Assessment of Phenolic Content, Antioxidant Activity, Colour and Sensory Attributes of Wood Aged “Tsipouro”

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Abstract
This study aimed at investigating the benefits of immersing oak sticks in the Greek pomace brandy named tsipouro, regarding its total phenolic content, antiradical-antioxidant activity, colour parameters and sensory profile. In order to induce rapid aging of tsipouro, alternative experimental conditions were applied. Results revealed that the phenolic migration from the wood to the tsipouro significantly increased both with temperature increase during aging as well as with the oak sticks/tsipouro ratio (w/v). The impact of oak wood diversity was also tested, by selecting different types of French oak (Quercus robur) and French and American oak mix (Quercus robur-Quercus alba) sticks. Results exhibited a significant effect of wood stick type on total phenolic content, antioxidant and antiradical activity, colour values and sensory attributes, especially on overall acceptance. High positive correlation among total phenolic content, antiradical and antioxidant activity and overall acceptance was found for all the extracts studied. Wood aging promoted the migration of phenolic compounds from the wood into the tsipouro, which, apart from the organoleptic characteristics, could possess health beneficial effects.

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Introduction

Tsiouro is a strong traditional grape marc distillate produced from residues of the winemaking process either from white or red grapes that abide by the Regulation (EC) No 110/2008. The production process of tsiouro includes the fermentation of the grape marc followed by two sequential distillations and the collection of the second and final distillate. Finally, this distillate is diluted with water to a final ethanol content of 40-45% (v/v), leading to the production of the, popular in Greece, “tsiouro” alcoholic drink.

Oak chips are progressively used by winemakers as an alternative method to the maturation of wines and distillates, instead of the aging process in oak barrels. Oak and chestnut woods were approved by the International Enological Codex of the International Organisation of Vin and Wine, for aging wine and then, in 2006 the European Union approved the use of wood fragments to age wine.

Wood is a complicated material, composed mainly of cellulose, hemicellulose, lignin and secondary compounds, such as pectins, proteins and inorganic elements. Each of those components does not have the same effect when interacting with a distilled spirit, favouring specific reaction paths in the wood–beverage system. Additionally, the extent of thermal degradation of wood leads to the formation of new chemical compounds, which are released during the aging process and improve the final quality and sensory characteristics of the spirit.

Among the oak species used for beverage maturation, the French (Quercus robur) and the American oak (Quercus alba) are the most commonly used. Oak supplements can be found in a variety of forms including sticks, cubes or beans, powder, pencil shavings or granulates, dominoes, blocks or segments.

The aging process improves the maturation of wines or distillates and is accompanied by changes in their taste, colour and flavour. Furthermore, the aging process using oak chips, results in the enrichment of the beverage with bioactive components derived from the oak, such as ellagitannins, wood tannins, oak lactones, vanillin, phenyl ketones, benzoic, cinnamic and furanic derivatives and volatile phenols. The final composition of the beverage depends on many factors such as the aging time, the ethanol volume, the wood size, the geographical origin and species as well as the level of the smoking treatment, the reactions among the oak compounds and the beverage constituents, the degradation of oak macromolecules, etc. Therefore, the choice of the oak species should be based on the desired characteristics of the final product. Moreover, the toasting conditions (method and intensity) significantly affect the chemical composition of the oak wood and the profile of the compounds which are migrated from the wood to the beverage. Oak wood is permeable and porous to a certain level, so pyrolysis-generated and other components of wood will migrate into alcoholic drinks over the aging period. The leached components are considered to add a desirable sensory and functional profile to the spirits. In the case of aged whiskies, it is generally accepted that their improved flavour and colour is partly due to the components extracted from oak by the strongly ethanolic and initially colourless spirit.

Many studies have investigated the impact of the application of oak chips on the phenolic composition, antioxidant activity, volatiles and sensory profile for several beverages such as wines, vinegars, brandy, cachaca, apple ciders, but to the best of our knowledge there is little information regarding the addition of oak sticks in the aging of tsiouro.

