International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:6, 2015

Influence of Maturation Degree of Arbutus (*Arbutus unedo* L.) Fruits in Spirit Composition and Quality

Goreti Botelho, Filomena Gomes, Fernanda M. Ferreira, Ilda Caldeira

***Abstract*—**The strawberry tree (*Arbutus unedo* L.) is a small tree or shrub from botanical *Ericaceae* family that grows spontaneously nearby the Mediterranean basin and produce edible red fruits. A traditional processed fruit application, in Mediterranean countries, is the production of a spirit (known as *aguardente de medronho*, in Portugal) obtained from the fermented fruit. The main objective of our study was to contribute to the knowledge about the influence of the degree of maturation of fruits in the volatile composition and quality of arbutus spirit. The major volatiles in the three distillates fractions (head, heart and tail) obtained from fermentation of two different fruit maturation levels were quantified by GC-FID analysis and ANOVA one-way was performed. Additionally, the total antioxidant capacity and total phenolic compounds of both arbutus fruit spirits were determined, by ABTS and Folin-Ciocalteau method, respectively. The methanol concentration is higher (1022.39 g/hL a.a.) in the spirit made from fruits with highest total soluble solids, which is a value above the legal limit (1000 g/hL a.a.). Overall, our study emphasizes, for the first time, the influence of maturation degree of arbutus fruits in the spirit volatile composition and quality.

***Keywords*—**Arbutus fruit, maturation, quality, spirit.

I. Introduction

THE strawberry tree (*Arbutus unedo L.*) is a forest specie present in the most of Portuguese continental territory, known for its great rusticity, resiliency, annual production and fast entry in production, that is to say, it is one of the Portuguese forest species with the biggest potential for progression.

The arbutus fruit is seldom consumed fresh and is mainly processed to give various products, including jam, jelly, vinegar, liquor and spirit [1]-[8]. The main application of arbutus has been in production for a known spirit in Portugal as *aguardente de medronho* [2], [9], in Spain as *aguardiente de madroño* [10], in Greece as *koumaro* [1] and in Italy as *corbezzolo* [11]. This alcoholic drink is a traditional product produced in family size units or small industrial units which makes the strawberry tree a shrub with a socio-economic relevance [1]-[3].

As far as we know from the scientific literature, there is still a lack of knowledge in the arbutus fruit spirit production process optimization in order to improve the sensory quality of the final product. Hence, the major goal of this research was to study the effect of initial ripening level of arbutus fruit on the obtained spirit volatile composition (and on the fractions head and tail), and, at last, quantify the total antioxidant capacity and total phenolic compounds from that same spirits.

Financial support: Fundação para a Ciência e a Tecnologia (FCT), project - PTDC/AGR-FOR/3746/2012 and PRODER program, Project nº 43748, 4.1, Cooperation for Innovation. PEst-OE/AGR/UI0681/2014 for CERNAS, PEst-OE/AGR/UI4033/2014 for CITAB and PEst-OE/AGR/UI0115/2014 for ICAAM being the three I&D Units co-funded by FEDER/Compete and National Budget.

Goreti Botelho is with CERNAS Research Unit and Coimbra College of Agriculture, Polytechnic Institute of Coimbra (IPC), 3045-601 Coimbra, Portugal (corresponding author to provide phone: +351239802940; fax: +351239802979 e-mail: goreti@esac.pt).

Filomena Gomes is with CERNAS Research Unit and Coimbra College of Agriculture, IPC, 3045-601 Coimbra, Portugal (e-mail: fgomes@esac.pt).

Fernanda M. Ferreira is with CITAB Research Unit, UTAD and Coimbra College of Agriculture, IPC, 3045-601 Coimbra, Portugal (e-mail: fferreira@esac.pt).

Ilda Caldeira is with ICAAM Research Unit, University of Évora and National Institute of Agricultural and Veterinary Research – INIAV, 2565-191 Dois Portos, Portugal (e-mail: ilda.caldeira@iniav.pt).

II. Materials and Methods

A. Samples and Experimental Design

Mature fresh arbutus fruits were used for the experiment in this study. All fruits were collected in a 16 ha instalation located in the center of Portugal, at high about 680 m from sea level. Two distinct sets of fruits were collected in October 2013: A – arbutus fruits collected directly from protections covering the floor under the plants after their natural fall; B – arbutus fruits collected directly from the plant. All the fruits A and B, were carefully examined to avoid the presence of dust, dirt, immature and damaged fruits and rapidly transported to a laboratory for alcoholic fermentation process.

