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THE EFFECT OF GRAPE SEED OIL FORTIFICATION WITH EXTRACTS OF NATURAL ANTIOXIDANTS

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Abstract. Grape seed oil (GSO) consists mostly of triglycerides of polyunsaturated fatty acids, which are sensitive to oxidation and to the high temperatures. The aim of this research was to study the effect of GSO fortification with hydroalcoholic extracts of antioxidants from grape seeds, up to and after 12 months. It was established that the immediate effect of the oil fortification was the increase of the total polyphenol content (TPC) in the oil, by up to 141.34 mg GAE/kg of oil, and the increase of the DPPH• antioxidant activity by up to 0.580 mmol TE/kg (in the control sample, refined grape oil, these values were 1.57 mg GAE/kg and 0.003 mmol TE/kg, respectively). After 12 months, TPC and DPPH• antioxidant activity decreased nearly 10-fold in all of the fortified oil samples. The difference between the oxidative status of the oil was assessed (UV-Vis). In the case of the GSO sample, treated with *n*-octyl gallate, the absorbances remained unchanged after 12 months. When compared to the synthetic antioxidant *n*-octyl gallate, grape seed polyphenolic extracts were found to have a less protective effect on the oil during storage under environmental conditions.

Keywords: *antioxidant activity, extract of polyphenols*, *grape seed*, *octyl gallate, oil, UV-Vis*.

Rezumat. Uleiul din semințe de struguri conține în mare parte din trigliceride ale acizilor grași polinesaturați, sensibile la oxidare și la acțiunea temperaturilor înalte. Scopul cercetărilor a fost de studia efectul fortificării uleiului cu extracte hidroalcoolice de antioxidanți din semințe de struguri. Fortificarea uleiului are ca efect imediat creșterea conținutului total de polifenoli (TPC) cu până la 141.34 mg GAE/kg de ulei și a activității antioxidante DPPH• cu până la 0.580 mmol TE/kg (în proba martor, ulei de struguri rafinat, aceste valori erau de 1.57 mg GAE/kg și 0.003 mmol TE/kg, respectiv). După 12 luni, TPC și activitatea antioxidantă DPPH• au scăzut aproape de 10 ori în toate probele de ulei fortificate. A fost observată diferența dintre starea oxidativă a uleiului (UV-Vis): în cazul probei GSO tratată cu *n*-octil galat, absorbanțele au rămas neschimbate după 12 luni. Extractul de polifenoli din semințe

de struguri are un efect protector mai redus asupra uleiului pe perioada depozitării, în condiții ambientale, comparativ cu antioxidantul sintetic *n*-octil galat.

Cuvinte cheie: *activitate antioxidantă, extract de polifenoli, octil galat, semințe de struguri, ulei, UV-Vis*.

1. Introduction

Grape seed oil is mainly composed of unsaturated fatty acids, which account for 85-90% of the total fatty acid content. Of these, linoleic acid (C18:2 *ω*-7) accounts for up to 70% [1, 2]. The glycerides composed from polyunsaturated fatty acids (PUFA) have a short shelf life as they are very sensitive to oxidation and to high temperatures [3]. Depending on the storage conditions, they can undergo oxidative degradation, hydrolysis, polymerization, etc. with the formation of unwanted products, the concentration of which increases with the period of oil storage [4]. Research shows that oil with an increased content of antioxidants, such as tocopherols, carotenes, and phenolic compounds, is more stable to oxidative processes and has a longer shelf life [5, 6].

The phenolic compounds in edible oils have several health benefits, being responsible for capturing free radicals and for antibacterial, antiviral, anti-inflammatory, antitumor, cardioprotective, neuroprotective and antidiabetic properties [7-11], nevertheless, grape seed oil contains a very low concentration of the aforementioned compounds. Maier et al. [12] determined a very low quantity of total polyphenols (TPC), of only 2.90 mg GAE/kg (which represents 0.0003%), in cold pressed grape seed oil; the content of catechins and epicatechins reached 1.30 mg/kg, and *trans*-resveratrol was of 0.30 mg/kg. Heat treatments and rigorous purification considerably reduce the content of antioxidants in the oil. At the same time, the amount of total extractable polyphenols, in the hydrophilic extracts of grape seeds, differs depending on the variety, ranging between 42.9 and 114.8 g per kilogram of dry seeds [13-15].

Grape seed oil also includes approximately 0.8-1.5% of compounds that do not undergo saponification - tocopherols and sterols (campesterol, beta-sitosterol, sigmasterol). The content of vitamin E, another important antioxidant, is between 1 and 53 mg per 100 g of oil [17]. In the grape oil extracted by the Soxhlet and Folch methods, researchers determined the following compounds: gallic acid 3.70 and 14.02 mg/kg; caffeic acid 5.20 and 8.42 mg/kg; ellagic acid 1.50 and 3.50 mg/kg, respectively, as well as chlorogenic, 4-hydroxybenzoic, 3,5-dihydroxybenzoic, protocatechuic, genistic acids, etc. [18].

