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Chemistry

THE IMPACT OF AGING ON THE ANTIOXIDANT PROPERTIES OF ARMENIAN GRAPE BRANDIES

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The antioxidant (AH) properties (antioxidant activity, effective concentration, stoichiometry, and the number of reduced DPPH$^•$**)** of Armenian grape brandy depending on the aging period and radical scavenging kinetics were studied using DPPH assay and the steady-state kinetics method. The relationship between the antioxidant capacity and total polyphenol content was evaluated. The pseudo-first-order rate constant, which describes the main reaction between antioxidant and DPPH$^•$, and the second-order rate constant were determined. It was shown that the AH properties and radical scavenging rate of Armenian grape brandy depend on the aging period noticeably for brandies aged for 3–7 years, subsequent aging shows a mild effect.

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Keywords**:** antioxidant, aging, brandy, radical scavenging.

Introduction. In the human body, free radicals and antioxidants, compounds that scavenge or inhibit the formation of free radicals and cellular oxidative damage, are permanently formed during cellular metabolism. The equilibrium between antioxidants and radicals could be disturbed due to internal/external reasons, leading to a state of oxidative stress and, as a result, cellular biomolecules (DNA, proteins, and lipids) could be damaged and even die [1]. Normally, the human body has defense mechanisms to neutralize the formed free radicals. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition [2, 3]. The majority of natural antioxidants are phenolic compounds, and the most important groups of natural antioxidants are the tocopherols, flavonoids, and phenolic acids. Phenolic compounds belong to the class of chain breaking antioxidants that readily accept an electron from or donate an electron to another molecule, preventing the further propagation of the chain reaction. The phenolic compounds found in brandy appear to have no impact *in vivo* on blood pressure, decrease the risk of cardiovascular (it could decrease the infarct size after an ischemia-reperfusion), neurodegenerative diseases and cancer mortality, insulin resistance, type 2 diabetes, obesity, and atherosclerosis [4–8]. During aging in wooden barrels, cognac (brandy) undergoes a number of physical,

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chemical and biochemical reactions, which have a great effect on their characteristics (color, astringency, content of antioxidants) and quality [9, 10]. Grapes generate relatively large amounts of antioxidant compounds while growing. Many studies have categorized the phenolic compounds present in most of the common grape varieties [11]. As mentioned in [12], according to legislation, grape brandy can be characterized by the geographical area where the grapes were produced, the grape variety used and the selected distillation and aging techniques. In this article, we present the results of our studies on impact of aging on the antioxidant activity of Armenian grape brandies.

Materials and Methods. Factory samples of brandy spirits aged for 3, 5, 7 and 10 years were used. All chemicals used were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) of 98% purity was purchased from "Sigma-Aldrich" (USA) and used without further purification. All experiments and measurements were carried out in triplicate and data were analyzed using OriginPro 8.5 and Excel 2010 software.

Determination of the Antioxidant Activity of Brandy (Spirit) by DPPH Analysis. The $DPPH^{\bullet}$ scavenging activity of the alcoholic solutions was monitored according to the method described by [12]. To determine the antioxidant (antiradical) activity (*Rs*, %) of brandy, an alcoholic solution was mixed with an ethanolic solution of $DPPH^{\bullet}$ and kept at room temperature for 30 *min*. The decrease in absorbance at λ=515 *nm* was monitored, caused by the color change from purple to yellow. Electronic absorption spectra were registered on spectrophotometer Specord 50PC (Germany). The $DPPH^{\bullet}$ scavenging activity was calculated using Eq. 1:

$$Rs = \frac{A_0 - A_s}{A_0} \cdot 100\ \%, \quad (1)$$

where A_0 is the absorbance of the sample in the absence, and A_S in the presence of alcoholic solution respectively.