The experimental design involved two sets of four fermentation containers in equal controlled experimental conditions: A1 and A2 containers with 4 kg of fruits from set A in spontaneous fermentation each; B1 and B2 containers with 4 kg of fruits from set B. After the end of the alcoholic fermentations, all the fermented fruits were distilled in a small size copper alembic (16 dm^3). The alcoholic fermentation of arbutus fruits was monitored throughout the time all the way through physical and chemical parameters analysis, pH, total soluble solids (TSS, ºBrix) and its temperature. After the end of fermentation, distillation took place, and three fractions were obtained out of it: head, heart and tail. The quantification of volatile compounds was performed towards the three fractions, using the gas chromatography technique and the obtained analytical data was compared to the legal limits established in the Decree-Law n.º 238/2000 [12].

The first part with approximately 5 % (above 70 % vol., with a strong, pungent and unpleasant flavour) of the distillates was collected as head fraction. The heart fractions obtained by single distillation, were collected when the ethanol concentration varied from 70 to 35 % v/v; finally, the tail fractions were obtained when the alcoholic content decreased below 35 % v/v. The ethanol content of distillates

was determined by electronic densimetry [13] by using an electronic densimeter (Antoon Paar DMA 5000, 2002, Austria).

B. Chemicals

Ethanol was purchased from Merck (Darmstadt, Germany). Distilled water was used to prepare the hydroalcoholic solutions.

GC-FID standards: Ethyl acetate [CAS Nº 141-78-6; purity ≥ 99.8 %] was purchased from Riedel-de-Haen (Seelze, Germany), methanol [CAS Nº 67-56-1; purity ≥99.9 %] was purchased from Merck (Darmstadt, Germany). 2-Methyl-1-butanol [CAS Nº 137-32-6; purity ≥ 98.0 %] 3-methyl-1-butanol [CAS Nº 123-51-3; purity ≥ 98.5 %], 1-butanol [CAS Nº 71-36-3; purity ≥ 99.5 %], 2-methyl-1-propanol [CAS Nº 78-83-1; purity ≥ 99.5%], 1-propanol [CAS Nº 71-23-8; purity ≥ 99.5 %], 2-propen-1-ol [CAS Nº 107-18-6; purity ≥ 98 %], 2-butanol [CAS Nº 78-92-2; purity ≥ 99.5 %], 4-methyl-2-pentanol [CAS Nº 108-11-2; purity ≥ 97 %] and acetaldehyde [CAS Nº 75-07-0; purity ≥ 99.5 %] were purchased from Fluka (Buchs, Switzerland).

ABTS and TPC standards: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), galic acid, potassium persulfate and Folin-Ciocalteau reagent were obtained from Sigma–Aldrich (St. Louis, MO, USA). All the chemicals used were of analytical grade.

C. ABTS Assay

$ABTS^{\bullet+}$ radical-scavenging activity of the hydrophilic fractions was determined by a procedure reported by [14] with slight modifications [15]. All experiments were performed three times and in triplicate at controlled temperature of 30±1ºC. The free-radical scavenging activity was expressed as micromoles of Trolox per milliliter of sample (μmol $TE.cm^{-3}$).

D. Phenolic Compounds Evaluation

Total phenolic compounds (TPC) were estimated by the Folin-Ciocalteau method, a colorimetric assay based on procedures described by [16] and adapted by [15]. All experiments were performed three times and in triplicate. Results were expressed as gallic acid equivalents per amount sample ($GAE.cm^{-3}$).

E. GC-FID Analysis

Gas Chromatography (GC) analysis was carried out using an Focus GC gas chromatograph (Thermo Scientific, USA) equipped with a flame ionisation detector (FID, 250ºC) and a fused silica capillary column of polyethylene glycol (DB-WAX, JW Scientific, Folsom, CA, USA), 60 m length, 0.32 mm i.d., 0.25 μm film thickness. The carrier gas was hydrogen (3.40 $cm^3.min^{-1}$). The samples were injected (~1.0 μL) on the injector (200 ºC) in split mode (split ratio 1:6). The oven temperature program was: 35 ºC (for 8 min), then increased at $10ºC.min^{-1}$ to 200ºC and held for this temperature for a further 9 min.

The compounds, in the spirit drinks, are determined by direct injection in the gas chromatograph of about 1.0 μL of the spirits. The concentration of each compound is determined with respect to the internal standard from response factors, which are obtained during calibration using the standard solutions. The concentration of the compounds are expressed as g per hectolitre of 100 % vol. alcohol, (g/hL a.a.) using the alcohol strength results, in order to verify the regulatory requirements [17]. This methodology was previously validated [18].