Nowadays, the following synthetic antioxidants are used to stabilize vegetable oils: butylated hydroxytoluene, butylated hydroxyanisole, tertiary butylhydroquinone, *α*-tocopherol, propyl gallate, *n*-octyl gallate (OG), etc. [19]. Concurrently, some synthetic antioxidants have been banned, as their negative effect on the human body has been demonstrated [20, 21]. In the last decades, to meet the demands of the modern consumer, research is being focused on replacing synthetic antioxidants with harmless, natural additives [22], such as tocopherols, polyphenols, carotenoids, pigments, etc., which are found in plants, in agro-industrial waste and can be extracted by different methods.

It was reported that the fortification of oils with hydrophilic and lipophilic antioxidants, of plant origin, has an oxidative stabilization effect, similar to that exhibited by synthetic antioxidants. Roschel et al. [23] proved that the hydrophilic mixture of sinapic, ascorbic, and citric acids, was more effective in stabilizing oxidatively the linseed oil, compared to the lipophilic mixture of tocopherol, ascorbyl palmitate, and citric acid. The hydrophilic mixture introduced into the oil had an effect comparable to the one of tertiary

butyl hydroquinone (TBHQ), in preventing the formation of primary and secondary oxidation products. Mohanan et al. [24] had also determined the effect of natural antioxidants, of various polarity (tannic acid, tocopherol and ascorbyl palmitate), on the improvement of the linseed oil stability during long-term storage. The antioxidant protection of the ascorbyl palmitate was comparable to TBHQ, while tannic acid did not prevent lipid oxidation. Gülmez et al. [25] assessed the oxidative stability of hazelnut oil treated with natural gallic acid or *β*-carotene. In the dark, for 80 days under environmental conditions, the gallic acid increased the shelf life of the enriched oil nearly 3 times, compared to the untreated sample. Moreover, TPC increased by 77%. Manzano et al. [26] fortified soybean oil with polyphenols (0.02% and 0.04% *w/w*) extracted from *Theobroma cacao* and determined that only higher concentrations (0.04%) of phenolic compounds inhibited the generation of free fatty acids and oxidation products. Oil fortification increased 20 times the antioxidant activity against the DPPH radical (2,2-diphenyl-1-picrylhydrazyl), after frying.

Other authors have observed that the antioxidant activity of extracts from *Rosa woodsii* fruits, added to canola oil, depends on the concentration of polyphenols. Adding 200 µ*g* GAE/g to the oil has a marginal efficiency, but at a higher concentration, of 500 µ*g* GAE/g (or 0.5%), the antioxidant activity is significantly greater, compared to butylated hydroxytoluene, under the rancimat conditions and during frying. Probably, the higher temperatures encourages a better distribution of the polyphenols in the oil, allowing all the phenolic components in the extract to display their antioxidant properties. The authors determined that the protective effect of phenolic antioxidants is not significant during the storage period [27]. However, research did not mention what quantity of hydrophilic antioxidants can be delivered from vegetable matter or from extracts of polyphenols in the hydrophobic phase.

The purpose of this research was to dose spectrophotometrically the total content of polyphenols in grape seed oil fortified with hydroalcoholic extracts of grape seeds, at the initial stage and after 12 months; to elucidate the influence of polyphenols on the antioxidant activity of the oil, and to observe the difference in the oxidative status of the oil according to absorbances in UV-Vis before and after storage.

2. Materials and Methods

2.1. Materials

Commercial refined grape seed oil, produced on 12.06.2020, valid until 12.06.2021.

Folin-Ciocalteu phenol reagent was provided by Chem-Lab NV (Belgium). The 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) were provided by Alpha Aesar (U.S.A.). Gallic acid (GA) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.); *n*-octyl gallate (OG) from Alpha Aesar (Germany). Ethanol, methanol, *n*-hexane, sodium carbonate, and chloroform purchased from Chemapol (Czech Republic). All reagents were of analytical or chromatographic grade.

2.2. Methods

2.2.1. Extraction of antioxidants from grape seeds

The hydroalcoholic extract of native grape seeds (GSE) was obtained in the following way: 50 g of grape seeds, separated from the fermented pomace and dried at 70°C for 2 hours (humidity 6.63±0.02%), were crushed and macerated at room temperature for 24 hours in 65% aqueous ethyl alcohol solution, with a liquid:solid ratio (LSR) of 5 (*v/m*). The macerated mixture was subjected to ultrasound-assisted extraction (ISOLAB Laborgeräte GmbH), for 30 min, at a temperature of 40°C (37 kHz). The next steps consisted of centrifugation (5000 rpm

for 10 min), collection of the supernatant, vacuum evaporation of the solvent at a temperature of 60°C, and drying of the residue in a vacuum oven at 60°C. As a result, 3.985 g of GSE, with a greasy appearance, were obtained.