Determination of Effective Concentration, the Stoichiometry of the Reaction and Number of Reduced $DPPH^{\bullet}$ Radicals. Effective concentration of the antioxidant needed to scavenge 50% of the $DPPH^{\bullet}$ (EC_{50}) was calculated from the regression analysis of the response curve of *Rs* as a function of the antioxidant concentration. The stoichiometry of the reaction (EC_{100}), which is the theoretically efficient concentration of the antioxidant needed to reduce 100% of the $DPPH^{\bullet}$ and the number of $DPPH^{\bullet}$ reduced by one molecule of antioxidant (*n*), were calculated from EC_{50} values according to Eqs. 2 and 3 [12, 13]

$$\mathrm{EC}_{100} = 2\ \mathrm{EC}_{50}\,, \quad (2)$$

and *n*, according to

$$n = \frac{[\mathrm{DPPH}^{\bullet}]_0}{2} \cdot \mathrm{EC}_{50}\,. \quad (3)$$

Kinetic Studies by the Steady-State Method. The steady-state method was used to monitor the reaction rate for 1/40, 1/160, 1/320, 1/480 1/640 and 1/920 diluted (v/v) brandy samples. The decrease in $DPPH^{\bullet}$ absorbance at 515 *nm* was monitored for 70 *min* at 25.0°C [14]. In fact, these reactions are second-order reactions, but at given conditions when the concentration of the antioxidant is very small compared to the radical, they were treated as pseudo-first-order reactions.

Results and Discussion.

Radical-Scavenging Activity. The total amount of antioxidants (tannins) for 3, 5, 7 and 10 years aged brandies was determined according to [15]. The total amount of tannins and the results obtained on radical scavenging properties are presented in Tab. 1.

Table 1

The total amount of tannins and antioxidant characteristics of brandies aged for different periods

Aging period, year	Total amount of AH (tannins), *g/L*	EC_{50}	EC_{100}	*n*
3	0.14	0.00165± (0.00002)	0.00330 (± 0.00002)	10.73
5	0.17	0.00120 (± 0.00002)	0.00240 (± 0.00002)	7.80
7	0.22	0.00103 (± 0.00001)	0.00206 (± 0.00001)	6.69
10	0.26	0.00077 (± 0.00001)	0.00154 (± 0.00001)	5.01

As follows from the data, with an increase in brandy aging period, the total amount of tannins increases and the antioxidant characteristics are improved.

Kinetic Studies. Antioxidant activity deals with reaction rate. The mechanisms of radical scavenging by polyphenols can be considered as direct scavenging of free radicals by hydrogen atom transfer (HAT) or single electron transfer (SET) mechanisms and as transition metal chelation (TMC) mechanism [16]. HAT mechanism, which occurs in the presence of polyphenols, is based on reaction 1, where $DPPH^{\bullet}$ leads to hydrogen atom delivery from the antioxidant (AH) to the radical, thus interrupting the oxidative chain reaction described.

$$DPPH^{\bullet} + AH \rightleftarrows DPPH-H + \boldsymbol{A}^{\bullet} \quad \text{(reaction 1)}.$$

Possible side reactions can occur between less reactive $\boldsymbol{A}^{\bullet}$ and another $DPPH^{\bullet}$ or $\boldsymbol{A}^{\bullet}$ according to reactions 2 and 3:

$$\boldsymbol{A}^{\bullet} + DPPH^{\bullet} \rightarrow \text{products} \quad \text{(reaction 2)},$$
$$\boldsymbol{A}^{\bullet} + \boldsymbol{A}^{\bullet} \rightarrow A-A \quad \text{(reaction 3)}.$$

The kinetics of $DPPH^{\bullet}$ scavenging reaction by brandies aged for 3–10 years was studied at 25°C. Kinetic curves of diluted brandies aged for 3 and 10 years are presented in Figs. 1 and 2.

