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Estimation of The Antioxidant Capacity of Traditional Greek Spirits Produced From "Koumaria" Fruits.

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Abstract : *Koumaria is the common Greek name of the bushy shrub Arbutus unedo L., also known as the strawberry tree. Its fruits are considered to be an organic product because it is widely grown without any agrochemical substances and are mainly used for the production of an aromatic traditional distillate, named Koumaro. The aim of the present study was to estimate total phenols and antioxidant capacity of Koumaro distillates from five mainland areas of Greece (Preveza, Ioannina, Thesprotia, Pieria and Aitoloakarnania). 25 different samples of Koumaro distillates were used (5 samples from each of the above mentioned areas). The antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and total phenols were determined by the Folin-Ciocalteu method. The antioxidant capacity for all samples was ranging between 48.41% and 58.84%. Moreover, total phenols in all samples were ranging between 5.97 mg/L and 9.99 mg/L expressed as gallic acid. Obtained results showed that Koumaro distillates exhibited a remarkable antioxidant capacity, which could possibly be increased by the addition of various kinds of berries during the distillation process.*

Keyword: *antioxidant capacity; DPPH; Folin-Ciocalteu; Koumaro distillate; total phenols;*

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I. Introduction

Arbutus unedo L. or Strawberry tree is an evergreen shrub very widespread in the Mediterranean region, especially in regions where frost is not very usual and summer dryness is not very intense. In Europe, this species grows in Portugal, Spain, France, Italy, Albania, Greece, Bosnia and Herzegovinia, Croatia, FYROM, Montenegro, Serbia and Slovenia and in the Mediterranean islands (Balearic, Corsica, Sardinia, Sicily and Crete). It is also able to adapt to conditions of the south-western coast of Ireland [1–4]. The vernacular name of Strawberry tree in Greece is "koumaria" and of its fruit "koumaro" [3]. Koumaria is considered as a bee-keeping plant. Honey from Koumaria is considered to have particularly beneficial properties for the human body: it is rich in trace elements and vitamins, it helps in the regulation of cholesterol and it contains natural antibiotics. However, due to its harsh taste, it is of little commercial value and the beekeepers usually leave it in the hive to feed the honeybees during winter [5]. Koumaro fruits are rounding, fleshly, relatively small (with a diameter of 1.5-2 cm) and have small conical protrusions which from the far side appear like thorns. Initially, their color is yellow, then turns yellow-orange and finally becomes red during maturation (Picture 1). According to some studies, phenolic compounds that have excellent antioxidant activity, have been identified in the Koumaro fruits as well as fatty acids, organic acids, sugars, various volatile compounds and minerals [2, 6]. It is considered to be an organic product because it is widely grown without any agrochemical substances. Koumaro fruits, as many kinds of berries, have a long list of health benefits. They are a good source of antioxidants, such as flavonoids, vitamins C and E, carotenoids and polyphenols, which help fight chronic diseases and cancer and are commonly consumed as processed products including preserves, jams, marmalades and jellies [7, 8]. It is important to mention that, depending on the location they are that thrive and the prevailing climatic conditions, Koumaro fruits show qualitative and quantitative differences [9, 10].

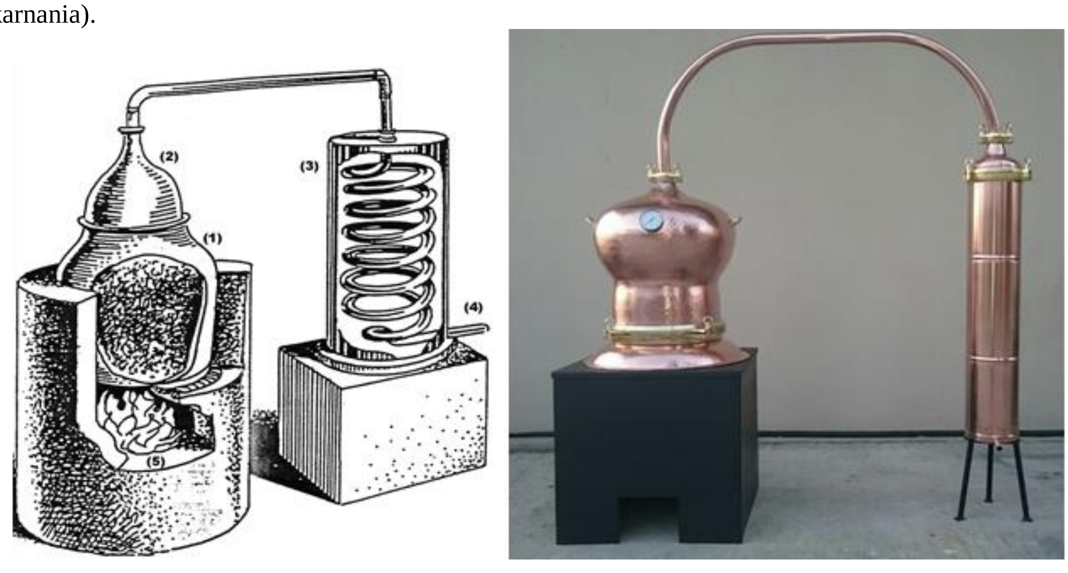
One of the main uses of koumaro fruits in Greece is the production of an aromatic traditional distillate, named "Koumaro". The production of this spirit has been documented since the early Byzantine times, although there are indications that it began even earlier [11, 12].