Considering the increased popularity throughout the years, there is a great interest to produce new and improved distillate products applying different methods, one of which is the acceleration of aging process or simulation of aging effects. To this extent, alternative experimental conditions were applied to induce accelerated aging of tsiouro. Therefore, the main scope of this study was to examine comparatively the influence of ten different oak sticks from French oak (Quercus robur) and French and American oak mix (Quercus robur- Quercus alba) on the final product quality, by means of the total phenolic content, antioxidant and antiradical activity, colour and sensory profile.
**Materials and Methods**

**Reagents and Standards**

All chemicals used were supplied from Sigma-Aldrich Chemie GmbH (Germany) and Mallinckrodt Chemical Works (St. Louis, MO, USA). Sodium persulfate, 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), ferrous sulphate heptahydrate, Iron(III) chloride hexahydrate, Folin-Ciocalteu’s reagent and Trolox (6-hydroxy-2,5,7,8-tetramethylenoxantin-2-carboxylic acid) were obtained from Sigma-Aldrich Chemie GmbH. Gallic acid (3,4,5-trihydroxybenzoic acid) was obtained from Alfa Aesar GmbH & Co (Karlsruhe, Germany). ABTS [2,2’-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] was obtained from Tokyo Chemical Industry Co. LTD (Japan).

**Samples**

Bottled grape marc distillate tisouro, containing 40% alcohol by volume, was kindly supplied by Domaine Babatzimopoulos (Ossa, Thessaloniki). The oak sticks (size 4.0 cm x 1.25 cm x 0.375 cm) used (Table 1) were originated from French oak (*Quercus robur*) and from French and American oak mix (*Quercus robur-* *Quercus alba*), kindly supplied by a commercial supplier (Ampelooeniki P.C, Thermi, Greece) and provided by Oak Solutions Group Inc (http://www.oaksolutionsgroup.com/).

**Table 1: Wood sticks type**

| Oak treatments          | Oak aroma abbreviation as shown in the text | Oak type           | Heat source/Toasting level   | Description                                                                                     |
|-------------------------|---------------------------------------------|--------------------|-------------------------------|-------------------------------------------------------------------------------------------------|
| High Extract series     | High Vanilla (HV)                           | French oak         | Convection / Heavy toast      | intense vanilla flavour                                                                      |
|                         | High Vanilla Pure (HVP)                     |                    |                               | creamy taste with waffle and vanilla flavour                                                  |
|                         | High Spice (HS)                             |                    |                               | cinnamon spice flavour                                                                       |
|                         | High Mocha (HM)                             |                    |                               | mocha flavour with notes of vanilla and toast                                                 |
| Latitude series         | Bordeaux (Bo)                               | French oak         | Infrared / Medium plus toast  | fruit flavour                                                                                  |
|                         | Burgundy (Bu)                               |                    |                               | butterscotch flavour                                                                         |
|                         | Rhome (Rh)                                  |                    |                               | coffee and toasted bread flavour                                                              |
| Cuvée series            | CNo1                                        | French oak blends  | Infrared / Medium toast       | coconut, honey and roasted almonds flavour                                                    |
|                         | CNo2                                        |                    |                               | vanilla and nougat flavour                                                                   |
|                         | CNo3                                        |                    |                               | coffee and bittersweet chocolate flavour                                                      |

**Tsipouro Aging**

Preliminary experiments with French oak sticks were performed at temperatures between 4 and 25°C, with or without stirring process, to select the optimum conditions regarding temperature and stirring. Furthermore, three different concentrations of oak sticks (1.0, 2.0 and 4.0 % w/v) into tisouro were tested. The tisouro samples were stored in closed flasks for 1, 2, 5, 7, 10 or 15 days, at room temperature (20°C). The experiment was performed three times for each sample (n=18 samples, for three concentrations and six storage periods). Concerning the final evaluation, ten different types of wood sticks were added to tisouro under the optimized conditions. The experiment was repeated five times for each wood stick type. At the end of each aging period, oak sticks were removed from the flasks to stop the aging process and flasks were kept closed in the dark, at 4°C, to be used for further analysis.

