Silva and Malcata, 1998). Nycanen and Nycanen (1991). For this reason, the European legislation demands minimum requirements for these aromatic substances higher than 140 g hL^{-1} AA. However, some alcohols (e.g. the amylic) in very high concentrations are responsible for certain toxicity. The levels of these compounds are influenced by variety, fermentation conditions and distillation and are concentrated mainly in the first fraction, named head, of the distillates (Silva and Malcata, 1998).

The most important higher alcohols of Opuntia ficus (fragosyco) distillate are, isobutyl alcohol, isoamyl alcohol, 1-butanol, 3-methyl-1-propanol. Silva *et al*. (1996) reported for grape pomace distillates mean values equal to 80 g hL^{-1} AA for 2-methyl-1-propanol and 5.1 g hL^{-1} AA for 1-butanol. Bauer-Christoph *et al*. (1997), for 2-methyl-1-butanol, presented the following concentrations for distillates coming from apple 67 g hL^{-1} AA, from pear 67 g hL^{-1} AA, from plum 53 g hL^{-1} AA, from cherry 48 g hL^{-1} AA and finally from grape marc 66 g hL^{-1} AA. For 1-butanol, they gave mean values of 11.4, 12.4, 11.8, 1.9 and 2.5 g hL^{-1} AA for the rementioned distillates. Soufleros and Bertrand (1991) and Soufleros (1987), for grape pomace distillates reported that 2-methyl-1-propanol varies from 29.2-65.2 g hL^{-1} AA. Fitzgerald *et al*. (2000), for whiskey, reported values as low as 0.96 g hL^{-1} AA for 2-methyl-1-propanol and 1.09 g hL^{-1} AA for 1-butanol.

The amylic alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) constitute quantitatively the biggest part of the higher alcohols and are considered-be predictors of sensory character in the distilled product (Silva *et al*., 1996). For these two compounds, Silva *et al*. (1996) gave for grape pomace mean values of 62.2 and 204.4 g hL^{-1} AA, higher than Opuntia ficus (fragosyco) distillate (Table 1), but Silva and Malcata (1998) presented concentrations similar-ours for 2-methyl-1-butanol, ranging from 39.5-50.3 g hL^{-1} AA and slightly lower values between 86.8 and 103.56 g hL^{-1} AA for 3-methyl-1-butanol. Similar concentrations were evaluated from Fitzgerald *et al*. (2000) demonstrated a little lower concentrations for whiskey, ranging from 7.5-20 and from 21.4-49.8 g hL^{-1} AA. On the other hand, Lehtonen *et al*. (1999) defined levels, respectively, from 0.19-0.20 and from 0.56-0.59 g hL^{-1} AA for whiskey, 0.03-0.07 and 0.19-0.28 g hL^{-1} AA for rum and equal-0.49 and from traces-2.32 g hL^{-1} AA for brandy. Generally, all our distillate samples satisfy the minimum limits of 140 g hL^{-1} AA that the European legislation demands. 1-Hexanol is an alcohol originating only from raw material (Soufleros and Bertrand, 1991). It is considered-be a favourable compound if its concentration is above 0.5 g hL^{-1} AA but not higher than 10 g hL^{-1} AA; otherwise, a grassy flavour is imposed, making the distillate product unpleasant both in aroma and taste (Tourliere , 1977). Ferreira *et al*. (1993,

1999) estimated that the presence of 1-hexanol in the above mentioned concentrations imparts-wines and distillates a fruity or liquorice aroma. 1-hexanol concentrations are presented in Table 1. According-Soufleros and Bertrand (1991) the concentrations of 1-hexanol in grape pomace distillates ranged from 1.6-4.3 g hL^{-1} AA and were lower than those in Opuntia ficus (fragosyco) distillates. Silva *et al.* (1996) observed for grape pomace distillates a mean value of 13.3 g hL^{-1} AA, while Silva and Malcata (1998) found concentrations from 11.4-21.89 g hL^{-1} AA and from 6.36-31.56 g hL^{-1} AA in 1999. Bauer-Christoph *et al.* (1997) demonstrated values of 10.2 g hL^{-1} AA for apple, 10.3 g hL^{-1} AA for pear, 3.2 g hL^{-1} AA for plum, 1.6 g hL^{-1} AA for cherry and 15.4 g hL^{-1} AA for marc distillates.

