Enhancing Wine Production Potential: A Comparative Analysis of Fermentation Using Fresh and Dried Grape-Blueberry Mixtures with Different Yeast Strains

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Posted Date: 10 October 2023

doi: 10.20944/preprints202310.0536.v1

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Article

Enhancing Wine Production Potential: A Comparative Analysis of Fermentation Using Fresh and Dried Grape-Blueberry Mixtures with Different Yeast Strains

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Abstract: Red fruits such as grapes and blueberries are known for their high content of bioactive compounds and antioxidants. In Mediterranean winemaking, traditional sun drying can be replaced by controlled airflow chamber drying which provides better quality, higher phenolic content, and increased antioxidants. This study aimed to increase the sugar content and phenolic compounds of the must by drying the fruits to 50% of their original moisture content. Two musts were prepared by combining fresh grapes and dried blueberries (M1), and the other using dried grapes and fresh blueberries (M2), followed by fermentation at 25 ºC with M05 Mead and X5 yeast strains. Must M2 showed the highest level of phenolic compounds, A520, total anthocyanins and antioxidant activity. During fermentation, the anthocyanin content increased mainly in the dried blueberry macerates, which raised between 4 to 5.5-fold. More bioactive compounds were extracted from wines produced by yeast inoculation despite shorter maceration times. Sensory analysis demonstrated consumer acceptance of the wines in terms of color, flavor and aroma. In conclusion, the use of red grapes in the production of blueberry red wine proved to be effective, providing sugars and higher must yields, while the dried fruits improved the fermentable sugar content and the levels of bioactive compounds, increasing the antioxidant capacity of the resulting red fruit wines.

Keywords: wines; chamber drying technique; grape-blueberry synergy; bioactive compounds; antioxidant activity

1. Introduction

Red berries, such as grapes and blueberries, are small berry-like fruits characterized by the color of their skin or pulp. Both grapes and blueberries are known for their high content of bioactive compounds and antioxidant activity [1–3]. These characteristics give them numerous beneficial properties for health, such as their anticancer, anti-inflammatory, and antimicrobial capacity, among others [4–6]. The presence of antioxidants in these fruits is particularly important, as they can react with reactive oxygen species (ROS,), which are unstable and highly reactive molecules present in our body. When oxidized, antioxidant compounds help reduce oxidative stress, an imbalance that occurs when ROS exceed the body's ability to neutralize them. Oxidative stress has been linked to premature aging and the development of various diseases, such as cardiovascular disease, neurodegenerative diseases, and some types of cancer. However, fresh blueberries and grapes present a challenge in terms of preservation, as they are highly susceptible to mechanical damage and microbial degradation. This leads to a short shelf life and unavoidable economic losses. Since these fruits have a seasonal availability and a limited storage period, derivative products have been developed that preserve the bioactive compounds present in these fruits, turning them into functional foods. In the case of blueberries, several products have been created to make the most of these fruits and reduce waste. These products include blueberry juice, blueberry wine, blueberry vinegar, blueberry jam,

 doi:10.20944/preprints202310.0536.v1

dehydrated blueberries, powdered blueberry pulp, dyes and flavor additives used in the production of cakes, cookies, bread, yogurt, and jelly [7].

Zhu et al. [8] carried out a study with 234 consumers to investigate consumer preferences for fruit wines as well as the descriptors associated with different types of fruit wines. The results revealed that grape wine and blueberry wine were most favored by the study participants. However, winemaking blueberries presents a challenge due to their low sugar content. As a result, after fermentation, beverages with an alcohol content of approximately 5-6 % v/v are obtained [9,10]. According to the official definition of the International Organization of Vine and Wine (OIV), it could not be called wine, since its alcoholic content cannot be less than 8.5% v/v. The most common method to compensate for this sugar deficiency is the direct addition of sucrose to the blueberry juice [11–13]. In a study by Liu et al. [14], the fermentation process of blueberry wines was studied by adding different amounts of sucrose. The results revealed that additional sucrose prolongs the total fermentation time and increases the acidity of the wine. In addition, the color of the wine is affected, as the added sugar darkens and yellows the final product. Interestingly, sucrose has a protective effect on anthocyanin levels, the compounds responsible for color in blueberries. However, despite this protective effect, total anthocyanin levels still decrease considerably after fermentation.

Another way to address this problem and increase sugar content is through the post-harvest dehydration process of blueberries. This process has been shown to have significant impacts on the composition and characteristics of blueberries and their resulting wines. First, postharvest dehydration has shown a decrease in the titratable acidity of both blueberries and the wines made from them. In addition, moderate dehydration has been observed to increase the levels of anthocyanins and phenols in both blueberries and wines, which contributes to a higher content of health-promoting compounds [15]. Drying, from an oenological point of view, is an important process since many wines are made from dehydrated grapes [16]. In Mediterranean areas, sun-drying of grapes is still used, as in the Montilla-Moriles appellation in southern Spain for the production of its sweet white wines. This type of drying usually extends over a range of 5 to 10 days depending on the weather conditions of each year. In addition, it presents a series of disadvantages as a consequence of working in the open air, such as occasional rains, high solar radiation, insect and animal attacks or microbial attacks by toxin-producing fungi, such as ochratoxin A, among others [16–19]. This type of drying can be replaced by drying with air flow in a temperature-controlled chamber, which would avoid all these types of inconveniences. Some authors have shown that the use of this type of drying allows obtaining higher quality dried products than sun-dried ones and have found that the phenolic content and antioxidant activity of the berries are increased, in addition to increasing the sugar content [17,20–22]. In a previous study, the vinification of sugar-enriched blueberry juice was investigated by pre-concentrating it through dehydration in a temperature-controlled drying chamber, achieving an alcoholic strength of 17% v/v [21].

Therefore, the aim of this work was to investigate the vinification of musts obtained from the combination of dried grapes or blueberries with fresh fruits, with the purpose that the dehydration of one of the fruits in the mixture would increase the sugar content and, consequently, the alcohol content of the resulting beverages. In addition, changes in phenolic content, antioxidant activity and acceptance of the wines produced by regular consumers were evaluated.