**Determination of Total Phenolic Content (TPC)**

The total phenolic content (TPC) of each sample was determined applying a modified micromethod of Folin–Ciocalteu’s colourimetric assay according
to Andreou et al. (2018). The absorbance was measured at 750 nm with a Vis spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed, Inc., USA). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per L of spirit drink.

**Determination of Antiradical Activity on (ABTS•+) Radical**
The antiradical activity of tsipouro samples was determined according to the method described by Lantzouraki et al. (2018) and was expressed as mg Trolox equivalents (TE) per L of aged tsipouro. Absorbance was measured at 734 nm with a Vis spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed, Inc., USA).

**Determination of Antioxidant Activity by Frap (Ferric Reducing / Antioxidant Power) Assay**
The ferric reducing antioxidant power assay (FRAP) was performed according to the method described by Lantzouraki et al. (2018) and the antioxidant activity was expressed as mg FeSO₄×7H₂O per L of aged tsipouro. The absorbance was measured at 595 nm, on a Vis spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed, Inc., USA).

**Colour Measurement**
The chromatic characteristics of tsipouro samples were defined by the colourimetric coordinates L* (lightness), a* (redness/greenness), b* ( yellowness/blueness), C ( chroma) and h (hue angle in degrees). The above values were measured with a tristimulus chromatometer (model CR-400, Minolta, Tokyo, Japan) calibrated with a white standard. Three random readings per sample were taken and averaged.

**Sensory Analysis**
The sensory analysis of the oak-aged samples took place in a special room which met the requirements of ISO 8589:2007 to enable the sensory panel to identify and score the main descriptors. Sensory properties were evaluated applying Quantitative Descriptive Analysis (QDA) with a 20-member trained panel and using an appropriate questionnaire with a scoring scale of 1-9, for main quality parameters. Since organoleptic assessment is of major importance, a special training was performed, and specific guidelines were given to assessors, including a detailed explanation of the terminology used. As far as visual assessment is concerned, the colour, clarity, brightness and viscosity of the alcoholic beverage are a few of the more important and often included variables when using sensory evaluation. Other visual observations, such as suspended particles, opacity, cloudiness or sediment could be of great practical importance. The second step in the traditional method is to smell. The identification of different odours, characteristic of such matured spirits, along with the scoring of their intensity and persistence, allows for the design of the aromatic profile of the alternatively aged tsipouro spirits. Taste is the last of the steps in the traditional method, giving a good perception of mouthfeel and aroma sense. Properties like acidity, astrinvcency, pungent taste or aftertaste, sweetness, extent of woody or smoked taste, etc. were assessed by the trained panel.

**Statistical Analysis**
Spectrophotometric assays were repeated three times. The values were averaged and reported along with their standard deviation (S.D). The data regarding TPC, antiradical-antioxidant activity and colour parameters were analysed with One-Way ANOVA Post Hoc Tests, using Tukey's test for pairwise multiple comparisons with statistical significance (P<0.05). Regarding sensory scores, for all attributes assessed, F-value and P-values of each effect of the ANOVA and of the post-hoc Duncan discrimination test were calculated. The correlation among the results was performed by Pearson correlation test. All statistical calculations including partial correlations were performed with the SPSS statistical software for Windows (IBM SPSS Statistics, version 19.0, Chicago, IL, USA).