Generally, fragosyco distillate has a low content of 1-Hexanol in relation-all other alcoholic products. This is probably due-the weak grassy character of this fruit. 2-Phenyl-ethanol introduces a pleasant aroma-distillates, resembling-rose (Stark *et al.*, 1998) and derives from L-phenylalanine through metabolic reaction of Saccharomyces cerevisiae during carbonic anaerobiosis (Silva and Malcata, 1998, 1999; Stark *et al.*, 1998).

Table 1 shows values from 2.4-6.1 g hL^{-1} AA. Silva *et al.* (1996) gave for grape pomace a mean value up-2.22 g hL^{-1} AA. Fitzgerald *et al.* (2000), for whiskey, presented a mean concentration of 1.44 g hL^{-1} AA and Rogerson *et al.* (2001) revealed values ranging from 0.55-1.8 g hL^{-1} AA for aguardiente. These amounts are more or less inferior-our results. Higher concentrations, for 2-phenylethanol, were demonstrated by Soufleros and Bertrand (1991) for grape distillates and varying from 2.8-23.4 g hL^{-1} AA.

The fatty acid esters contribute-the distillates a flavour with a pleasant fruity and flowery smell (Karagiannis and Lanaridis, 2002), indicative of the quality of the spirit (Silva and Malcata, 1999; Soufleros *et al.*, 2004, 2005). Ethyl hexanoate is the most abundant of all esters (Bartley and Schwede, 1989). The ethyl esters hexanoate, octanoate and decanoate, which are produced during the raw materials fermentation (Silva and Malcata, 1998), pass-the spirits and increases during aging (Silva and Malcata, 1999; Soufleros *et al.*, 2004). Through the distillation process, the heat releases a significant amount of these esters from the yeast cells where they remain bond after fermentation (Caumeil, 1983). Schumacher *et al.* (1998). These three compounds have quantitatively a small participation compared-the other volatile compounds (e.g. higher alcohols) and determine a vastly profiled aromatic character for the spirits (Ferreira *et al.*, 1999).

According to Table 1, ethyl hexanoate, ethyl octanoate and ethyl decanoate are given at mean values of 0.3-1.5 g hL^{-1} AA, respectively. For the same esters, concerning the grape pomace samples, Silva *et al.* (1996) indicated mean values of 0.89, 21.44 and 2.44 g hL^{-1} AA correspondingly. Silva and Malcata (1998) presented lower concentrations with mean values varying from 0.18-0.24, from 0.10-0.45 and from 0.16-0.4 g hL^{-1} AA, respectively, while in 1999, they presented almost double values, varying from 0.22-2.25, from 0.25-1.84 and from 0.08-1.04 g hL^{-1} AA for the aforementioned esters, depending on grape variety.

Fitzgerald *et al.* (2000) determined for whiskey values ranging between 0.04-0.22, 0.03-0.13 and 0.02-0.13 g hL^{-1} AA for these three esters respectively, which are lower compared-our results (Table 1). From these two articles it is concluded that both Rogerson *et al.* (2001) and Fitzgerald *et al.* (2000) gave such values due-the influence of the initial raw materials being used for the distillates. Furthermore, Soufleros and Bertrand (1991) reported for grape pomace distillates, for these ethyl esters, values ranging respectively from 0.4-1.2, from 0.8-4.0 and from 0.3-4.1 g hL^{-1} AA.

Isoamyl acetate, hexyl acetate and phenyl-ethyl acetate constitute the acetic acid ester group, which are mostly responsible for the flowery and fruity aroma of the distillates (Ferreira *et al.*, 1999; Silva and Malcata, 1999). Table 1 shows that isoamyl acetate has the highest concentration among these three acetates.

According to Silva *et al.* (1996) the mean value of isoamyl acetate for bagaceiras was 1.33 g hL^{-1} AA. Silva and Malcata, 1998 reported concentrations from 0.4-0.96 g hL^{-1} AA for isoamyl acetate and in 1999, values slightly higher ranging from traces-0.91 g hL^{-1} AA, depending mostly on the 2 grape varieties used and the extraction time. Rogerson *et al.* (2001) reported for aguardiente very surprising values of isoamyl acetate, varying from 183.5-398 g hL^{-1} AA, while Soufleros and Bertrand (1991) gave for grape pomace values lower than 10 g hL^{-1} AA. For whiskey, much lower mean concentrations were given from Fitzgerald *et al.* (2000) for this same compound, ranging from 1.43-4.92 g hL^{-1} AA. Ethyl acetate derive mainly from bacterial spoilage of the distilled marc (Silva and Malcata, 1998, 1999; Soufleros and Bertrand, 1991). Ethyl acetate is the ester with the higher concentration, which above the perception threshold of 180 g hL^{-1} AA gives-the spirit an acidic character (Ferreira *et al.*, 1999).