F. Statistical Analysis

ANOVA one-way analysis was performed using the software IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

III. Results and Discussion

The analytical characterization of the arbutus fruit samples is summarized in Table I.

TABLE I
ANALYTICAL CHARACTERIZATION OF ARBUTUS FRUITS A AND B USED IN THE STUDY

Samples	Total Soluble Solids (TSS)	pH
Arbutus fruits A	21.4 ± 0.0	3.493 ± 0.022
Arbutus fruits B	19.8 ± 0.0	3.304 ± 0.047

Average values of three determinations (mean±sd).

Table I shows that the arbutus fruits A present a greater total soluble solids value than the than the arbutus fruits B indicating that the first ones presented a higher maturation level. The pH values were also higher in the fruits A which were collected after their natural fall.

The determination of volatiles was carried out for three parts of distillate (head, heart and tail) obtained for each sample, A and B. The volatile composition of the different types of spirits (fruit, marc, cereals, etc.) is legislated by [17], while the arbutus fruit spirit is legislated, in Portugal, by [12]. According to this last Decree-Law the arbutus fruit spirit must possess certain specifications with regard to some chemical characteristics in order to be consumed.

The chemical and volatile characteristics analyzed in arbutus spirits A and B of the different fractions are reported in Table II.

TABLE II
COMPOSITION OF ARBUTUS SPIRITS RESULTING FROM FERMENTATIONS A AND B

Composition	Unit	Legal limit*	Results		F	Sig.
			Arbutus spirit A ($\bar{x} \pm$ dp)	Arbutus spirit B ($\bar{x} \pm$ dp)		
Head						
pH			4.20 ± 0.04	4.25 ± 0.24	0.208	0.664
Ethanol strenght	% vol.		46.95 ± 1.21	45.75 ± 2.02	1.037	0.348
Acetaldehyde	g/hL a.a.[a]		47.52 ± 7.79	31.48 ± 2.52	15.329	0.008
Ethyl acetate	g/hL a.a.		327.03 ± 68.44	506.03 ± 10.07	26.787	0.002
Methanol	g/hL a.a.		854.89 ± 12.55	711.80 ± 34.49	60.794	0.000
2-Butanol	g/hL a.a.		0.00 ± 0.00	0.00 ± 0.00		
1-Propanol	g/hL a.a.		19.33 ± 1.33	11.46 ± 1.26	74.201	0.000
2-Methyl-1-propanol (Isobutanol)	g/hL a.a.		82.81 ± 7.07	65.01 ± 8.47	10.395	0.018
1-Butanol	g/hL a.a.		0.00 ± 0.00	0.00 ± 0.00		
2+3-Methyl-1-butanol	g/hL a.a.		278.12 ± 23.68	221.62 ± 10.25	19.181	0.005
Total higher alcohols	g/hL a.a.		380.26 ± 32.08	298.08 ± 19.90	18.952	0.005
Isobutanol/1-propanol	g/hL a.a.		4.28 ± 0.08	5.66 ± 0.17	233.65	0.000
Heart						
pH			3.50 ± 0.00	3.72 ± 0.32	1.238	0.328
Ethanol strenght	% vol.	≥ 42	37.40 ± 0.00	39.45 ± 2.37	1.333	0.312
Acetaldehyde	g/hL a.a.	≥ 5 ≤ 40	39.84 ± 1.28	32.32 ± 0.87	76.925	0.001
Ethyl acetate	g/hL a.a.	≤ 300	145.98 ± 2.58	359.62 ± 11.96	558.234	0.000
Methanol	g/hL a.a.	≥ 500 ≤1000	1022.39 ± 0.27	825.90 ± 20.87	157.656	0.000
2-Butanol	g/hL a.a.	≤ 2	0.00 ± 0.00	0.00 ± 0.00		
1-Propanol	g/hL a.a.	≥ 10 ≤ 40	18.79 ± 0.05	11.03 ± 0.68	227.668	0.000
2-Methyl-1-propanol (Isobutanol)	g/hL a.a.	≥ 30 ≤ 70	66.96 ± 0.52	56.07 ± 2.88	25.100	0.007
1-Butanol	g/hL a.a.	≤ 3	0.00 ± 0.00	0.00 ± 0.00		
2+3-Methyl-1-butanol	g/hL a.a.	≥ 80 ≤ 185	230.25 ± 0.34	197.06 ± 5.16	73.550	0.001
Total higher alcohols	g/hL a.a.	≥ 130 ≤ 300	315.99 ± 0.91	264.17 ± 1.87	1260.218	0.000
Isobutanol/1-propanol	g/hL a.a.	≥ 1.5 ≤ 4	3.56 ± 0.02	5.08 ± 0.06	1221.007	0.000
Tail						
pH			3.29 ± 0.06	3.36 ± 0.22	0.381	0.560
Ethanol strenght	% vol.		23.30 ± 0.12	24.25 ± 0.40	20.434	0.004
Acetaldehyde	g/hL a.a.		36.01 ± 2.57	34.71 ± 3.16	0.408	0.547
Ethyl acetate	g/hL a.a.		140.77 ± 36.48	241.19 ± 43.99	12.350	0.013
Methanol	g/hL a.a.		1215.30 ± 16.56	947.20 ± 21.62	387.624	0.000
2-Butanol	g/hL a.a.		0.00 ± 0.00	0.00 ± 0.00		
1-Propanol	g/hL a.a.		16.32 ± 0.69	9.64 ± 1.32	79.577	0.000
2-Methyl-1-propanol (Isobutanol)	g/hL a.a.		52.96 ± 2.85	45.36 ± 7.05	3.983	0.093
1-Butanol	g/hL a.a.		0.00 ± 0.00	0.00 ± 0.00		
2+3-Methyl-1-butanol	g/hL a.a.		187.14 ± 6.65	161.27 ± 11.40	15.366	0.008
Total higher alcohols	g/hL a.a.		256.41 ± 10.17	216.27 ± 19.78	13.034	0.011
Isobutanol/1-propanol	g/hL a.a.		3.25 ± 0.05	4.69 ± 0.09	781.448	0.000