**Results and Discussion**
**Aging Process Evaluation**
Preliminary experiments were performed in order to obtain the maximum total phenolic content (TPC) after the oak sticks immersion in tsipouro. Results showed that stirring during the aging process didn't affect the extractability of the oak phenolic compounds. Additionally, the increase of temperature resulted in a significant positive effect (P<0.05) in total phenols extractability. According to these results and acknowledging that these conditions could be easily applied on a pilot scale, a
temperature of 20 °C without stirring was selected for the sample preparation. Two independent variables, namely immersion time (days) and oak stick/solvent ratio, were investigated. Considering the results of TPC in tsipouro samples after the aging process, the optimal preparation period was 5 days. More specifically, TPC of aging samples reached the highest (P<0.05) values at five days, and afterwards a slight but significant (P<0.05) reduction occurred (Table 2). According to the above results, it is obvious that the contact time between oak sticks and distillate affected the profile of the extracted compounds. A possible explanation could be that the increase of contact time promotes the reactions among the oak compounds or the decomposition of oak macromolecules\textsuperscript{28}. Considering the Oak stick/solvent ratio, the increase of the amount of oak sticks also increased the total phenols extractability. According to Balcerek et al.,\textsuperscript{29} the temperature as well as the dose and type of oak chips significantly increased the extractable compounds of matured plum distillate. However, the optimum recommended ratio was 2% w/v, as a compromise between the sensory and the antioxidant profile of the end product.

| Days | Oak sticks / tsipouro ratio % w/v |
|------|----------------------------------|
| 1    | 58.73±4.51a 135.47±3.06a 394.80±15.87a |
| 2    | 104.07±5.51b 184.80±5.29b 470.73±3.21b |
| 5    | 250.07±6.66c 571.47±9.00c 1046.13±18.53c |
| 7    | 248.07±9.07c 545.47±11.02d 991.47±12.10d |
| 10   | 237.52±5.69c 534.73±4.73d 949.40±7.94e |
| 15   | 244.81±7.08c 530.80±4.28d 941.13±9.08e |

Results depict average values ± S.D. Different letters in the same column reveal significant differences (P<0.05).

Spectrophotometric Profile of Wood Aged Tsipouro Products

Tsipouro samples before the immersion of the sticks were evaluated for the total phenolic content, antiradical and antioxidant activity showing non-significant results. To a further step, the positive characteristics induced in tsipouro after being aged with a variety of oak wood chips under the optimal conditions, found in the previous section, were investigated. Wood aged tsipouro samples were prepared by immersing ten commercial oak chips (Table 1), representative of different toasting levels, including medium and high toast, and of different heat source (convection and infrared), into the tsipouro. Chips were added at 2% w/v to tsipouro and then samples were stored in closed flasks for 5 days at 20 °C. At the end of the aging process, samples were characterized in terms of their TPC, antiradical and antiradical activity (Table 3). Results showed that average values of TPC (expressed as gallic acid equivalents in mg/L of tsipouro) ranged from 139.73 to 777.40 mg/L. Results for ABTS and FRAP assays, expressed as mg of trolox and Fe(II) equivalents respectively, per L of wood aged tsipouro, ranged from 483.87 to 4564.57 mg TE/L and from 1367.96 to 11511.93 mg Fe(II)E/L of tsipouro. Significantly (P<0.05) higher TPC, antiradical and antioxidant activity were found when Rhome, High Spice, Cuvee No3 and Bordeaux chips were used, compared to the rest of the chips. The above results indicate that the oak sticks led to significant differences in phenolic composition and antioxidant activity of the final product, namely the wood aged tsipouro. The sensory attributes of the examined sticks, as provided by the manufacturer (Table 1), seem to play a predominant role in contributing to phenolic content and antioxidant activity. According to Llodrá\textsuperscript{30}, the toasting method significantly affects the quality and the quantity of the phenolic compounds produced in the wood sticks. Moreover, the different
toasting recipes used for the individual oak sticks, as declared by the manufacturer, influence the lignin degradation degree. According to Soares et al.,\textsuperscript{31} toasting conditions probably cause lignin and tannins degradation, releasing simple phenolics such as phenolic aldehydes and phenolic acids. This observation may justify the fluctuation of TPC and antioxidant-antiradical activity values for the studied samples (Table 3), since these assays determine the total of bioactive compounds irrespective of their specific group. Therefore, the heat source used and the toasting level and/or duration cannot be directly correlated with the final antioxidant capacity.