For ethyl acetate, Silva *et al.* (1996) presented for bagaceiras mean values of 44.4 g hL^{-1} AA, respectively. In addition, Silva and Malcata (1998) gave 2 mean values of 314.7 and 494 g hL^{-1} AA for ethyl acetate, related-the

grape variety used for the distillates. In 1999, Silva and Malcata gave, for the same spirit, two mean values of 45.1 and 853.8 g hL^{-1} AA for ethyl acetate, depending on the grape variety and the extraction time. Soufleros and Bertrand (1991), for tsipouro, demonstrated concentrations up-58 g hL^{-1} AA for ethyl acetate lower than ours. For ethyl acetate, many other authors gave results with a high diversity. Lafon *et al.* (1973) and Cordonnier (1987) gave for marc values of 100-280 g hL^{-1} AA and 230-330 g hL^{-1} AA, respectively, almost twice as much as ours. For a different distillate, whiskey, Fitzgerald *et al.* (2000) and Lehtonen *et al.* (1999) gave significantly lower values, ranging from 1.59-20.6 g hL^{-1} AA and from traces-0.19 g hL^{-1} AA, respectively. On the other hand, Lehtonen *et al.* (1999) recorded for rum concentrations with a lower mean value ranging between 0.06 and 0.12 g hL^{-1} AA and for brandy slightly higher up-0.41 g hL^{-1} AA.

About ethyl acetate, comparing all the above results we conclude that in our samples, with two exceptions, he is present at concentrations commonly acceptable.

Comparing all above mentioned values we conclude that among the pomace distillates, ours samples have the lowest concentrations of these three acetates. Long chain fatty acids, hexanoic, octanoic, decanoic and dodecanoic acid, are of smaller flavour effect-the distillates (Silva and Malcata, 1999; Soufleros *et al.*, 2004, 2005) and are usually found at low concentrations but with an odor similar in strength string smell equal-acetic acid (Silva and Malcata, 1999; Soufleros *et al.*, 2004, 2005) and consequently, with an important contribution-the aromatic character of the distillates (Ferreira *et al.*, 1999; Silva and Malcata, 1999). Karagiannis and Lanaridis (2002) note that there are probably released as intermediate products via yeast metabolism of carbohydrates and can be influenced by insoluble grape solids during fermentation presenting a soapy odour.

The results in Table 1 shows that, from these two fatty acids (hexanoic acid and octanoi acid), are quantitatively the most important.

Also Table 1 shows that hexanoic acid has the highest mean value of all these acids, followed by octanoic acid, decanoic acid and dodecanoic acid According to Silva *et al.* (1996) the mean concentrations for bagaceiras corresponding-the pre-mentioned four acids were 0.44, 0.89, 0.89 and 0.22 g hL^{-1} AA, respectively. Silva and Malcata (1998) determined mean values ranging from 0.21-0.36, from 0.16-0.36, from 0.17-0.36 and from 0.08-0.14 g hL^{+} AA, respectively, while Silva and Malcata (1999) defined results from 0.12-3.0, from 0.09-1.06, from 0.04-1.6 and from 0.02-0.37 g hL^{-1} AA, respectively for these four fatty acids, directly related-grape variety and extraction time. Rogerson *et al.* (2001) from the aguardiente evaluation gave concentrations for hexanoic, octanoic and dodecanoic acid from traces-518 g hL^{-1} AA, from 45.5-1662.5 g hL^{-1} AA and finally from 0-116.5 g hL^{-1} AA, respectively. Soufleros and Bertrand (1991), for tsipouro, estimated values ranging from 0.3-1.2 g hL^{-1} AA for octanoic, from 0.2-1.4 g hL^{-1} AA for decanoic and from 0.1-0.4 g hL^{-1} AA for dodecanoic acid, concentrations similar-those given from other authors and more or less close-those gathered at Table 1.

The pH values were given in Table 2. Caumeil (1983) gave for aguardiente pH mean value similar-ours (4.34). Lafon *et al.* (1973) determined that this pH usually characterizes wine and fruit pomace distillates 1-2 years old. Soufleros and Bertrand (1991) presented for tsipouro similar pH values varying from 4.15-7.0, like our results, while Lehtonen *et al.* (1999) gave for brandy much lower pH mean values, equal-3.50. These last authors gave for whiskey and rum intermediary values of 3.95 and 3.89, respectively.