[a]g per hectolitre of 100 % vol. alcohol, (g/hL a.a.); $p \leq 0.05$; *According to Portuguese legislation "Decree-Law nº. 238/2000".
(N = 2; mean ± standard deviation)

A. Effect of Maturation Level of Arbutus Fruits on Heads and Tails Volatile Composition

Based on Table II, in which the volatile composition, pH and alcohol content of different fractions of the distillate are described it can be seen that in the head portion there are statistically significant differences in the concentrations of acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, 2+3-methyl-1-butanol, total higher alcohols and in the ratio isobutanol/propanol. Moreover, there were no statistically significant differences in pH and alcohol content (95 % confidence interval).

In tail fraction there are statistically significant differences in the ethanol content, concentration of ethyl acetate, methanol, 1-propanol, 2+3-methyl-1-butanol, total higher alcohols and ratio isobutanol/propanol. No statistically significant differences in pH, acetaldehyde and isobutanol concentration were found.

The rejection of the first and the last fraction (heads and tails of the distillate) in the production of spirits is a common practice, as the acetaldehyde is aplenty in the heads and methanol found in large quantities in tails [19]. Comparing the concentration of volatile compounds of heads and tails, in Table II, it was found that the concentration of acetaldehyde is higher in the distillate fraction designated as head, not being able, however, to say the same about the concentration of methanol, as it exists in larger amount in the tail.

The tail has still low concentration of ethanol (alcohol responsible for the alcohol content of a fermented beverage) also leading to its rejection.

B. Effect of Maturation Level of Arbutus Fruits on Heart Fractions Volatile Composition

The heart fraction corresponds to the arbutus fruit spirit.

The alcohol content does not show significant differences, whether the fruit is harvested from the floor or tree, for a 95% confidence interval. Thus, for the sample A, the alcohol content is 37.40% vol. and sample B presents 32.32% vol., which is, in both cases, below the legal limit value (≥ 42.00% vol.) (Table II). This variation shows that the alcoholic spirit fractionation stages require systematic production and standardization in order to ensure the homogeneity and quality of the final product [1]. We worked with small quantities of fruits during fermentation, to obtain the various distillate fractions and may have been a heart cut fraction slightly delayed in time which depresses the alcohol content of the heart fraction below the legal limit.