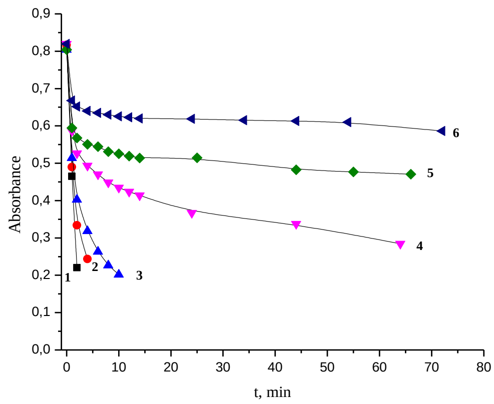

Fig. 1. Kinetic curves of $DPPH^{\bullet}$ scavenging by brandy aged for 3 years. Brandy dilution, v/v: 1) 1/40; 2) 1/160; 3) 1/320; 4) 1/480; 5) 1/640; 6) 1/920. $[DPPH^{\bullet}] = 6.5 \cdot 10^{-5}$ *mol/L*, $T = 25$°C.

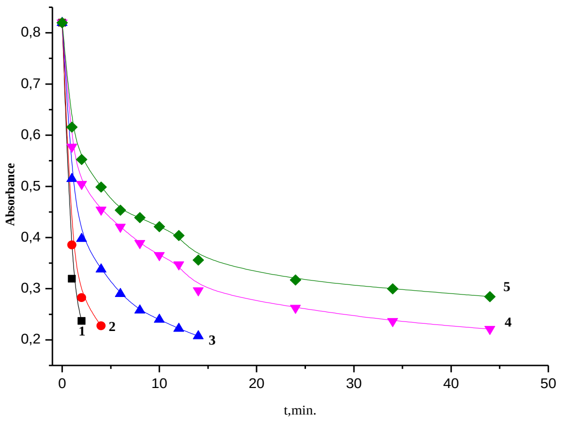

Fig. 2. Kinetic curves of DPPH$^\bullet$ scavenging by brandy aged for 10 years. Brandy dilution, v/v: 1) 1/40; 2) 1/160; 3) 1/320; 4) 1/480; 5) 1/640. [DPPH$^\bullet$]=6.5·10^{-5} *mol/L*, T = 25°C.

The rate of these reactions practically depends on the concentration of the antioxidants, and the reaction proceeds from a few minutes to approximately an hour. The absorbance quickly decays over 1–3 *min* as a result of the most labile H atoms transfer (fast step). This is followed by a much slower decrease in absorbance, which could be featured to other processes (slow step). Different kinetic models are used for analyzing the H-atom-transfer between DPPH$^\bullet$ and a given antioxidant during the fast step [17]. The simplest model makes no hypothesis about the mechanism of antioxidant degradation. Hence, the reaction rate is described by Eq. (4)

$$V = -\frac{d[\mathrm{AH}]}{dt} = -\frac{d[\mathrm{DPPH}^\bullet]}{dt} = k_2[\mathrm{AH}][\mathrm{DPPH}^\bullet], \quad (4)$$

where k_2 is the second-order rate constant. Since H-atom-transfer is the fast step reaction, it follows pseudo-first-order kinetics as shown in Eq. (5):

$$\ln[\mathrm{DPPH}^\bullet]_t = \ln\,[\mathrm{DPPH}^\bullet]_0 - k_{obs}\,t\ , \quad (5)$$

where $[\mathrm{DPPH}^\bullet]_0$ is the radical concentration at t = 0; $[\mathrm{DPPH}^\bullet]_t$ is the radical concentration at time t, and k_{obs} is the pseudo-first-order rate constant. Using Eq. (5), k_{obs} were calculated and from the linear plot of k_{obs} versus the concentration of the antioxidant k_2 were determined, and the rate of the reaction is expressed as

$$\frac{d[\mathrm{DPPH}^\bullet]}{dt} = -k_{obs}\,[\mathrm{DPPH}^\bullet]_t = -k_2[\mathrm{AH}][\mathrm{DPPH}^\bullet]_t. \quad (6)$$

Obtained results are presented in Tabs. 2 and 3.