CONCLUSION

The aroma profile of Opuntia ficus alcoholic beverage was successfully characterized by means of head space GC-MS and major and minor compounds were identified at the trace level. Those compounds where differences occur among samples having different cultivation areas, basically constituted by ethyl esters and C_{13} norisoprenoids.

This research, for the important macro and micro volatiles that contribute the typical aroma of the Opuntia ficus(fragosyko)-the produced alcoholic beverage, such 2-phenyl ethanol ethyl 2-phenyl acetate, in combination with the other volatiles, are in higher concentration in comparison with other spirits. Methanol is lower than the internationally limit for alcoholic beverages. The distillates from Opuntia ficus (fragosyko)shows that this spirit has a very rich and unique organoliptic fingertip with all this macro and micro volatiles which contains The obtained results have shown that the fruit of Opuntia ficus (fragosyko) has significant influence on aroma of produced distillates. In this study it is demonstrated that using specific fruit and the procedures of distillation can be produced one unique alcoholic beverage.

REFERENCES

Amerine, O., 1980. Methods forAnalysis of Musts and Wines, Wiley, New York, pp: 74-152.

Association of Official Analytical Chemists ,1984. Official methods of analyses. Washington, DC: Association of Official Analytical Chemists.

Association of Official Analytical Chemists, 1990. Official methods of analyses. Washington, DC: Association of Official Analytical Chemists.

Bartley, J.P. and A.M. Schwede, 1989. Production of volatile compounds in ripening kiwi fruit (Actinidio chinensis). J. Agric. Food Chem., 37: 1023-1025.

Bauer-Christoph, C., H. Wachter, N. Christoph, A. Roßmann and A. Ludwig, 1997. Assignment of raw material and authentication of spirits by gas chromatography, hydrogen- and carbon-isotope ratio measurements. Zeitschrift fur Lebensmittel Untersuchung und Forschung A., 204: 445-452.

Bertrand, A., 1975. Recherches sur l'analyse des vins par chromatographie en phase gazeuse. Thëse Docteur en Sciences. Universitè de Bordeaux II, France. Bertrand, A., 1994. Recueil des mèthodes d' analyse des boissons spiritueuses, des alcohols, de la fraction aromatique des boissons. O.I.V., Paris, France.

Boidron, J.N., P. Chatonnet and M. Pons,1988. Influence du bois sur certaines substances odorantes des vins. Connaissance Vigne et Vin., 22: 275-294.

Bustos, E.O., 1981. Alcoholic beverage from Chilean Opuntia ficus indica. Am. J. Enol. Viticul., 32 (3): 228-229.

Cantagrel, R., L. Lurton, J.P. Vidal and B. Galy, 1997. From Vine-Cognac. In: Lea, A.G.H. and J. R. Piggott (Eds.), Fermented Beverage Production. 1st Edn. London: Blackie Academic and Professional, pp: 208-228.

Caumeil, M.,1983. Le Cognac. Revue Pour la Science, Edition: Francaise de. scientific Americain. France, pp: 48-57.

Cordonnier, R., 1987. Le methanol et ses origines dans le vin. Progres Agricole et Viticole, 104: 315-318.

Danilatos, N. and A. Harvala, 1981. Evaluation of wine distillates Greek grape varieties Greek Enological Chronicles, v.1, pp: 88-93.

Da Porto, C., 1998. Grappa and grape-spirit production. Crit. Rev. Biotechnol., 18: 13-24.

Di Cesare, L.F. and R. Nani, 1992. Analysis of volatile constituents of prickly pear juice (Opuntia ficus indica var. Fructa sanguineo). Fruit Processing, 2 (1): 6-8.

El Kossori, R.L., C. Villaume, E. El Boustani, Y. Sauvaire and L. Mejean, 1998. Composition of pulp, skin and seeds of prickly pears fruit (*Opuntia ficus* indica sp.). Plant Foods for Human Nutrition, 52: 263-270.

Espinosa, J., R. Borrocal, M. Jara, C. Zorilla and J. Medina, 1973. Quelques proprietes et essais preliminaires de conservation des fruits et du jus de figue de barbarie (*Opuntia ficus* indica). Fruits, 28: 285-289.

Ewaidah, E.H. and B.H. Hassan, 1992. Prickly pear sheets: A new fruit product. Int. J. Food Sci. Technol., 27: 353-358.

FAO/WHO Recommendations methods of sampling, 1986. Codex Alimentarius Commission of the joint FAO/WHO Food Standards Program: Food and Agricultural Organization/World Health Organization, Rome.