2. Materials and Methods

2.1. Materials

Grapes (Vitis vinifera), Tempranillo variety, were provided by the Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Cabra, Spain. Blueberries (Vaccinium corymbosum), Ventura variety, provided by the company PlusBerries, harvested in the province of Huelva, Spain. Both fruits were harvested at their optimum ripeness for consumption, frozen at -18 ºC until the time of analysis. Subsequently, to begin the experiments, they were thawed at 25 ºC for 24 hours.

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2.2. Dehydration process

The starting grapes had 23 ºBrix and the blueberries 13 ºBrix. Both grapes and blueberries were dehydrated in a Frisol Climatronic drying chamber, with a relative humidity of 20 % and an air current at a constant temperature of 50 ºC. The drying process was controlled by the loss of fruit weight, with periodic weighing, and was maintained until both fruits lost 50 % of the initial moisture. After the drying process, the fruits were blended using the same amount of undried fruit (2.5 kg) and dried fruit, thus obtaining two different musts. One from fresh grapes and dried blueberry (M1) with a sugar content of 21.2 ° Brix; and the other from dried grapes and fresh blueberry (M2) with a sugar content of 18 ° Brix.

To calculate the dry matter content of both fruits, they were dried in an oven at 100 ºC until constant weight.

2.3. Selection and preparation of yeast inocula

Two Saccharomyces cerevisiae yeast strains were selected for must fermentation, X5 (CECT131015) yeast isolated from partially sun-dried Pedro Ximenez grape musts [23], which are resistant to high concentrations of sugar and alcohol; and the commercial strain M05 Mead from Mangrove Jack's (M), normally used in mead fermentation.

The preparation of pre-inocula of the different strains was carried out in YPD culture medium (1 % w/v yeast extract, 2% peptone, 2% w/v D-glucose) at 28 °C and 150 rpm on the New Brunswick Scientific orbital shaker (Edison, NJ, USA) for 24 h.

2.4. Fermentation process

For the development of the fermentation process, the musts previously mentioned in section 2.2 (M1 and M2) were used. After blending the fruits, they were pressed together in a manual vertical press, performing two pressing cycles, with a maximum pressure of 300 bar. 100 mL of the resulting musts together with 50 g of solid parts from the pressing were inoculated with the selected yeasts, with a cell concentration of 5x106 cells/mL, and introduced into thermostatized baths at 25 ± 0.2 ºC. The monitoring of the fermentations was carried out by weight difference, with periodic weighing and estimation of the CO_2 released, since according to reaction 1 it is stoichiometric with the production of ethanol in the medium.

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 \quad (1)$$

An aliquot of each must (M1 and M2) was used as a control and allowed to ferment spontaneously with the indigenous yeasts of the fruits used. The fermentation process was maintained until the alcoholic strength estimated by the sugar-alcohol correlation tables was reached, with which 12.5 % v/v ethanol was estimated for the M1 must and 10.6 % v/v for the M2 must. To control the evolution of fermentation, periodic weighings were carried out, which allowed us to approximate the alcohol content of our wines at each moment, related to the loss of CO_2. Once the expected alcoholic content of the fermentations was reached, the yeast residues were removed from the medium by centrifugation and filtration.

Fermentations of each must (M1 and M2) and in the three conditions (control, M05 Mead yeast and X5 yeast) were carried out in duplicate (a and b), obtaining the following wines:

	W1		W2	
	a	b	a	b
Control	W1C_a	W1C_b	W2C_a	W2C_b
M05 Mead yeast	W1M_a	W1M_b	W2M_a	W2M_b
X5 yeast	W1X_a	W1X_b	W2X_a	W2X_b

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2.5. Alcohol content

The procedure followed was the one proposed by Crowell and Ough, based on steam entrainment of the ethanol contained in the sample and subsequent reaction, at controlled temperature, with a solution of potassium dichromate in an acid medium, performing a spectrophotometric measurement at 600 nm in a Beckman DU 640 UV-visible spectrophotometer, and comparing the absorbance with those obtained in a standard line of ethanol [24].

2.6. Volatile acidity

The isolation of volatile acids was carried out according to the OIV method by entrainment with water vapor and rectification of the vapors [25]. The sample was acidified before the entrainment, taking care to ensure that it was free of carbon dioxide gas. The acidity was determined on the distillate, titrating with NaOH and using phenolphthalein as an indicator.

2.7. Spectrophotometric determinations

Using a UV-visible spectrophotometer, Beckman DU 640, and quartz cuvettes with an optical pitch of 1 mm, the absorbance of the musts was measured at 420, 520, and 620 nm to estimate the contribution to color of brown, red, and blue compounds, respectively. In addition, the hue parameter was calculated with formula 2 to determine the existence of a greater contribution from brown or red compounds. All absorbances were performed in triplicate for each independent fermentation and were corrected to an optical step of 1 cm.

$$\text{Hue} = \frac{\text{Abs 420 nm}}{\text{Abs 520 nm}} \quad (2)$$

2.8. Total phenolic compounds

The Folin-Ciocalteu method [26] was used for the determination of total phenolic compounds in triplicate for each independent fermentation. For this, to 1.25 mL of Folin Ciocalteu's reagent diluted 1:5 with distilled water, 50 µL of sample was added, shaken vigorously and allowed to stand for 1 min. Then 1 mL of 10 % w/v sodium carbonate was added and left in the dark for 30 min. After this time, the blue coloration produced by the Folin-Ciocalteu reagent oxides related to the concentration of phenolic compounds was measured at 760 nm in a Beckman DU 640 spectrophotometer. A calibration curve for gallic acid was performed using different concentrations of standard in a range between 0.01 and 1 g gallic acid/L.

2.9. Total flavonoids

For the determination of total flavonoids in triplicate for each independent fermentation, the aluminum trichloride method is used. For this, 300 µL of sample filtered through 0.45 µm were taken in each case, to which 120 µL of 2 % (w/v) $AlCl_3$ was added and the volume was made up to 3 mL with 5 % acetic acid in methanol. It is then allowed to stand in the dark for 30 min and finally the absorbance is measured at 425 nm with a Beckman DU 640 UV-vis spectrophotometer. The data are expressed as mg quercetin/L. For this purpose, a quercetin calibration line between 0 and 700 mg quercetin/L is made.