The acetaldehyde is derived from the fermentation of raw material and increases during distillation of spirits, and aging. It is also considered the main result of spontaneous oxidation and/or microbial action [20]-[22]. For a 95% confidence interval, it was found that there are significant differences between the acetaldehyde concentration values in samples. Samples A and B contain an acetaldehyde concentration of 39.84 g/hL a.a. and 32.32 g/hL a.a., respectively. These values are in agreement with the values legally allowed. This means that the fermentation and distillation took place under favorable conditions and without interaction of unwanted bacteria [20]-[22]. It was also found that in the spirit of arbutus, from fruits with higher degree of maturation, the concentration of acetaldehyde was superior (Table II). Watkins et al. [23] and Zaldivar et al. [24] observed that the presence of acetaldehyde in higher concentrations in fruits is an indicator of advanced ripening stage, as with the fruit ripening occurs cellular disorganization and membrane degradation. Such events may impair oxidative phosphorylation (in mitochondria membrane) and the production of NAD^+, which is essential for the production of glycolysis and energy. To meet this need, it is possible that the pyruvate is oxidized but not decarboxylated in the fermentative metabolism, forming acetaldehyde and ethanol [25].

Ethyl acetate is the main ester in several distillates [26], which is formed in greater amounts by the yeast during fermentation and also as result of esterification reactions that are favoured by the oxygen presence [27]. This volatile compound provides the characteristic flavour of adhesives varnish and glue [28], contributing negatively to the flavour characteristics of the final product [29], [30]. According to [31], the arbutus spirit presents higher values of ethyl acetate than other distillates. It was found by statistical analysis performed on different samples (A and B) that the acetate concentrations are statistically different at 95% confidence interval. The sample A has the concentration of ethyl acetate 145.98 g/hL a.a., which is in line with current legislation [12]. In sample B, the value obtained from the concentration of acetate was higher than allowed, 359.62 g/hL a.a. This event can be explained by contacting the fermented fruits with atmospheric oxygen, once a day for successive sample collections (throughout the fermentation period) for the analytical control of the fruits under fermentation which certainly enhanced the appearance of this compound. The daily opening of fermenters and its exposure to air, even for a few seconds, was a limitation of the experimental work and certainly contributed to the increase in ethyl acetate values obtained in spirits.

Thus, in industrial conditions, it is recommended that the opening of deposits in fermentation must be a practice very well controlled during the fermentation process of arbutus fruits, and above all, the temporal phase between the end of fermentation and the distillation process must be avoided. These recommendations have already been previously suggested by [11] and [32]. Santo [32] obtained an ethyl acetate concentration of 349.3 g/hL a.a. which also lies above the legally allowed, which also possibly enhanced by contact with oxygen because he also conducted daily analysis of fermented fruits. Galego [33] determined ethyl acetate concentration of 143.0 g/hL a.a.

Methanol is formed by the degradation of pectic substances in fruit. For this reason the concentration of methanol in the final distillate increased with time of extraction [20]-[22]. According to Portuguese legislation [12] methanol concentration should be between 500 to 1000 g/hL a.a. In this study, it was found that the concentrations of methanol in samples A and B are statistically different with a 95%

confidence interval. For sample A (with fruits from natural fall and average of 21.4 °Brix of TSS) a concentration of methanol 1022.39 g/hL a.a. was found which is a value more than required by the legislation. In contrast, in Sample B (arbutus fruits harvested with a TSS of 19.8 ° Brix) the methanol content was 825.90 g/hL a.a. (Table II), which is within the legal limit. This means that on the one hand, handling of the raw material and distillation procedures were carried out carefully and with great sensitivity [22] and, secondly, the degree of ripening of fruits used in the fermentation of sample B have been the most adequate to maintain relatively low levels of methanol in the resulting spirits.

Santo [32] obtained methanol concentrations between 763.9 and 895.0 g/hL a.a. in six arbutus spirit samples, while [33] obtained 723.0 g/hL a.a. methanol present in arbutus spirit. Bauer-Christoph et al. [34] found methanol values for apple distillate of 359 g/hL a.a., cherry distillate of 457 g/hL a.a., pear distillate 765 g/hL a.a. and plum distillate of 866 g/hL a.a., in accordance to Regulation (EC) nº 110/2008 [17].

The higher alcohols are the group with the highest concentration in distillate, accounting for to impart the characteristic aroma [21], [35], [36]. The most important of the spirit alcohols are 2-butanol; 1-propanol; isobutanol (2-methyl-1-propanol), 1-butanol and (2 + 3-methyl-1-butanol). The levels of these compounds are mainly resulting from yeast and bacteria metabolism during the fermentation stage [32], [37], and are concentrated mainly in the first distillate fraction [20].