Ferreira, A., A., Rap, J.F. Cacho, H. Hastrich and I. Yavas, 1993. Fast and quantitative determination of wine flavor compounds using microextraction with freon 113. J. Agric. Food Chem., 41: 1413-1420.

Ferreira, V., P. Hernandez-Orte, A. Escudero, R. Lopez and J. Cacho, 1999. Semipreperative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. J. Chromatography A., 864: 77-88.

Fitzgerald, G., K.J. James, K. Macnamara and M.A. Stack, 2000. Characterization of whiskeys using solid-phase microextraction with gas chromatography-mass spectrometry. J. Chromatography A., 896: 351-359.

Flath, R.A. and J.M. Takahashi, 1978. Volatile constituents of prickly pear (*Opuntia ficus* indica Mill.), de Castilla variety. J. Agric. Food Chem., 26: 835-837.

Garcia-Jares, C., S. Garcia-Martin and C. Cela-Torrijos, 1995. Analysis of some highly volatile compounds of wine by means of purge and cold trapping injector capillary gas-chromatography. Application-the differentation of Rias Biaxas Spanish white wines. J. Agric. Food Chem., 43: 764-768.

Hardy, P., 1969. Extraction and concentration of volatiles from dilute aqueous and aqueous-alcoholic solution using trichloromethane. J. Agric. Food Chem., 17: 656-658.

Hassan, M., P.J. Blanc, A. Pareilleux and G. Goma, 1995. Production of cocoa butter equivalents from prickly pear juice fermentation by an unsaturated fatty acid auxotroph of Cryptococcus curvatus grown in batch culture. Process Biochem., 30 (7): 629-634.

Hegwood, D.A., 1990. Human health discoveries with *Opuntia* sp. (Prickly pear). J. Horticultural Sci., 25: 1515-1516.

Kana, A., A. Kanellaki and A. Koutinas, 1991. Cause of and methods-reduce methanol content of tsicoudia and ouzo. Int. J. Food Sci. Technol., 26: 241-247.

Karagiannis, S. and P. Lanaridis, 2002. Insoluble grape material present in must affects the overall fermentation aroma of dry white wines made from three grape cultivars cultivated in Greece. J. Food Sci., 67: 369-374.

Lafon, J., P. Couillaud and F. Gay-Bellile, 1973. Le cognac, sa Distillation. 5th Edn. Bailliere, Paris, -VI. 288 France.

Lanikanra, O. and O. Richard, 2002. Effect of storage on some volatile aroma compounds in fresh-cut Cantaloupe Melon. J. Agric. Food Chem., 50: 4043-4047.

Lehtonen, P.J., L.A. Keller and E.T. Ali-Mattila, 1999. Multimethod analysis of matured distilled alcoholic beverages for brand identification. Zeitschraft f-ur Lebensmittel-Untersuchung und- Forschung A., 208: 413-417.

McGarvie, D. and H. Parolis, 1979. The mucilage of Opuntia ficus indica. Carbohydrate Research, 69: 171-179.

Medina-Torres, L., E. Brito-De La Fuente, B. Torrestiana-Sanchez and R. Katthain, 2000. Rheological properties of the mucilage gum (*Opuntia ficus* indica). Food Hydrocolloids, 14: 417-424.

Moio, I., F. Chamberllant, I. Leschaeve, S.P. Issanchou and X.P. Etievant, 1995. Production of representative wine extracts for chemical and olfactory analysis. Italian J. Food Sci., 3: 265-278.

Nycanen, L and I. Nycanen, 1991. Volatiles compounds in Food and Beverages. Edited by Henk Maarse. New York-Basel. Hong Kong.

Orriols-Fernandez, I., 1994. Tecnologia de la distilation en los aguardientes de orujo. Proc. Congresso International de la Viticultura Atlantica (Spain), pp: 291-234.

Priser, C., X.P. Etievant, S. Nicklaus and R. Brun, 1997. Representative champagne with exracts for gas chromatography-olfactometry analysis. J. Agric. Food Chem., 45: 3511-3514.

Rapp, A., H. Hanstrich and H. Engel, 1976. Gas-chromatographic investigations on the aroma constituents of grape. Concentration and separation by capillary glass column. Vitis, 15: 29-36.

Rogerson, F.S.S. and V.A.P. de Freitas, 2001. Quantitative analysis of volatiles present in wine spirits used for port production. American Society of Enology and Viiiculture fifty 2nd Annual meeting, San Diego, CA.

