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Influence of the storage in bottle on the antioxidant activity of wine spirit aged by sustainable technology of micro-oxygenation with Limousin oak staves

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The traditional ageing technology (TAT) has always been associated with oak barrels ageing and involves staging the wine distillate (WD) in wooden barrels with a continuous innate diffusion of oxygen through the wood and space between staves, under which the beverage spirit undergoes positive changes that contribute to the enhancement of its chemical composition and sensory properties. It is worth mentioning, that these physicochemical and sensory characteristics of aged wine spirit (WS) are prompted by direct extraction of wood components, decomposition of wood macromolecules and subsequent extraction, reactions between wood and wine distillate constituents, and reaction involving wood extractable compounds and distillate components, among other events.[1]

Furthermore, due to its good permeability, oak wood has been most commonly used in ageing process of beverage spirits, allowing trace oxygen to enter the vascular bundle of oak and cracks between the boards slowly and steadily, causing slow and continuous oxidation of WD, and thus extracting the target compounds. However, TAT is costly and time consuming, which seriously affect the production capacity and economic benefits of wineries. For these reasons, our research team has studied the use of the alternative ageing technology (AAT) for the ageing of WS,[2] using oak wood staves combined with micro-oxygenation (MOX) applied to wine spirit (WS) stored in tanks, with the goal of simulating the ageing process that occurs in wooden barrel (TAT), but in a more sustainable way: lower environmental impact, less time and lower cost.

In this context, despite the interesting results attained on some physicochemical and sensory properties of the WSs during the ageing process using AAT, it is also essential to assess the overall quality of the aged WSs during the storage in bottle to select the best MOX strategy. Some factors, including the closure, temperature, exposure to light, bottle position and the availability of oxygen in headspace height, may change the characteristics of the aged WS during this stage.[3] Thus, this study aimed to analysed, for the first time, the influence of the storage in bottle over 12 months on the evolution of antioxidant activities (DPPH and FRAP assays) and total phenolic content (TPI) of the WSs aged through three modalities (MOX levels: O15, O30 and O60) and one control (N) from AAT. The work was conducted as part of the Oxyrebrand project (https://projects.iniav.pt/oxyrebrand), which includes a detailed explanation about the experimental design. Briefly, the samples were aged with Limousin oak wood by an alternative technology, using 50 L glass demijohns with wood staves with the total of 48 samples (4 modalities × 2 replicates × 2 sampling bottles × 3 storage times).

Figure 1 depicts average values of total phenolic index (TPI) and the antioxidant activity of the aged WSs from the four ageing modalities (O15, O30, O60, N) during storage time (0, 6, 12 months) in bottle. The results show that TPI values of WS from four ageing modalities were not significantly different in the beginning of storage (t0), however the antioxidant activity of ageing modalities (O60 and N) were higher than O15 and O30 (**Figure 1B**). The differences in antioxidant activity between the ageing modalities at t0 could be ascribed to the MOX level, the wood variability (even with the same surface to volume ratio used) and variability of the phenolic profile extracted. After six months (t6), an increase significant in the antioxidant activities by DPPH and FRAP assays for the WSs in all modalities was observed. These changes in the antioxidant activities are presumably due to differential transformation of phenolic

compounds into polymeric and condensed forms, under different oxygen content resulting from the MOX levels combined with the action of factors ruling the storage in bottle (light, temperature, oxygen transfer from the cork, among others), being possible showed by little reduction of TPI values **(Figure 1A**). Polymeric phenolic compounds exhibit different chemical properties and reactivity towards the organic radical reagent (DPPH assay) and with a Fe(III) complex (FRAP assay), according to their degree of hydroxylation and extent of conjugation.[3] After 12 months (t12), the antioxidant activity by FRAP assays decreased significantly compared to t6, however were not significantly different in the beginning of storage (t0) for ageing modalities (O30, O60, N), except for O15 WS. Thus, O60 modality resulted in higher preservation of the phenolic contents and antioxidant activity of aged WS, assuring the aged WS quality. According to this study, this technological alternative (AAT) appears to be the most suitable for wine spirit quality and ageing sustainability.

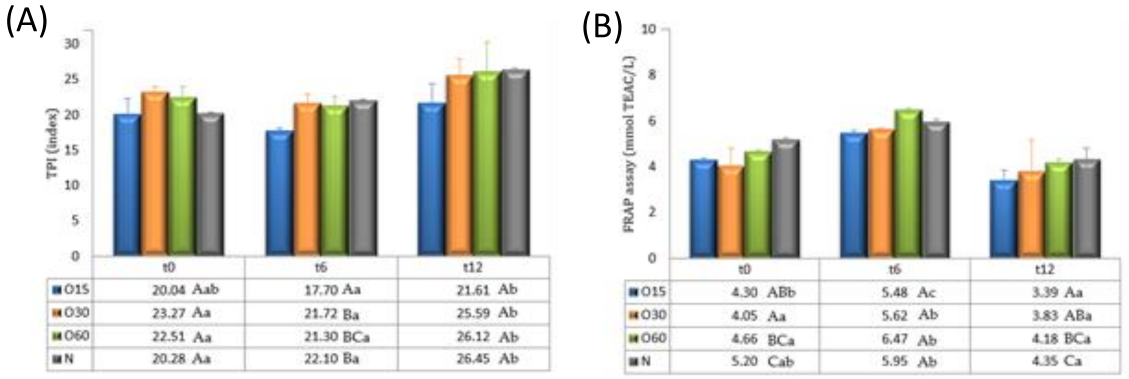

Figure 1: Average values of TPI and antioxidant activity of aged WS in each storage time (0, 6, 12 months) according to ageing modalities (O15, O30, O60 and N). (A) Total phenolic index (TPI); (B) Antioxidant activity using FRAP assay. For each analytical determination: different uppercase letters (A, B, C) in the same column denote significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$); different lowercase letters (a, b, c) in the same row denote significant differences between storage times for each ageing modality by Tukey's test ($p < 0.05$).

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