2.10. Total anthocyanins

To determine the total anthocyanin content in triplicate in each independent fermentation, the differential pH method was used [27]. For this, two 1:10 dilutions of the sample were prepared in two different buffers: KCl at pH 1 and $NaCH_3COO$ at pH 4.5. After a resting period of 20 minutes, the absorbance was measured using a Beckman DU 640 UV-vis spectrophotometer at 520 nm and 700 nm. The total anthocyanin content was calculated using formulae 3 and 4, taking into account the following parameters: Mw (molecular weight of cyanidin-3-glucoside, 449.2 g/mol), D (dilution factor), ε (molar absorptivity of cyanidin-3-glucoside, 26900 L/mol-cm) and PL (optical light path).

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$$\text{Total anthocyanins (mg/L)} = \frac{A \cdot M_W \cdot D \cdot 1000}{\varepsilon \cdot PL} \quad (3)$$

$$A = (A_{520} - A_{700})_{pH1} - (A_{520} - A_{700})_{pH4,5} \quad (4)$$

2.11. Antioxidant activity

2.11.1. DPPH assay

The DPPH assay was used to determine the antioxidant activity of musts and wines obtained in triplicate for each independent fermentation, according to the method used by Katalinic et al [28], under some modifications. To 3 mL of DPPH 45 mg/L solution, 200 µL of sample diluted 1:10 or 200 µL of water in the case of the control was added. The absorbance of the control was measured immediately at 517 nm in a Beckman DU 640 spectrophotometer, while the sample was measured after 30 min incubation at room temperature and darkness. A calibration curve was performed with Trolox in the range of 10-200 mg Trolox/L and the percentage inhibition was calculated according to formula 5.

$$\text{Percentage inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \text{ x } 100 \quad (5)$$

2.11.2. ABTS assay

The ABTS assay was developed according to the method proposed by Re et al [29], but under some modifications. The $ABTS^{\cdot+}$ radical was formed by oxidation of 7 mM ABTS solution with 2.45 mM potassium persulfate, the mixture was kept in the dark for 12 h to complete the reaction. Subsequently, the $ABTS^{\cdot+}$ radical solution was diluted with ethanol until the absorbance at 734 nm reached a value of 0.700 ± 0.020. Next, 900 µL of this diluted solution was taken and 100 µL of the sample or 100 µL of distilled water in the case of the control was added. The absorbance of the control was measured immediately at 734 nm in a Beckman DU 640 spectrophotometer, while that of the sample was measured after 6 min at room temperature and darkness. The percentage inhibition was calculated with formula 6 and the antioxidant activity was established with the help of a calibration line of Trolox in the range 10-100 mg Trolox/L.

$$\text{Percentage inhibition} = \frac{Abs_{control} - Abs_{muestra}}{Abs_{control}} \text{ x } 100 \quad (6)$$

2.12. Sensory analysis

In the sensory analysis, a tasting card was prepared to evaluate the color, flavor and aroma parameters of the different wines obtained, according to the scales proposed by ISO 4121:2003. The different parameters were evaluated with the following options: desirable (5-6), acceptable (3-4) and undesirable (1-2).

2.13. Statistical analysis

The results of all samples were subjected to analysis of variance at a 99.0 % confidence level. Homogeneous groups were calculated to establish significant differences between means. A simple linear correlation has been made between antioxidant activity values and total phenolic compounds and anthocyanins content. The data obtained from the drying processes were adjusted to different mathematical models frequently used to model drying curves. The software used was Statgraphics Centurion XVI.

3. Results

The dehydration process of grapes and blueberries was developed to increase the sugar and phenolic compound content in the must to be fermented. The temperature chosen for both drying

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processes was 50 ºC, based on what was reported by other authors [22,30] who, studying the drying of these fruits, had concluded that 50 ºC was the temperature at which the dehydration process was faster, maintaining a greater amount of phenolic compounds and antioxidant activity. On the one hand, the drying process was monitored by measuring moisture loss and expressing it in kg water/kg drying matter (Figure 1). As can be seen, the blueberry initially contained a greater initial amount of water than the grape (5.87 vs. 2.70 kg w/Kg dm). Considering that both dryings were maintained for 16 h until the fruits lost 50 % of their initial moisture, this implies that the dried blueberry was left with a higher moisture content than the dried grape (2.88 vs. 1.40 Kg w/Kg dm). On the other hand, it is important to consider in the drying processes the variation of the moisture ratio, determined as the ratio of the moisture content at each time to the initial moisture content, versus the drying time (Figure 1).

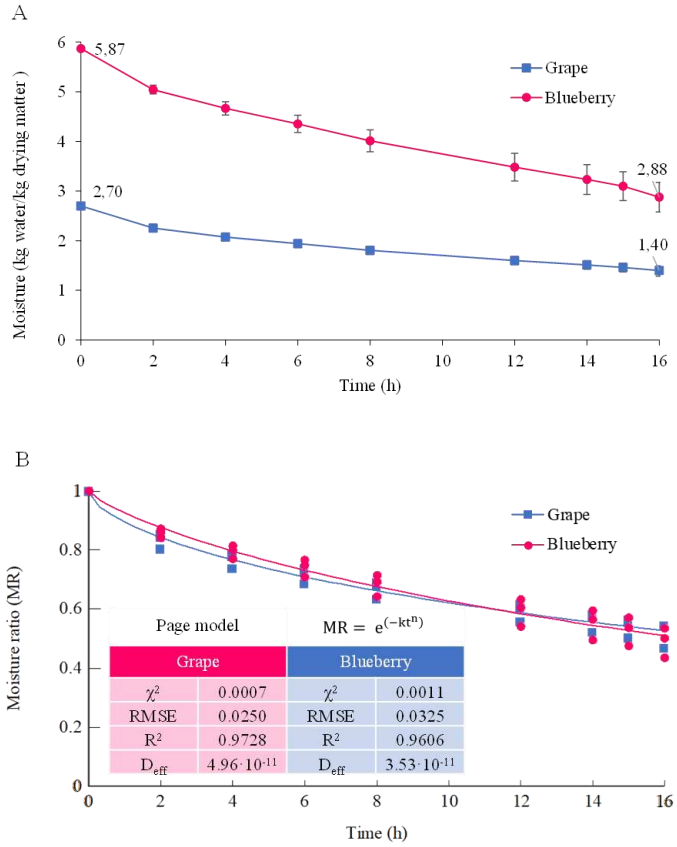

Figure 1. Dying curves of blueberries and red grapes, Moisture vs time (A), and Moisture ratio vs time (B).