Saenz, C., 2000. Processing technologies: An alternative for cactus pear (*Opuntia* sp.) fruits and cladodes. J. Arid Environ., 46: 209-225.

Salinas, M.R., G.L. Alonso and F.J. Javier-Infantes., 1994. Adsorption thermal desorption gas-chromatography applied-the determination of wine aromas. J. Agric. Food Chem., 42: 1328-1331.

Salvo, F., E.M. Galati, S. Lo Curto and M.M. Tripodo, 2002. Study on the chemical characterization of lipid composition of Opuntia ficus-indica L. seed oil. Rivista Italiana delle Sostanze Grasse, 79 (11): 395-398.

Sawaya, W.N. and P. Khan, 1982. Chemical characterization of prickly pear seed oil, Opuntia ficus indica. J. Food Sci., 47: 2060-2061.

Sawaya, W.N., H.A. Khatchadorian, W.M. Safi and H.M. Al-Mohammad, 1983. Chemical characterization of prickly pear pulp. Opuntia ficus indica and the manufacturing of prickly pear jam. J. Food Technol., 18: 183-193.

Stintzing, F.C., A. Schieber and R. Carle, 2001. Phytochemical and nutritional significance of cactus pear. Eur. Food Res. Technol., 212: 396-407.

Schumacher, K, S. Asche, M. Heil, F. Mittelstaedt, H. Dietrich and A. Mosandl, 1998. Methyl-branched flavour compounds in fresh and processed apples. J. Agric. Food Chem., 46: 4496-4500.

Silva, M.L., F.X. Malcata and G. Revel, 1996. Volatile contents of grape marcs in Portugal. J. Food Composition and Anal., 9: 72-80.

Silva, M.L. and F.X. Malcata, 1998. Relation between storage conditions of grape pomace and volatile composition of spirits obtained therefrom. Am. J. Enology and Viticulture, 49: 1.

Silva, M. L. and F.X. Malcata, 1999. Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs. Zeitschrift fur Lebensmittel Untersuchung und Forschung A., 208: 134-143.

Soufleros, E. and A. Bertrand, 1991. Etude sur le tsipouro eau de vie de marc traditionelle, precurseur de l 'ouzo. Connaissance Vigne Vin. 21 (2): 93-111.

Soufleros, E., 1987. La production artisanale du antinasale du Tsipouro a Naoussa (Greece). In Les Eaux-de vie Traditionelles d' Origine Viticole .Bertrant (Ed). 19-27.

Soufleros, E.H., S.A. Mygdalia and P. Natskoulis, 2004. Characterization and safety evaluation of the traditional Greek fruit distillate. Mouro. by flavor compounds and mineral analysis. Food Chem., (86): 625-636.

Soufleros, E.H., S.A. Mygdalia and P. Natskoulis, 2005. Production process and characterization of the traditional Greek fruit distillate. Koumaro. by aromaticand mineral composition. J. Food Composition and Anal., 18: 699-716.

Stark, D., T. Munch, B. Sonnleitner, I.W. Marison and U. von Stocker, 1998. Inhibition kinetics of 2-phenylethanol on the growth of Saccharomyces cerevisiae. Symposium international ESBES-2, Porto, Lausanne.

Tourliere, S., 1977. Commentaires sur la pr-esence d-un certain nombre de compos-es accompagnant l-alcool dans les distillats spiritueux rectifi-es et eaux-de-vie. Int. Alimenti Agric., 94: 565-574.

Trachtenberg, S. and A.M. Mayer, 1981. Composition and properties of Opuntia ficus indica mucilage. Phytochemistry, 20: 2665-2668.

Trachtenberg, S. and A.M. Mayer, 1982. Biophysical properties of Opuntia ficus indica mucilage. Phytochemistry, 21(12): 2835-2843.

Uchoa, A.F., P.A.S. Souza, R.M.Z. Zarate, E. Gomez-Filho and F.A.P. Campos, 1998. Isolation and characterization of a reserve protein from the seeds of Opuntia ficus indica. Brazilian J. Med. Biolog. Res., 31: 757-761.

Usseglio, T., 1971. Le caracteristiche della grappa . Vini d' Italia, 453-462.

Vernin, G., C. Boniface, J. Merzger, D. Fraisse, D. Doan and S. Alamercery, 1987. Aromas of Syrah wines: Identification of volatiles compounds by GC-MS spectra data bank and classification by statistical methods. In: Frontiers of flavors, Proceedings of the 5th International Flavor Conference, Porto Karras, Chalkidiki, Greece, pp: 1-3.