Picture 1: Koumaro fruits

Usually for the production of distillate in Greece, only the fully matured koumaro fruits were collected, piece by piece, in order to ensure the most appropriate raw material for this distillate. In most cases, because of the traditional character of this product, the fruits are carried to the farmer's installations into small wooden flasks of 5–10L capacity or into fabric sacks wrapped around their waists.

In the farmer's installations, they are transferred the collected Koumaro fruits into wooden barrels of 30–50L and up to 50hL capacity sometimes. The barrels are filled with the product up to 30–45cm below the surface, covered up with leaves and distaff corn or wool blankets, and left to ferment. Then, small quantities of water are added progressively in order to assist the fermentation procedure. According to local producers, the fermentation period lasts from 5 to 6 months because of the gradual collection of the raw material, the low winter temperatures (usually below 20^{o}C) and the fact that, unlike grapes, the fruits are not crushed before fermentation. The fermented raw material is transferred to the traditional copper alembics (Picture 2) of 130L up to ¾ of its capacity in order to be distilled. Before heating, the alembics are sealed with dough or cinder pulp in order to prevent any vapor leakage. As the temperature reaches 80–90^{0}C, the liquid spirit starts to run from the funnel and is gathered in glass bottles of varying capacity (30–100L). The first 0.5–1L of the distilled product corresponds to the beginning of the distillation procedure and is removed as ''head''. It usually presents a very high alcoholic title, traditionally defined as 36 grada (Grado-Grada: is the Descartes-degree grades) or 90%vol approximately. Then, and for about 2.5h, a pure spirit—commonly called single distillation or first pitcher—is transferred into egg-shaped glass bottles (15–50L) known as damitzanes. The distillation product is sequentially obtained until 11 grada (5% vol) and it is led back to the alembic for a second distillation or ''metavrasma'' along with 5.5–6 kg of Pimpinella anisum spores, commonly called Glycanissos. This plant is responsible for the production of a smoother, clearer and more aromatic spirit. During the second distillation, the ''head'' and ''tail'' are separated while the ''heart'' of the spirit, which has an alcoholic title between 60% and 70% vol, is gathered. The ''tails'' from the two distillations or—as commonly known—"aporaka", "ahamko" or "hamko" are used for the next single distillation process with the new lot of the fermented koumaro pomace (Picture 3). Finally, the various lots of the second distillations are mixed and placed, before the dilution with water, into oak barrels and left to age 1 year before consumption [12].

The aim of the present study was to estimate total phenols and antioxidant capacity of Koumaro distillates produced in five mainland areas of Greece (Preveza, Ioannina, Thesprotia, Pieria and Aitoloakarnania).



Picture 2: Traditional alembic: (1) kettle, (2) cover, (3) cooler (serpentine), (4) distillation receptacle, (5) heater.

Picture 3: Different ranges of distillation, head, heart, tail, flavor range, pure alcohol.

II. Materials And Methods

2.1 Samples

Several samples of Koumaro distillates were used, from different producers and from five different mainland areas of Greece (Preveza, Ioannina, Thesprotia, Pieria and Aitoloakarnania). More specifically, 25 different samples of Koumaro distillates were used, 5 samples from each of the above mentioned areas. All distilled samples were placed into glass bottles of 1/2 L capacity and stored in the dark at 4 ^{o}C until they were analyzed.

2.2 Determination of alcoholic title and pH

The ethanol content of the spirits was determined after distillation with the official method of Office International de la Vigne et du Vin (OIV, 1994). The pH of samples was determined with a Delta 320 pH meter (Mettler, Germany). Ethanol content and pH values are given in Table 1.