From the analysis of Table II, it can be seen that the concentration of higher alcohols are no statistically significant different (with 95% confidence interval) between the samples A and B. Samples A and B presented concentrations of isobutanol (2-methyl-1-propanol) of 66.96 g/hL a.a. and 56.07 g/hL a.a., respectively. The 1-propanol concentrations were 18.79 g/hL a.a. for sample A and 11.03 g/hL a.a. for sample B. In all distillate fractions was not detectable the presence of 1-butanol and 2-butanol. All these higher alcohols are within the legal limit established [12]. The same was not true with the concentration of 2 + 3-methyl-1-butanol, which present the value of 230.25 g/hL a.a. in sample A and 197.06 g/hL a.a. in the sample B, values that are out of range established by Decree-Law nº. 238/2000 [12]. Taking into account the importance of fermentation step in the production of these alcohols it could be hypothesized that the fermentation process occurs with a microbiota flora very different from Algarve [38], [39]. This region was the first, in Portugal, that has been studied [2], [9], [31]-[33], [38]-[40]. So, the legislation was based in the experimental assays from arbutus spirit from Algarve, and that could be the reason for this disagreement with Portuguese legislation.

C. Total Antioxidant Capacity and Phenolic Compounds

In this study the total antioxidant capacity was determined using the $ABTS^{\bullet+}$ reagent and the determination of phenolic compounds with the Folin-Ciocalteu reagent. Quantification of total antioxidant capacity and total phenolic compounds is presented in Table III.

From the analysis of Table III, it was found that the spirit of arbutus (sample A), from the natural fall, contains lower total antioxidant capacity, 111.6 µmol Eq GAE/mL. In turn, the sample B, from the direct collection of shrub has a higher antioxidant capacity, with an average of 157.3 µmol Eq GAE/mL. The concentration of phenolic compounds in sample A was also lower, showing an average value of 290.6 µmol Eq GAE/mL, compared to sample B containing 423.9 µmol Eq GAE/mL of total phenolics.

TABLE III
TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT OF ARBUTUS FRUIT SPIRITS

Samples	Total antioxidant capacity ABTS (µmol Eq TE/mL)	Total phenolic compounds (µmol Eq GAE/mL)
Arbutus spirit A	110.6 ± 4.3	290.6 ± 6.8
Arbutus spirit B	157.3 ± 5.0	423.9 ± 8.0

Average values of three determinations (mean±sd).

Thus, depending on the degree of maturity of fruits, the spirit of arbutus has different total antioxidant capacity and total phenolic compounds.

In our work the antioxidant activity is strongly related to the content of phenolic compounds, which has also been demonstrated by several authors [41]-[45].

IV. CONCLUSION

For the production of high quality arbutus fruit spirit within the legal requirements concerning its volatile composition, the fruits should not present an advanced maturation level, since the more mature the fruit is, higher methanol levels will have and, therefore, the resulting spirit will be probably not suitable for commercialization.

Different total antioxidant capacity and total phenolic compounds amount were found, according to the arbutus fruits maturation level being the most mature the poorest in terms of total antioxidant capacity and total phenolic compounds. Moreover, these results seem to indicate that antioxidant activity of arbutus fruit spirit is strongly related to total phenolic content.

ACKNOWLEDGMENT

The authors wish to thank the MSc student Carolina Santos by her laboratory work and Mr. Jorge Simões for his arbutus fruit kind supply. The authors also thank to Amelia Soares, Deolinda Mota and Otília Cerveira for their technical support.

REFERENCES

[1] E. H. Soufleros, S. A. Mygdalia, and P. Natskoulis, "Production process and characterization of the traditional Greek fruit distillate "Koumaro" by aromatic and mineral composition". *Journal of Food Composition Analysis* vol. 18, pp. 699–716, 2005.

[2] L. R. Galego, A. N. Martins, and V. R. Almeida, "Valorização da Aguardente de Medronho - Estudo do Processo Tecnológico". *Proceedings of VIII Congresso do Algarve, Vilamoura,* 1995.

[3] M. Alarcão-e-Silva, A. E. B. Leitão, H. G. Azinheira, and M. C. A. Leitão, "The Arbutus Berry: Studies on its color and chemical characteristics at two mature stages". *Journal of Food Composition Analysis* vol. 14 pp. 27–35, 2001.

[4] F. A. Ayaz, M. Kucukislamoglu, and M. Reunanen, "Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. ellipsoidea) fruits". *Journal of Food Composition Analysis* vol. 13 pp. 171–177, 2000.

[5] K. Pallauf, J. C. Rivas-Gonzalo, M. D. del Castillo, M. P. Cano, and S. Pascual-Teresa, "Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits". *Journal of Food Composition Analysis* vol. 21 pp. 273–281, 2008.