As can be seen the moisture ratio decreased exponentially over time in both processes in the same way, indicating that they have been carried out at the same rate. Specifically, considering that the optimal criteria to evaluate the best fit and the quality of the fit to the mathematical models is to have the highest R^2 value and the lowest χ^2 and RMSE values. The best fit in both processes was "Page model" with R^2 values of 0.9728 and 0.9606 for blueberry and grape drying respectively, in addition to possessing the lowest χ^2 and RMSE values, indicating that with this model the changes in fruit moisture content with drying time could be predicted. Being the same model that was previously recorded for blueberry drying at that temperature [30].

Figure 2 shows the fermentation progress of musts M1 (fresh grapes+dried blueberry) and M2 (dried grapes+fresh blueberry) inoculated with the selected yeasts (M05 Mead and X5) and the fermentation of a control must (not inoculated), which was left to ferment spontaneously with the indigenous yeasts present in the fruits.

The fermentations carried out with the M2 must were the first to finish (29 hours) because they consumed all the sugar content, both the musts inoculated with the M05 Mead and X5 yeasts, with a

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final alcohol content of 10.3 and 10.5 % v/v, respectively (Table 1). Then, after 45 hours, the four fermentations inoculated of M1 musts were finished with a final alcohol content between 10.8 and 11.6 % v/v. Finally, the control fermentations carried out without inoculum ended at 51 hours those from M2 with an alcohol content of approximately 9.5 % v/v and at 67 hours those from M1 with an alcohol content of approximately 11.4 % v/v.

Table 1. Enological parameters of initial musts and all elaborated wines studied (n=3, mean ± standard deviation).

	Ethanol	Volatile acidity	Total phenolic compounds	Total flavonoids	Total Anthocyanins	Antioxidant activity	
						DPPH assay	ABTS assay
M1	0	0	845±2.28	27.9±2.62	2.91±0.094	432±2.72	824±43.1
W1C_a	11.4±0.012	7.64±0.246	917±0.228	37.5±0.131	11.47±0.155	560±13.3	1093±3.29
W1C_b	11.3±0.067	6.17±0.246	912±4.53	39.4±1.19	10.64±0.106	561±28.0	1157±15.2
W1M_a	10.5±0.056	3.73±0.248	1043±7.09	41.1±0.758	14.18±0.149	612±8.52	1300±15.9
W1M_b	11.6±0.281	3.95±0.000	1063±4.48	47.9±0.845	16.04±0.291	600±3.84	1380±2.86
W1X_a	10.8±0.080	1.74±0.248	957±2.00	40.3±1.22	13.58±0.235	626±2.84	1264±25.0
W1X_b	10.8±0.080	1.74±0.248	1019±4.33	40.4±0.675	13.77±0.072	575±15.5	1231±23.7
M2	0	0	988±3.22	24.1±0.611	9.08±0.384	532±0.620	1106±9.61
W2C_a	9.6±0.059	7.15±0.246	988±1.70	30.6±0.007	9.96±0.181	533±1.43	1393±13.4
W2C_b	9.4±0.065	7.40±0.000	979±4.74	33.1±0.801	11.19±0.073	540±0.562	1205±10.9
W2M_a	10.5±0.106	2.73±0.248	1109±6.48	36.4±0.420	11.81±0.127	634±4.20	1155±23.2
W2M_b	10.3±0.049	2.96±0.000	1025±51.8	33.1±0.210	12.29±0.068	583±0.580	1146±11.2
W2X_a	10.4±0.039	1.74±0.248	1037±5.96	34.9±0.250	10.84±0.162	606±0.926	1466±40.5
W2X_b	10.5±0.112	1.99±0.000	1066±9.15	36.5±1.230	15.53±1.399	610±9.38	1294±9.3

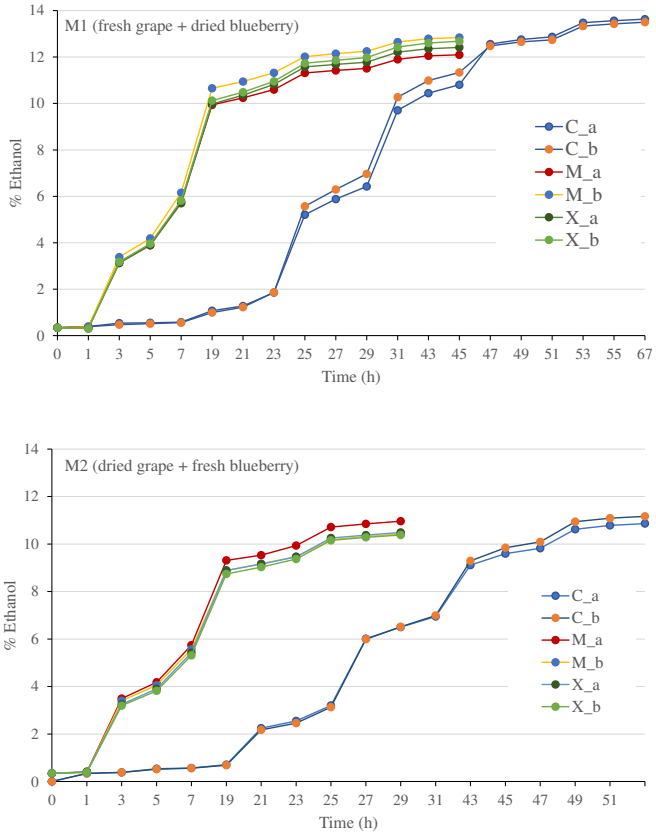

Figure 2. Fermentation curves of fresh grapes and dried blueberries (M1) and dried grapes and fresh blueberries (M2).