Table 1. Ethanol content and pH (average values) in the spirits studied.

Origin of spirits	Ethanol % vol.	pH
Preveza	41.85 ± 3.52	5.72 ± 0.83
Ioannina	39.25 ± 3.28	5.08 ± 0.77
Thesprotia	37.95 ± 2.99	6.03 ± 0.91
Pieria	44.13 ± 4.01	4.82 ± 0.71
Aitoloakarnania	40.41 ± 3.64	4.99 ± 0.84

2.3. Antioxidant Capacity

The antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH (2,2-difenyl-1-picrylhydrazyl) is one of the few commercially available nitrogen radicals with visible and ultraviolet absorptions with a maximum wavelength of 515nm [13]. It has a deep purple color and when it reacts with other roots, electrons, or hydrogen atoms, leads to a loss of color. It is hydrophobic and so all reactions have to be done in organic solvents. DPPH reactions are extremely sensitive to the conditions of the reaction system, i.e. water and solvent, pH, oxygen, and light exposure [14].

To prepare DPPH solution, 5 mg DPPH was added to a 25 ml volumetric flask containing methanol (CH_3OH), thus preparing a 0.5 mmol concentration solution. Thereafter, the 25ml was placed in a 125ml round bottom flask and diluted with methanol. The solution, a concentration of 0.1mmol, was placed in a dark place at -20 °C, after shaking. A volume of 3.8 ml of 0.1 mmol/L DPPH radical solution in methanol was added to 0.2 ml of each sample and the free radical capacity of the sample was evaluated by measuring the absorbance at 517 nm immediately after the addition of DPPH (t=0) and at 1 min intervals, until the radical scavenging reaction reached a plateau (after 45 min). The results were expressed as the percentage of reduction of the DPPH (Q), by the following expression:

$$Q=[(A_0-As)/A_0] \times 100$$

Where A_0= the initial absorbance and As= the value of absorbance after the reaction time.
All determinations were performed in triplicate.

2.4 Total phenols

Total phenols were determined by the Folin-Ciocalteu method. This is a photometric method based on the oxidation of phenolic compounds from the Folin-Ciocalteu reagent [15].

Each sample (0.2 ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent. After 1 min 1.5 ml of 7.5% w/v sodium carbonate solution and 7.8 ml distillated water were added and thoroughly mixed. The samples were left to stand for 2h at room temperature and the absorbance at 760 nm was measured. The results were expressed in milligram gallic acid equivalents (GAE) per liter.

Standard solutions of gallic acid were used to make the standard reference curve. From a stock solution of gallic acid, a working solution of 1 mg/ml was prepared by dilution. From the working solution, solutions of 100, 200, 300, 400, 500, 600, 700, 800 and 900 μg/ml were prepared. Then, the experimental procedure referred to in the first paragraph was followed by the addition of 0.2 ml of the above solutions (Figure 1). The instrument was reset using a blank sample prepared in the same manner.
All determinations were performed in triplicate.

2.5 Statistical analysis

The Minitab Statistical Program Release 13.20 (US registered trade Mark of Minitab Inc.) was used for determination of mean and SD values.

III. Results And Discussion

The antioxidant capacity for all samples was ranging between 48.41% and 58.84%, and more specifically between 49.03% and 53.93% in the samples from Preveza (Figure 2), between 53.36% and 58.84% in the samples from Ioannina (Figure 3), between 51.52% and 53.15% in the samples from Thesprotia (Figure 4), between 48.41% and 51.93% in the samples from Pieria (Figure 5) and between 53.52% and 55.28% in the samples from Aitoloakarnania (Figure 6). Moreover, total phenols in all samples were ranging between 5.97 mg/L and 9.99 mg/L expressed as gallic acid equivalents, and more specifically between 6.32 and 6.35 mg/L in Preveza samples (Table 2), between 5.97 and 6.61 mg/L in Ioannina samples (Table 3), between 8.29 and 9.99 mg/L in Thesprotia samples (Table 4), between 6.41 and 7.84 mg/L in Pieria samples (Table 5) and between 7.41 and 8.33 mg/L in Aitoloakarnania samples (Table 6).