Strong positive correlations were found between the TPC and the antiradical activity (0.931, P<0.01), the TPC and the antioxidant activity (0.956, P<0.01), and between the antiradical and antioxidant activity (0.980, P<0.01). Therefore, it can be concluded that the phenolic compounds measured by Folin–Ciocalteu’s assay contribute significantly to the antiradical and antioxidant activity of the tsipouro samples. In accordance to the above result, previous studies\textsuperscript{32,33} reported that the antioxidant activity of aged with wood distillates, brandies and vinegars was significantly correlated with total phenolic content.

| Samples | TPC (mg gallic acid E / L) | ABTS (mg Trolox E /L) | FRAP mg FeSO\textsubscript{4}·7H\textsubscript{2}O / L |
|---------|---------------------------|----------------------|-----------------------------------------------|
| HV      | 139.73±20.79h             | 607.45±35.25g        | 1712.39±111.24f                              |
| HVP     | 229.07±5.51g              | 824.15±19.60f        | 1692.31±7.08f                                |
| HS      | 562.40±40.00b             | 2908.45±5.22b        | 8742.49±393.85b                               |
| HM      | 451.40±23.00d             | 850.25±37.77f        | 3743.40±125.10d                               |
| Bo      | 482.40±32.00c,d           | 2429.80±39.19d       | 7613.46±147.21c                               |
| Bu      | 314.40±2.00e              | 992.11±16.30e        | 2856.86±184.02e                               |
| Rh      | 777.40±11.00a             | 4564.57±32.85a       | 11511.93±417.66a                              |
| C No1   | 257.73±5.03f              | 982.54±13.40e        | 2906.29±99.63e                                |
| C No2   | 147.73±17.93h             | 483.87±26.28h        | 1367.96±143.96g                               |
| C No3   | 507.40±18.00c             | 2558.60±67.88c       | 7848.22±294.66c                               |

Results depict average values ± S.D. Different letters in the same column reveal significant differences (P<0.05).

Colour Parameters

The colour of the aged tsipouro is a critical visual feature for consumers’ acceptance. The results showed a great influence of the oak wood sticks’ description on the colour parameters of the final products (Tables 1 and 4). The oak type and the toasting method did not seem to affect the colourimetric coordinates in a specific way. The highest (P<0.05) value for lightness (L*) was found when High Vanilla Pure chips were used. The samples matured with Bordeaux, Burgundy, Rhone and High Vanilla Pure sticks showed higher h values (more than 90 degrees), compared to the rest of the samples. Hue angles that correspond to 60-90° and to 90-100° exhibit hue values which are related to light brown and greenish yellow, respectively\textsuperscript{34}. The tsipouro samples aged with the Bordeaux, Burgundy and Rhome sticks exhibited negative a* values, indicating a notable green contribution. In agreement to this result, Balcerek et al.,\textsuperscript{29} reported that negative a* values were found in plum distillates aged with oak wood chips. Furthermore, significant differences were observed for b* values, with aged tsipouro using High Spice, High Mocha and Cuvee No3 sticks, being significantly more yellow compared to the other samples.

The high positive correlation found between the TPC and the b* values (0.740, P<0.05), pointed to an impact of phenolic compounds to the aged tsipouro...
yellow colour. Moreover, the positive correlation found among the b* values and the antioxidant activity determined by FRAP assay (0.635, P<0.05), indicate the significant contribution of the compounds with antioxidant capacity in the yellowness of the aged tsipouro. In accordance to this result, Zhang et al.,\textsuperscript{35} reported that hydroxybenzoic and hydroxycinnamic acids as well as ellagitannins, which are the main oak polyphenolic compounds, could significantly affect the colour of alcoholic beverages. Based on Garcia et al.,\textsuperscript{36} findings, the colour values could also depend on the presence of products of browning reactions occurring during the toasting treatment, which lead to the degradation of polysaccharides and polyphenols.