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The wines produced in this work do not exceed the allowed volatile acidity limit of 7.9 meq/L for grape wines [31], since they all presented lower values (Table 1). However, it can be seen that the control wines produced by spontaneous fermentation showed higher volatile acidity values than the wines produced after inoculation. This may be due to the fact that in the control musts the fermentation process was longer, so that a greater number of secondary reactions could occur. In addition, the wines made with the X5 yeast (W1X and W2X) had the lowest volatile acidity values, which could mean that this type of yeast causes the sugars to go mainly to the metabolic route for the production of alcohol and do not participate in secondary reactions, such as the formation of acetic acid, or do so to a lesser extent.

The color of a fruit is the result of a complex mixture of pigments, which do not remain the same qualitatively or quantitatively, but change over time, due to chemical reactions that occur between the pigments with oxygen or other compounds present in the wine [32]. In wine, color is an important quality parameter, since it can be one of the first attributes that define it organoleptically. Table 2 shows the values of the absorbances at 420, 520 and 620 nm and the color intensity and hue of the initial musts and the different wines obtained. Comparing the two starting musts, the must obtained from the mixture of dry grapes and fresh blueberries (M2) had a higher absorbance at 520 nm than the M1 must (2.30 vs. 1.68 a.u. respectively). This confirms what has been reported in previous works [22,30], that fresh blueberries contribute more red compounds than fresh grapes, namely 0.495 versus 0.087 a.u., respectively.

Table 2. Color parameters of initial musts and all elaborated wines studied (n=3. mean ± standard deviation).

	A420	A520	A620	Color intensity	Hue
M1	1.85±0.010	1.68±0.003	0.410±0.003	3.94±0.009	1.10±0.004
W1C_a	1.59±0.005	2.58±0.000	0.358±0.003	4.52±0.002	0.615±0.002
W1C_b	1.61±0.001	2.50±0.005	0.345±0.005	4.45±0.001	0.646±0.001
W1M_a	2.11±0.008	3.66±0.023	0.497±0.004	6.26±0.034	0.578±0.001
W1M_b	2.23±0.029	3.83±0.043	0.526±0.020	6.59±0.092	0.583±0.001
W1X_a	2.05±0.004	3.47±0.011	0.511±0.001	6.03±0.015	0.592±0.001
W1X_b	1.84±0.004	3.14±0.006	0.436±0.002	5.41±0.008	0.586±0.000
M2	1.47±0.007	2.30±0.034	0.400±0.003	4.17±0.044	0.642±0.006
W2C_a	1.23±0.004	1.84±0.019	0.272±0.001	3.34±0.024	0.667±0.005
W2C_b	1.24±0.006	1.94±0.021	0.253±0.002	3.43±0.029	0.641±0.004
W2M_a	1.50±0.007	2.32±0.015	0.316±0.004	4.13±0.025	0.643±0.001
W2M_b	1.49±0.002	2.44±0.011	0.343±0.002	4.27±0.015	0.607±0.002
W2X_a	1.65±0.005	2.64±0.017	0.385±0.001	4.67±0.023	0.622±0.002
W2X_b	1.65±0.001	2.70±0.007	0.419±0.001	4.76±0.005	0.611±0.002

The absorbance at 420 nm is related to the coloration provided by brown compounds, and previous studies have shown that fresh grapes provide a lower concentration of these compounds than fresh blueberries [22,30]. The M1 must made with dried blueberry showed a higher value of A420 than when the blueberry was fresh, indicating that during the drying process the brown compounds increased due to the concentration effect of water evaporation and enzymatic and non-enzymatic browning reactions.

The hue is a ratio between the contribution of brown compounds versus red compounds, so values above 1 would indicate a higher contribution of brown compounds. In the case of young red wines, it is in the range of 0.5 - 0.7 and increases with aging to a range of 1.2 - 1.3 [33]. The great difference in tonality between the two starting musts can be observed, with the value of M1 being the only one above 1 and with a value almost double that of M2 (1.10 vs. 0.642 respectively). This is a consequence of the higher value of A420 and lower value of 520 in the M1 must.

After fermentation of the M1 must, an increase in absorbance at 520 nm was observed in all the wines (Table 2). The W1M wines presented the highest absorbance values and the W1C control the

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smallest values, indicating that when the maceration was with dried blueberry, the extraction of red pigments was much higher because of the fact that in this case, for the same weight of dried fruit, the proportion of skins in the blueberry is higher. Figure 3 shows the variation of A520 from the initial musts to the final wines. It can be seen that this occurs in both the control wine and those that were inoculated with yeast. Consequently, when macerated with fresh blueberry (M2), a loss of color could be observed compared to the starting must (W2C) and a slight increase in the other two wines obtained (W2M and W2X). This marked increase in A520 in the vinification of the M1 must caused a considerable decrease in hue, and the corresponding wines were found to have a hue between 0.578 and 0.646. However, during fermentation of the M2 must, the hue was maintained, so that the finished W2 wines showed hues similar to those of W1.Table 1 shows the anthocyanin content of the initial musts and of all the wines obtained after fermentation. It can be seen that, as mentioned for the A520, the M2 must obtained from the mixture of dry grapes and fresh blueberries presented a concentration that was slightly more than three times that of the M1 must (9.08 vs. 2.91 mg/L), indicating that the red color mentioned is due to the anthocyanin derivatives, confirming that the fresh blueberry has a higher concentration of these compounds than the fresh grape [22,30]. Figure 3 shows the variation in anthocyanin content from the initial musts to the final wines. It can be seen that, in the three fermentations carried out, the highest extraction occurred in obtaining wine 1 (W1) as a consequence of the fact that since the maceration was carried out with the same weight of fruit, when the blueberry is dry it is macerated with a much higher proportion of skins and justifies extracting 280 vs 16.5% in the control wines, 419 vs 32.8% in the wines obtained with M05 Mead and 370 vs 45.3% in the wines obtained with X05.