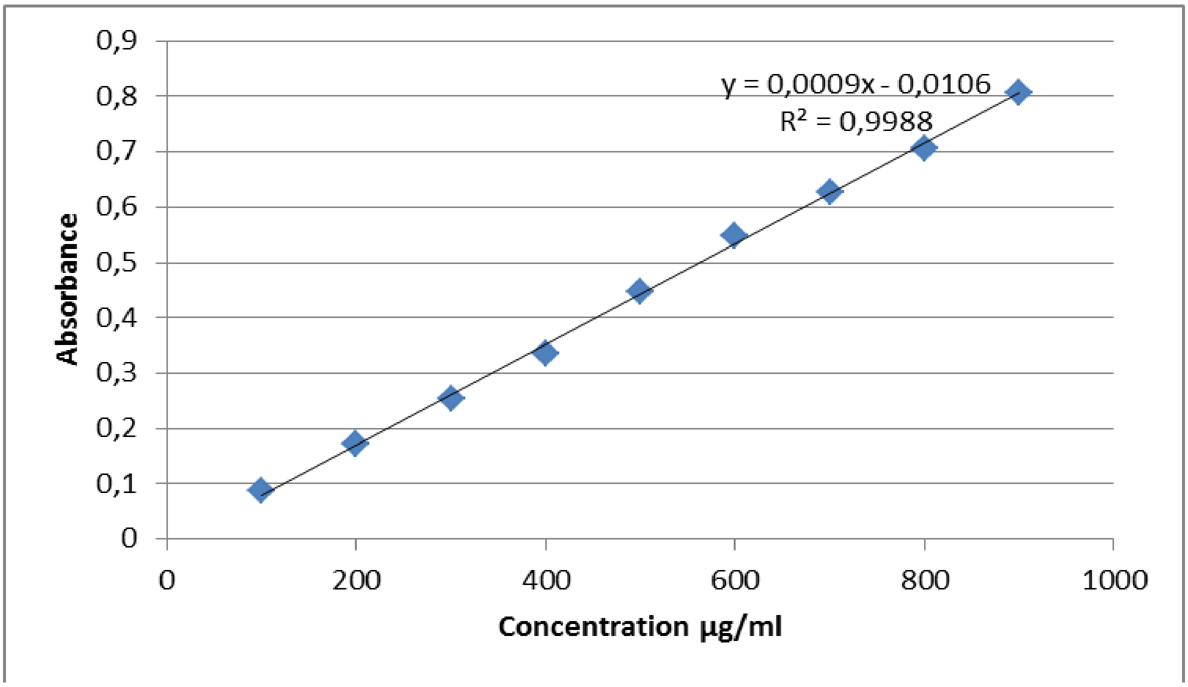


Figure +1: Standard reference curve of gallic acid

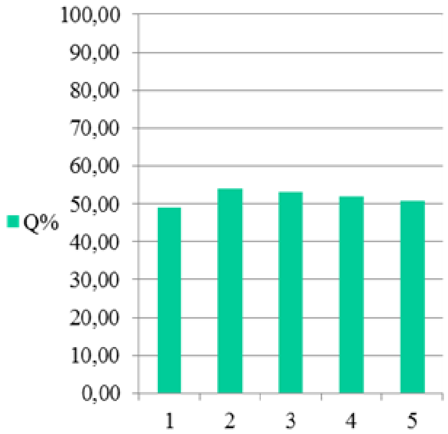


Figure 2: Antioxidant capacity of samples from Preveza

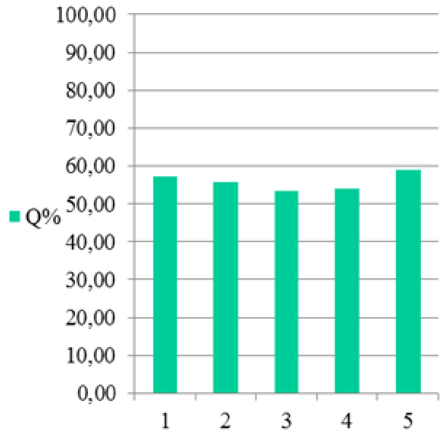


Figure 3: Antioxidant capacity of samples from Ioannina

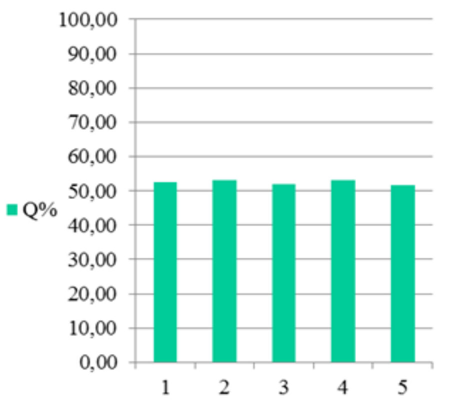


Figure 4: Antioxidant capacity of samples from Thesprotia

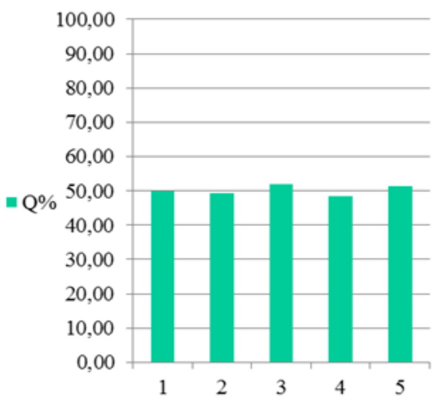


Figure 5: Antioxidant capacity of samples from Pieria

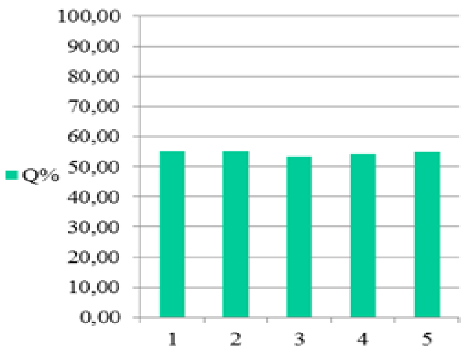


Figure 6: Antioxidant capacity of samples from Aitoloakarnania

Table 2: Total phenols (mg GAE/L) ± SD (n=3) of samples from Preveza

Sample No	Total phenols (mg GAE/L)
1	6,33 ±0,14
2	6,32 ±0,11
3	6,35 ±0,24
4	6,34 ±0,16
5	6,34 ±0,27

Table 3: Total phenols (mg GAE/L) ± SD (n=3) of samples from Ioannina

Sample No	Total phenols (mg GAE/L)
1	6,61 ±0,21
2	6,42 ±0,34
3	6,56 ±0,23
4	5,97 ±0,14
5	6,19 ±0,19

Table 4: Total phenols (mg GAE/L) ± SD (n=3) of samples from Thesprotia

Sample No	Total phenols (mg GAE/L)
1	9,68 ±0,72
2	9,33 ±0,68
3	8,29 ±0,54
4	9,99 ±0,79
5	8,84 ±0,66

Table 5: Total phenols (mg GAE/L) ± SD (n=3) of samples from Pieria

Sample No	Total phenols (mg GAE/L)
1	7,79 ±0,41
2	7,84 ±0,45
3	6,93 ±0,33
4	6,41 ±0,30
5	6,78 ±0,38

Table 6: Total phenols (mg GAE/L) ± SD (n=3) samples from Aitoloakarnania

Sample No	Total phenols (mg GAE/L)
1	7,94 ±0,65
2	8,33 ±0,71
3	8,17 ±0,77
4	7,41 ±0,72
5	7,55 ±0,75

Differentiations between samples are probably due to differences in the composition of the soil, the microclimate of each region, the degree of maturation at harvesting, the differentiations during fermentation process (container's material -wood, plastic or inox-, environmental conditions during fermentation, etc.), the distillation process (heating method, cleaning of the distillation system, possible second distillation, etc.), as well as the storage of the distillate (container's material and conditions).

A number of experimental studies involving small fruits, berries, have been carried out to determine their qualitative characteristics, such as total phenolics, total flavonoids, anthocyanins and their total antioxidant capacity [10, 16-18]. In the present study it was chosen to study Koumaro distillates. In general, it is easy to see that there are many factors that can influence the concentration of the various components, which is the main reason why studies dealing with the same subject matter as Koumaro fruits differ from place to place. The results identified in other studies carried out on Koumaro fruit show that it is a good source of antioxidants, ω3 and ω6 fat, vitamins and sugars [7, 8, 11, 12, 16-18]. The intake of these berries can positively affect the health of consumers either because of their antioxidant action or because of their vitamin, mineral and trace minerals content. Moreover, according to the literature, since the Koumaro fruits exhibit differences in the contents of their constituents during their various stages of maturity, the most appropriate stage with the most beneficial ingredients should be determined [7].

IV. Conclusion

In conclusion, Greek koumaro distillates exhibited a remarkable antioxidant capacity, which could possibly be increased by the addition of various kinds of berries during the distillation process. This finding, combined with the fact that strawberry tree fruits contain a number of ingredients that are beneficial for health, leads to the need for further investigation of all parameters needed to differentiate high-quality spirits and to create an identity for each geographical origin or country.

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