Table 4: Colour parameters of aging tsipouro samples

| Samples | L*  | a*   | b*   | h    | C    |
|---------|-----|------|------|------|------|
| HV      | 19.59±0.45c | 0.61±0.08b | 1.54±0.07e | 70.84±2.21g | 1.68±0.07e |
| HVP     | 21.52±0.22a | 0.33±0.04c | 4.05±0.08c | 96.94±0.07b | 4.13±0.09c |
| HS      | 18.05±0.72d | 0.37±0.03c | 6.30±0.39a | 86.62±0.44e | 6.37±0.39a |
| HM      | 16.56±0.18e | 0.81±0.02a | 6.79±0.12a | 83.21±0.09f | 6.83±0.12a |
| Bo      | 20.38±0.30b | -0.18±0.15e | 5.04±0.08b | 91.85±1.18c | 5.04±0.05b |
| Bu      | 21.08±0.14a | -0.57±0.05g | 3.78±0.06d | 98.77±1.02a | 3.82±0.05d |
| Rh      | 18.61±0.75c,d | -0.32±0.11f | 4.80±0.21b | 93.75±1.10c | 4.81±0.22b |
| C No1   | 19.51±0.10c | 0.49±0.08b | 1.45±0.12e | 70.25±4.21g | 1.56±0.08e |
| C No2   | 20.62±0.20b | 0.53±0.02b | 1.10±0.06f | 62.94±1.78h | 1.23±0.08g |
| C No3   | 16.42±0.32e | 0.13±0.08d | 6.44±0.24a | 89.24±0.85d | 6.45±0.24a |

Results depict average values ± S.D. Different letters in the same column reveal significant differences (P<0.05).

Sensory Evaluation

The first purpose of the sensory analysis was to obtain a first approach to the sensory nomenclature for this alcoholic drink, that include the most important and representative words that are used to describe the sensory space related to the product\textsuperscript{37}.

By evaluating the different tsipouro samples, as explained in materials and methods, a list of 12 attributes were obtained that are scored as shown in the characteristic radar plot (Figure 1). The starting spirit was colourless, without any characteristic taste and flavour related to the attributes studied.

![Fig. 1: Sensory profile of aged tsipouro](image-url)
One-way ANOVA analysis of the 12 attributes used, revealed that only two sight attributes and three nose/taste attributes, together with overall quality, differed significantly (P<0.05) among samples (Table 5). According to Delgado de la Torre et al., the thermal treatment of oak chips introduces hydrolisis of insoluble polymers, leading to increased levels of compounds with affect significantly the main sensory properties of spirits. In order to better evaluate the results, each sensory attribute was correlated with TPC, antiradical-antioxidant activity and colour results.

The appearance evaluation, through ‘dark colour’ and colour intensity scoring, was in agreement with instrumentally measured colour perception, as high positive Pearson correlation with b* values (0.805 and 0.832, respectively, P<0.01) as well as high negative correlation with L* values (-0.807 and -0.872, respectively, P<0.01) were observed. Interestingly, high positive correlations were also noticed among the overall acceptance grading, TPC and antiradical-antioxidant activity (0.962, 0.890 and 0.942, respectively, P<0.01). Therefore, it worth's mentioning that the overall acceptance grading revealed the sensory superiority of Bordeaux, Rhome, High Spice and Cuvee No3 samples, that also possessed an elevated biological activity (Table 3). This finding allows for considering these wood aged spirits quite advantageous, not only from a functional / nutritional viewpoint, but also owing to their sensory characteristics. The tsipouro samples aged with the Cuvée series sticks exhibited the lowest (P<0.05) pungent taste, compared to the other samples (Table 5). A possible explanation could be the fact that Cuvée series sticks are manufactured using French and American oak blends, or that these sticks were toasted by infrared heat at lower temperatures and for a shorter duration than the other sticks. Moreover, samples aged with the high extract series sticks showed significantly (P<0.05) higher toasted and wood-like flavour than the other samples (Table 5). This differentiation could be attributed to the heat source used and the toasting level. Specifically, high extract series sticks were toasted by convection heat for a long time, instead of using infrared heat at lower temperatures or for a shorter duration. In this sense, Caldeira et al., reported that the increase of toasting levels accelerates the ageing process and affects the sensory attributes of brandies.