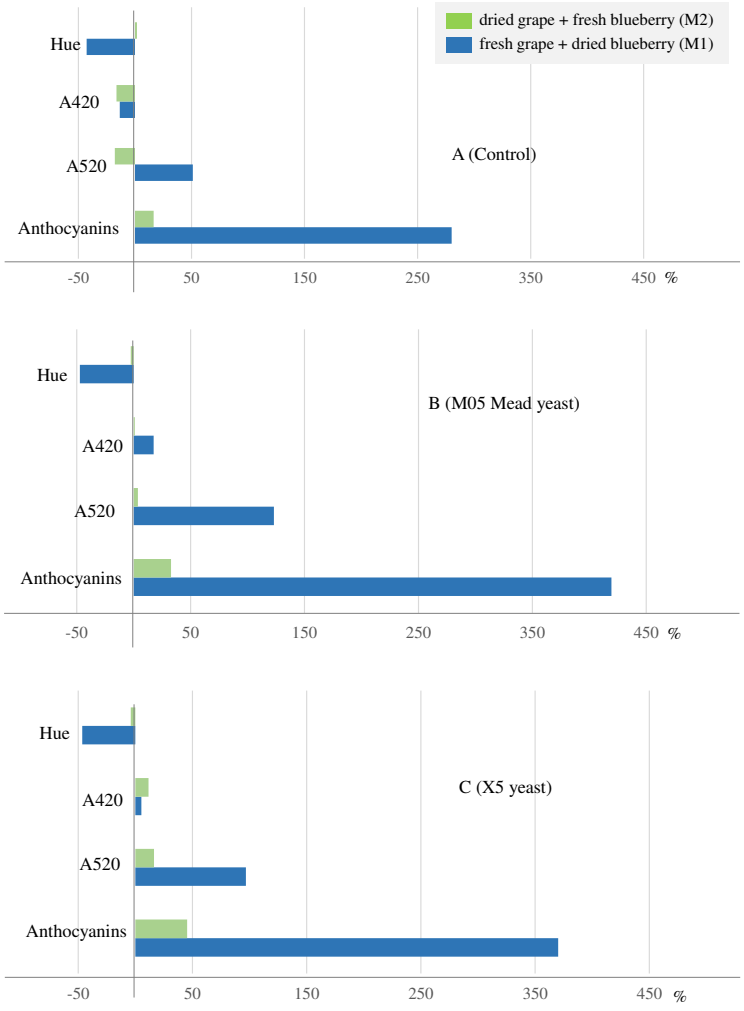

Figure 3. Percentage of change of anthocyanin content and color parameters in the control fermentations (A) and with the two selected yeasts (B and C).

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The Folin-Ciocalteu index is a widely used method to measure the total phenolic compound content of wines, although other compounds could be included to a lesser extent. Table 1 shows the values of the Polyphenol Index of the initial musts and of the different wines obtained.

First of all, it can be seen that the musts coming from the pressing of a mixture of dried grapes and fresh blueberry (M2) presented a higher phenolic content than those coming from fresh grapes and dried blueberry (M1), 988 vs 845 mg/L, possibly and as mentioned above as a consequence of their much higher content in red pigments. In addition, it can be seen that these contents increased during vinification of the M1 must, due to the extraction of these compounds by the presence of the fruit skins during fermentation. Of these, the wines obtained by inoculation with M05 Mead yeast (W1M_a W1M_b) had the highest total phenolic content, followed by those inoculated with X5 (W1X_a W1X_b) and, finally, the control wines (W1C_a and W1C_b) with values of 917 and 912 mg/L respectively, which despite being the wines that underwent the longest fermentation process, i.e. the longest maceration, did not acquire a higher content.

In the vinification of the M2 must, as in the previous case, the wines fermented with pre-inoculum showed higher contents of total phenolic compounds. The capacity of yeasts to adsorb phenolic compounds during fermentation at the same time as they are extracted from the fruit skins is a well-known fact. It is also known that at the end of fermentation the yeasts experience a process of autolysis and subsequent settling to the bottom of the vessel, carrying away the coloring and bioactive compounds that had remained adhered to the cell walls of the yeasts [34]. Therefore, it could be concluded that, although the control fermentations also had the presence of yeast, their duration was much longer, so that, probability, after a certain fermentation time, the extraction was less than the adsorption by the yeast.

All the wines obtained after the fermentation process of the mixed blueberry and grape musts (W1 and W2), presented a content in total phenolic compounds within the range, 912-1068 mg gallic acid/L, values that are similar to those studied by other authors in blueberry wine such as Su and Chien [35], whose content in total phenolic compounds is in the range of 858-1150 mg gallic acid/L; or those studied by authors Jonhson and Gonzalez de Mejia [36], which are in the range of 966-2510 mg gallic acid/L. Even the wines produced showed a higher phenolic content than the blueberry wines produced by the authors Jonhson et al. [37], whose content was between 375 and 657 mg gallic acid/L; or those elaborated by Zhang et al. [13] with 10 different blueberry varieties with a content in the range of 506 - 888 mg/L with the exception of the Gardenblue variety with a content of 1205 mg gallic acid/L.

In relation to flavonoid content, once again, the musts from the pressing of a mixture of dried grapes and fresh blueberries (M2) showed a higher content than those from fresh grapes and dried blueberries (M1), 30.6 vs 27.9 mg/L. During vinification, the contents increased in all the wines, although the increases in the W1 wines were greater, with the result that the W1 wines had a higher flavonoid concentration.

Berries such as grapes and blueberries have a high antioxidant activity, which gives them important beneficial properties for health [38,39]. Antioxidant activity was measured by two methods whose name is given to the colored molecule used as a proton or electron scavenger. The DPPH assay reaction consists of an electron transfer followed or preceded by a proton transfer, known as a coupled proton-electron transfer reaction [40]; whereas the ABTS reaction consists only of an electron transfer [29]. Therefore, the results obtained by both methods cannot be directly compared, since they present different reaction mechanisms [41].

Firstly, it can be seen that the musts from the pressing of a mixture of dried grapes and fresh blueberry (M2) showed higher antioxidant activity than those from fresh grapes and dried blueberry (M1), both for the DPPH assay (532 vs 432 mg Trolox/L) and for the ABTS (1106 vs 824 mg Trolox/L).