Table 2

Values of pseudo-first-order reaction rate constant at different brandy dilution

Brandy dilution ×10^3, v/v	Period of aging, year			
	3	5	7	10
	Rate constant k_{obs}·10^4, s^{-1}			
1.09	1.58	2.55	4.11	5.45
1.56	2.69	3.87	5.54	6.68
2.08	4.54	4.89	7.63	7.98

Table 3

Values of second-order reaction rate constants at different period of brandy aging

Period of aging, year	k_2, $M^{-1} \cdot s^{-1}$	Correlation coefficient, R
3	0.195 ± 0.015	0.99087
5	0.240 ± 0.004	0.99968
7	0.367 ± 0.007	0.99943
10	0.417 ± 0.027	0.99364

As can be seen from the data presented in Tabs. 2 and 3, with the increase in brandy aging period, the rate constants of pseudo-first-order and second-order reactions increase approximately three and two times, respectively. Moreover, the difference between the rate constants of seven and ten years aged brandies decreases, indicating that further aging may already have little effect on brandy antioxidant properties.

Conclusion. The antioxidant properties (antioxidant activity, effective concentration, stoichiometry, and the number of reduced $DPPH^{\bullet}$) of Armenian grape brandy depending on the aging period and radical scavenging kinetics show that the aging period of 3–10 years noticeably affects the brandy antioxidant properties, subsequent aging shows a mild effect.

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ՀՆԱՑՄԱՆ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ՀԱՅԿԱԿԱՆ ԽԱՂՈՂԻ ԿՈՆՅԱԿՆԵՐԻ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԻ ՎՐԱ

Ուսումնասիրվել է հայկական խաղողից պատրաստված կոնյակի հակաօքսիդանտային հատկությունները (հակաօքսիդանտային ակտիվություն, արդյունավետ կոնցենտրացիա, ստեխիոմետրիա և որսված DPPH˙ թիվ) և ռադիկալների որսման կինետիկան՝ կախված հնացման ժամանակամիջոցից

DPPH˙ անալիզի և ստացիոնար վիճակի կինետիկական մեթոդի օգտագործմամբ: Գնահատվել է կոնյակների հակաօքսիդանտային ունակության և ընդհանուր պոլիֆենոլների պարունակության միջև կապը: Որոշվել են կեղծ առաջին կարգի ռեակցիայի արագության հաստատունը, որը նկարագրում է հակաօքսիդանտի և DPPH˙-ի միջև հիմնական ռեակցիան, և երկրորդ կարգի ռեակցիայի արագության հաստատունը: Ցույց է տրվել, որ հայկական խաղողից պատրաստված կոնյակի հակաօքսիդանտային հատկությունները և ռադիկալների որսման արագությունը կախված են հնացման ժամանակամիջոցից. նկատելիորեն փոխվում է 3–7 տարի հնացված կոնյակների համար, հետագա հնացումը ունի արդեն մեղմ ազդեցություն:

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ВЛИЯНИЕ ВЫДЕРЖКИ НА АНТИОКСИДАНТНЫЕ СВОЙСТВА АРМЯНСКИХ ВИНОГРАДНЫХ БРЕНДИ

Методами DPPH˙-анализа и кинетики стационарного состояния изучены антиоксидантные (АО) свойства (антиоксидантная активность, эффективная концентрация, стехиометрия и количество восстановленных DPPH˙**)** и кинетика поглощения радикалов для бренди с различным временем выдержки, изготовленных из армянского винограда. Оценена взаимосвязь между АО свойствами и общим содержанием полифенолов бренди. Определены константа скорости реакции псевдопервого порядка, описывающая основную реакцию между АО и DPPH˙ и константа скорости реакции второго порядка. Показано, что АО свойства и скорость поглощения радикалов зависят от срока выдержки армянского виноградного бренди: выдержка 3–7 лет влияет значительно, а последующая выдержка оказывает незначительный эффект.