[6] A. M. Pawlowska, M. De Leo, and A. Braca, "Phenolics of *Arbutus unedo* L. (Ericaceae) fruits: identification of anthocyanins and gallic acid derivatives". *Journal of Agricultural and Food Chemistry* vol. 54 pp. 10234–10238, 2006.

[7] B. M. Ruiz-Rodríguez, P. Morales, V. Fernández-Ruiz, M.C. Sanchez-Mata, M Cámara, and C. Díez-Marqués, "Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data". *Food Research International* vol. 44 pp. 1244-1253, 2011.

[8] M. Simonetti, F. Damiani, L. Gabrielli, L. Cossignani, F. Blasi, and F. Marini, "Characterization of triacylglycerols in *Arbutus unedo* L. Seeds". *Italian Journal of Food Science* vol. 20 pp. 49-56, 2008.

[9] T. Cavaco, C. Longuinho, C. Quintas, and I.S. de Carvalho, "Chemical and microbial changes during the natural fermentation of strawberry tree (*Arbutus unedo* L.) fruits". *Journal of Food Biochemistry* vol. 31 pp. 715-725, 2007.

[10] R. Morales, "El madroño y sus usos. Plantas y cultura popular: La etnobotánica en España". *Quercus* vol. 12 pp.8-10, 1995.

[11] G. Versini, R. Seeber, A. D. Serra, G. Sferlazzo, J. H. B. Carvalho, and F. Reniero, "Aroma compounds of Arbutus distillates". In: Charalambous G. (Ed.). Food flavors: Generation, Analysis and Process Influence, *Elsevier Science, London*; pp. 1779-1790, 1995.

[12] Decree-Law nº. 238/2000 de 26 de Setembro. *Diário da República I série A, Imprensa Nacional* 223 pp. 5145-5147, 2000.

[13] OIV. Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin. *OIV, Paris,* 2014.

[14] N. Miller, and C. Rice-Evans, "Factors influencing the antioxidant activity determined by the ABTS radical cation assay". *Free Radical Research* vol. 26 pp. 195-199, 1997.

[15] C. Santos, G. Botelho, I. Caldeira, A. Torres, and F. M. Ferreira, "Antioxidant activity assessment in fruit liquors and spirits: methods comparison". *Ciência Técnica Vitivinícola* vol. 29 pp. 28-34, 2014.

[16] V. L. Singleton, and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents". *American Journal of Enology and Viticulture* vol. 16 pp. 144-158, 1965.

[17] Regulation EC nº 110/2008 of the European parliament and of the council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89 *Official Journal of the European Communities* vol. L39, pp. 16-54, 2008.

[18] A. Luís, D. Mota, O. Anjos, and I. Caldeira, "Single-laboratory validation of determination of acetaldehyde, ethyl acetate, metanol and fusel alcohols in wine spirits, brandies and grape marc spirits using GC-FID". *Ciência Técnica Vitivinícola* vol. 26 pp. 69-76, 2011.

[19] C. Da Porto, "Grappa and grape-spirit production". *Critical Reviews in Biotechnology* vol. 18 pp. 13-24, 1998.

[20] M. L. Silva, and F. X. Malcata, "Relationships between storage conditions of grape pomace and volatile composition of spirit obtained thereform". *American Journal of Enology and Viticulture* vol. 49 pp. 56-64, 1998.

[21] M. L. Silva, and F. X. Malcata, "Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs". *Zeitschrift für Lebensmittel Untersuchung und Forschung A* vol. 208 pp. 134-143, 1999.

[22] M. L.Silva, F. X. Malcata, and G. Revel, "Volatile Contents of Grappe Marcs in Portugal". *Journal of Food Composition and Analysis* vol. 9 pp. 72-80, 1996.

[23] C. B. Watkins, J. F. Nock, and B. D. Whitaker, "Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions". *Phostharvest Biology and Technology* vol.19 pp. 17-32, 2000.

[24] C. P. Zaldivar, J. B. Abda, S. E. Ebeler, and A. A. Kader, "Quality and chemical changes associated with flavor of camarosa strawberries in response to a CO_2 enriched atmosphere". *Hortscience* vol. 42 pp. 299-303, 2007.

[25] L. Taiz, and E. Zeiger, "Fisiologia vegetal". 3ª Edição, *Artmed, Porto Alegre,* 2004.

[26] L. Nykänen, and H. Suomalainen, "Aroma of beer, wine and distilled alcoholic beverages". *D. Reidel Pulbishing Company, Dordrecht,* 1983.

[27] G. H. Reazin, S. Baldwin, H. S. Scales, H. W. Washington, and A. A. Andreasen, "Determination of the congeners produced from ethanol during whisky maturation". *Journal of AOAC* vol. 59 pp. 770-776, 1976.