It should be highlighted that during fermentation of the M1 must, it can be seen in Figure 4, that there is a large increase in antioxidant activity for both the DPPH and ABTS assay, giving higher values in the ABTS assay. For this assay, the wines obtained by fermentation with M05 Mead yeast showed the highest antioxidant capacity, registering an increase of more than 60% (62.5%), followed

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by the W1X wines (increase of 51.4%), and finally the W1C control wines with an increase of 36.5% with respect to the starting must, which showed the lowest value.

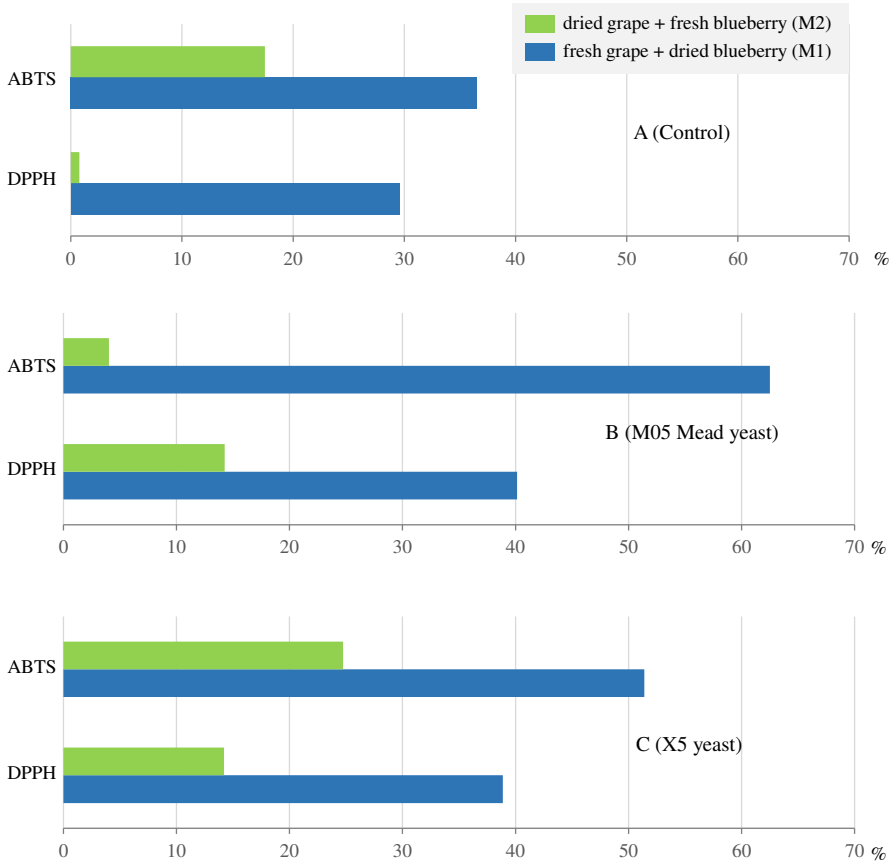

Figure 4. Percentage variation of antioxidant activity values in the control fermentations (A) and with the two selected yeasts (B and C).

In order to determine the relationship between antioxidant activity and phenolic compounds, a simple linear regression adjustment was carried out, measuring Pearson's r correlation coefficient, both for the ABTS assay and for DPPH (Figure 5). Likewise, and due to its great influence on these fruits, a correlation study was carried out between antioxidant activity and total anthocyanin content (Figure 5). For the 12 degrees of freedom of the analysis (number of samples -2), the linear correlation coefficient has a significance of 95% ($p<0.05$) when $r\geq0.532$, of 99% ($p<0,01$) when $r\geq0.661$ and of 99.9% ($p<0.001$) when $r\geq0.780$. As can be seen, the variation of antioxidant activity is related to the total anthocyanin content, both when determined with the ABTS assay ($p<0.01$), as with the DPPH assay even at an even higher significance level ($p<0.001$). This supports what has been reported in the literature that these phenolic compounds are highly valued for their antioxidant properties, both as free radical scavengers and metal chelators, which are the reason for their benefits to human health. When the correlation is made with the content of phenolic compounds, it can be seen that it only presented a significance higher than 95% when performed with the DPPH assay ($p<0.01$). It seems reasonable to think that the ABTS radical may have an affinity for other families of non-phenolic compounds and hence its lower significance in the correlation with antioxidant activity.

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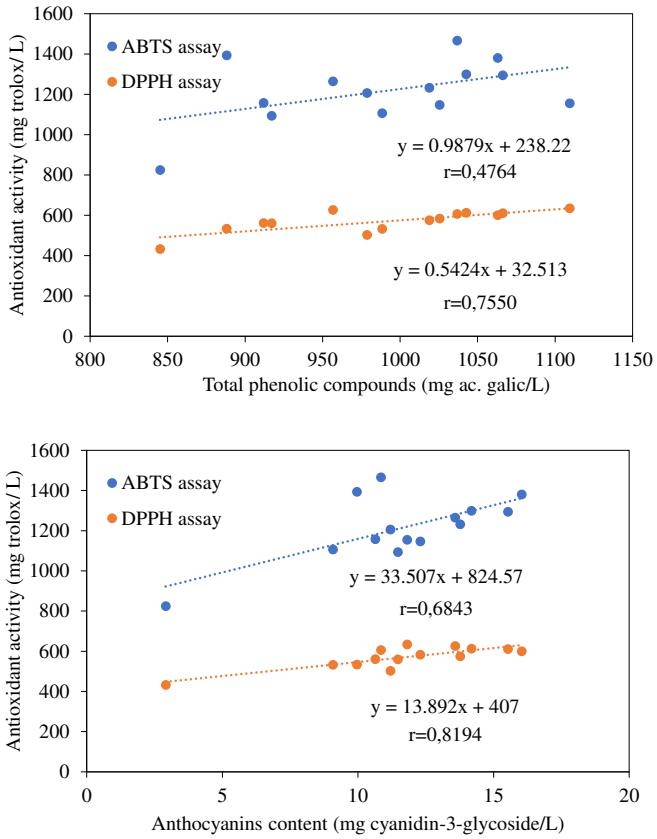

Figure 5. Correlations between antioxidant activity and total polyphenol and anthocyanin content.