| Samples | Dark Colour | Colour intensity | Toasted flavour | Wood-like flavour | Pungent taste | Overall acceptance |
|---------|-------------|------------------|-----------------|------------------|--------------|--------------------|
| HV      | 2.3±0.6e    | 4.4±1.0c         | 4.4±0.5b        | 4.8±0.7a         | 6.3±0.5ab    | 4.4±0.9c           |
| HVP     | 2.3±0.5e    | 4.3±1.2c         | 4.3±0.4b        | 4.8±0.7a         | 6.3±0.4ab    | 4.8±1.5c           |
| HS      | 6.0±0.9bc   | 6.3±1.4ab        | 5.0±0.6a        | 4.7±0.5a         | 5.8±0.4b     | 6.5±0.6ab          |
| HM      | 8.0±0.6a    | 7.7±1.0a         | 5.2±1.1a        | 4.7±0.8a         | 5.7±0.5b     | 6.0±0.7b           |
| Bo      | 5.2±0.7c    | 5.5±1.0bc        | 2.7±0.5b        | 3.2±0.4b         | 5.7±0.3b     | 6.2±0.5ab          |
| Bu      | 4.0±1.3d    | 4.7±1.2c         | 3.2±0.4b        | 3.7±0.5b         | 6.3±0.5ab    | 4.9±0.6c           |
| Rh      | 6.7±0.8b    | 6.7±1.2ab        | 3.3±0.3b        | 3.3±0.5b         | 7.2±0.7a     | 7.1.0.8a           |
| C No 1  | 3.5±0.8d    | 4.5±1.2c         | 3.3±0.3b        | 3.2±0.2b         | 4.7±0.4c     | 5.5±0.4bc          |
| C No 2  | 3.7±0.5d    | 4.7±1.0c         | 3.5±0.2b        | 3.6±0.2b         | 4.8±0.3c     | 4.7±0.5c           |
| C No 3  | 6.5±0.5b    | 6.8±1.0ab        | 3.0±0.5b        | 3.5±0.3b         | 4.8±0.4c     | 6.4±0.6ab          |

Results represent mean ± standard deviation (n = 5) of sensory assessments
In conclusion, the use of oak sticks could be a fast and efficient process for the enrichment of grape marc distillates with bioactive compounds, enhancing their colour, taste and aroma.

Different letters within a column reveal different (P<0.05) mean values for each considered attribute, applying Duncan post hoc test.

Conclusions
The current study intended to explore the acceleration of tsipouro aging to produce a high quality new product of improved colour, flavour and taste, significantly enriched with bioactive compounds, using oak sticks treated with different toasting recipes. The relationship among the total phenolic content, the antiradical-antioxidant activity, the colour parameters and the sensory character of wood aged tsipouro, was investigated. Sticks originated from French oak (Quercus robur) and from French and American oak mix (Quercus robur- Quercus alba), produced by alternative toasting methods, including convection and infrared heat source, were immersed to tsipouro to induce rapid aging. The results indicated that the toasting conditions mainly influenced the flavour and taste attributes of the aged tsipouro, whereas colour parameters, TPC and biological activity of the samples were not equivalently affected. Interestingly, high positive correlation among TPC, antiradical-antioxidant activity, yellowness and overall acceptance grading was confirmed. The superior quality of the new tsipouro product intends to convey health benefits for consumers and to match with economic profits for the producers.

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