[28] I. Caldeira, S. Canas, S. Costa, E. Carvalho, and A. P. Belchior, "Formação de uma câmara de prova organoléptica de aguardentes velhas e selecção de descritores sensoriais". *Ciência Técnica Vitivinícola* vol. 14 pp. 21-30, 1999.

[29] A. M. Vera-Guzmán, R. I. Guzmán-Geronimo, and M. G. López, "Major and minor compounds in a Mexican spirit, young mezcal coming from two *Agave* Species". *Czech Journal of Food Science* vol. 28 pp. 127-132, 2010.

[30] P. Ribéreau-Gayon, D. Dubourdieu, B. Donèche, and A. Lonvaud, "Traité d'Oenologie - Microbiologie du vin. Vinifications". Editions Dunod, Paris, 1998.

[31] L. Galego, "Traditional algarvian distillats and liqueurs historic scientific aspects". *Conference Proceedings of Traditional Food Processing and Technological Innovation in Peripheral Regions. Faro,* 2006.

[32] D. M. C. E. Santo, "Controlo e análise do processo de produção de Aguardente de Medronho na Serra do Caldeirão". Dissertação de Mestrado. *Instituto Superior de Engenharia, Universidade do Algarve. Faro,* 2010.

[33] L. R. Galego, "Optimização de parâmetros para a aguardente de medronho". Dissertação de Mestrado. *Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa. Lisboa,* 1995.

[34] C. Bauer-Christoph, H. Wachter, N. Christoph, A. Roßmann, and L. Adam, "Assignment of raw material and authentication of spirits by gas chromatography, hydrogen- and carbon-isotope ratio measurements". *Zeitschrift fur Lebensmittel Untersuchung und Forschung A* vol. 204 pp. 445-452, 1997.

[35] V. Ferreira, P. Hernández-Orte, A. Escudeiro, R. López, and J. Cacho, "Semipreparative reserved-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages". *Journal of Chromatography A* vol. 864 pp. 77-78. 1999.

[36] I. Caldeira, R. Bruno-de-Sousa, A. P. Belchior and M. C. Clímaco, "A sensory and chemical approach to the aroma of wooden aged Lourinhã wine brandy". *Ciência Técnica Vitivinícola* vol. 23 pp. 97-110, 2008.

[37] L. Nykänen, and I. Nykänen, Distilled beverages. Volatile compounds in food and beverages. *Marcell Dekker Inc., New York,* 1991.

[38] T. M. G. Cavaco, "Caracterização química e bioquímica dos frutos de "*Arbutus unedo* L." e de "*Rubus fruticosus* Agg." - Contribuição para a sua valorização". Dissertação de Mestrado. *Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Faro,* 2007.

[39] D. E. Santo, L. Galego, T. Gonçalves, and C. Quintas, "Yeast diversity in the Mediterranean strawberry tree (*Arbutus unedo* L.) fruits' fermentations". *Food Research International* vol. 47 pp. 45–50, 2012.

[40] L. Galego, and V. Almeida, "Aguardente de frutos e licores do Algarve. História, técnica de produção e legislação". *1ª Edição, Edições Colibri, Lisboa,* 2007.

[41] F. M. Abdoul-Latif, R. G. Bayili, L-C. Obame, I. H. N Bassolé, and M. Dicko, "Comparison of phenolic compounds and antioxidant capacities of traditional sorghum beers with other alcoholic beverages". *African Journal of Biotechnology* vol. 81 pp.14671-14678, 2012.

[42] S. Canas, V. Casanova, and A. P. Belchior, "Antioxidant activity and phenolic content of Portuguese wine aged brandies". *Journal of Food Composition and Analysis* vol. 21 pp.626-633, 2008.

[43] S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, and J. M. Mérillon, "Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays". *Journal of Agricultural and Food Chemistry* vol. 57 pp. 1768–1774, 2009.

[44] A. Floegel, D-O. Kim, S-J. Chung, S. I. Koo, and O. K. Chun, "Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods". *Journal of Food Composition and Analysis* vol. 24 pp. 1043-1048, 2011.

[45] S. Gorinstein, R. Haruenkit, S. Poovarodom, S. Vearasilp, P. Ruamsuke, J. Namiesnik, M. Leontowicz, H. Leontowicz, M. Suhaj, and G. P Sheng, "Some analytical assays for the determination of bioactivity of exotic fruits". *Phytochemical Analysis* vol. 21 pp. 355–362, 2010.