Figure 6 shows the results of the sensory analysis carried out where the organoleptic quality parameters such as color, aroma and flavor were evaluated using the following evaluation criteria and scores: undesirable (1-2), acceptable (3-4) and desirable (5-6). Figure 6A shows the scores given by three expert tasters, who concluded that in aroma the best wine was the one made from fresh grapes and dried blueberry (M1) and inoculated with M05 Mead yeast (W1M). They also found that the W1M wine had notes of ripe fruit, strawberry, and banana, and the W2M wine had notes of ripe fruit, blueberry jam, licorice and banana. In relation to the wines made with the X5 yeast, the experts found that the W1X5 wine gave notes of dried fruit skin, herbal, menthol, and toasted aromas, and the W2X5 wine with notes of ripe fruit, peach, passion fruit and licorice. Finally, the worst scores were for the spontaneously fermented wines, although to a lesser extent for the W1C wine. In them, the only aroma notes that could be appreciated were ethyl acetate, due to the amount of acetic acid present. In flavor, the scores were not very high due to the acidity of the wines in which an excess of malic acid was sensorially detected, with the exception of wines W2M and W2X5 which were within the acceptable range. On the other hand, in color, all the wines scored very similarly within the desirable range, due to their violet and burgundy hues characteristic of both fruits.

Figure 6B shows the data and scores obtained by the 20 tasters, who are regular wine consumers. As with the expert tasters, the wines made with pre-inoculums had the highest scores. In all the wines, color was the best evaluated parameter, with scores that classified it as acceptable. On the other hand, the best evaluated aroma was that of the wines that had been inoculated with M05 Mead yeast (W1M and W2M) and classified them as acceptable. In terms of flavor, all the wines showed very similar scores in the acceptable range. It should be taken into account that the wines produced are new products that do not exist in the market, making the tasting more complex for regular red wine consumers. Perhaps the most interesting thing is that in no case were they rejected for any of their attributes.

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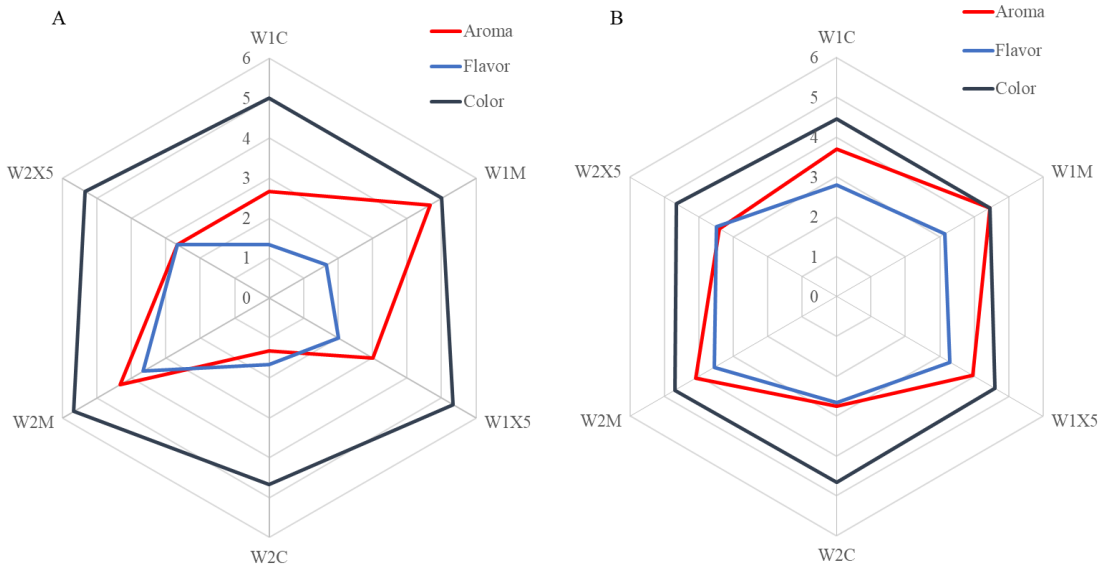

Figure 6. Scores obtained for the wines by the panel of expert tasters (A) and by the panel of regular consumers.

4. Conclusions

Despite the fact that the blueberry initially contained a greater amount of water than the grape, both dried plants experienced the same kinetics according to the same regression model (Page model), using the same time to reach 50% of their initial moisture content.

The must obtained from the mixture of dried grapes and fresh blueberry had the highest content of phenolic compounds, A520, total anthocyanins and antioxidant activity measured by both ABTS and DPPH assays. On the other hand, the must obtained with fresh grapes and dried blueberry had the highest content in reducing sugars, flavonoids and A420 and tonality. Therefore, it could be concluded that the use of blueberry has favored the higher content of bioactive compounds in the musts, while grape is the one that provides the highest amount of sugars.

During fermentation, the concentration of anthocyanins increased in all the wines obtained, being those macerated with dried blueberries those that multiplied their concentration between 4 and 5.5 times, so that the drying of this fruit is the one that contributes the reddest color to the wine. Wines obtained by yeast inoculation extracted the most bioactive compounds, even though the maceration time was shorter. However, a longer fermentation time may cause the extracted phenolic compounds to be adsorbed by the yeasts in the autolysis process.

The sensory analyses carried out on the wines obtained show that, in all cases, they are accepted by consumers in terms of color, flavor and aroma, with the wines obtained by inoculation with M05 Mead being the most highly valued.

Therefore, the use of red grapes to produce blueberry red wine was appropriate because they provided sugars and higher yields of must. In addition, the use of dried fruit increased the content of fermentable sugars to a greater extent and maceration with the solid parts, particularly dried blueberries, increased the content of bioactive compounds and, consequently, the antioxidant capacity of the red fruit wines obtained.

Author Contributions: Conceptualization, J.M-G., T.G-M, J.M. and M.P.S.; methodology, J.M-G. and M.A.V.; investigation, J.M-G., T.G-M, M.A.V., J.M. and M.P.S.; resources, T.G-M, J.M. and M.P.S.; data curation, J.M-G., T.G-M, J.M. and M.P.S.; writing—original draft preparation, J.M-G., J.M. and M.P.S.; writing—review and editing, J.M-G., J.M. and M.P.S.; visualization, J.M. and M.P.S.; supervision, J.M. and M.P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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