The hydroalcoholic extract of defatted grape seeds (DGSE) was obtained as follows: 50 g of seeds, dried and crushed, were extracted with *n*-hexane, LSR of 3 (*v/m*), whilst heated on a water bath at 50°C, for 60 min. The mixture was filtered through filter paper, the extraction was performed in two repetitions. The degreased sample was further extracted with hydroalcoholic solution, according to the procedure described above. As a result, 2.792 g of DGSE were obtained. The total polyphenol content of GSE and DGSE, was determined (Table 1).

2.2.2. Sample preparation

Six samples of refined GSO were weighed (100 g). Grape seed antioxidant extracts were added to four oil samples. The GSE and DGSE extracts in dry form and in dissolved form (GSE_{EtOH}, $DGSE_{EtOH}$) in 96% ethyl alcohol, were added, with the theoretically calculated concentration of 4 g GAE/kg of oil (or 0.4% TPC in the oil sample). For a better distribution of the biologically active substances from the extracts in the oil, the ultrasound-assisted method (ISOLAB Laborgeräte GmbH, Germany) was applied (30 min, 50°C, 37 kHz); followed by centrifugation (5000 rpm for 10 min) and sediment separation. Ethyl alcohol was distilled under vacuum at a temperature of 60°C. Refined grape seed oil (GSO) and oil with addition of synthetic antioxidant *n*-octyl gallate (GSO+OG), in a concentration of 200 mg GAE/kg of oil (or 0.02% in the oil sample), served as reference samples.

In June of 2021, the oil samples were scanned with a DR 5000 spectrophotometer (HACH- Lange GmbH, USA-Germany), the TPC was dosed and the DPPH• antioxidant activity was determined. The samples were stored at room temperature, in a cupboard (without access to light), in polystyrene containers, closed with plugs. After 12 months of storage in ambiental conditions (June of 2022), the same determinations were made.

2.2.3. Extraction of the polyphenols from oil

The hydrophilic fractions were obtained with 1.5 mL of aqueous solution of 60% MeOH from the 1.25 g of oil dissolved in 2.5 mL of *n*-hexane [28]. Hydrophilic and hydrophobic phases were separated by centrifugation (3500 rpm for 10 min). The procedure was repeated twice. The methanolic extracts were combined and analyzed.

2.2.4. UV-Vis spectroscopic analysis of oil samples

The absorbances of the oil samples (2% of oil in hexane) were recorded on a DR5000 spectrophotometer (HACH- Lange GmbH, USA-Germany).

2.2.5. Determination of the total content of polyphenols (TPC)

The TPC was dosed by the Folin-Ciocalteu method [29, 30] according to the calibration curve (0-500 mg/L, R^2= 0.9977) of the gallic acid (GA) standard. The results were expressed in milligrams equivalent of GA per gram of grape seed extract dry weight (mg GAE/g DW) or per kilogram of oil (mg GAE/kg).

2.2.6. DPPH• Free Radical Scavenging Activity

The antioxidant activity of the hydrophilic fractions was determined according to the procedure [31]. The calibration curve was made with Trolox in various concentrations (3.95 - 500 µmol/L). The absorbance of the solutions was measured at 515 nm, using the spectrophotometer mentioned above, the results were expressed in millimoles Trolox

equivalent per kilogram of oil (mmol TE/kg). DPPH• radical scavenging ability was calculated using formula (1):

$$DPPH \bullet \ (\%\ inhibition) = \frac{(A_0 - A_1)}{A_0} \cdot 100\ \% \qquad (1)$$

where A_0 is the absorbance of the control sample and A_1 is the absorbance of all extract samples and reference samples. All assays were performed in triplicate and results were averaged.

2.2.7. Statistical processing of experimental data

The statistical analysis of the results was carried out with the application of IBM SPSS Statistics 23 and Microsoft Excel 2010 programs. The level of significance of the results was 95% (q<0.05). To exclude results with accidental errors and those with high levels of uncertainty, three parallel measurements were performed [32].

3. Results and Discussion

In this study, attempts were made to fortify refined grape seed oil (GSO) with extracts from native grape seeds (GSE) and defatted grape seeds (DGSE), separated from fermented pomace. Research has shown that hydrophilic and lipophilic extracts of grape seeds are rich in phenolic antioxidants [33], with valuable potential for different fields [34]. Hydroalcoholic extracts from grape seeds contain polyphenolic compounds mostly in the form of monomers - gallic acid, catechin, epicatechin; in the form of oligomeric proanthocyanidins (PAC) - dimers, trimers and tetramers of catechin, epicatechin, epigallocatechin, and epicatechin gallate [16, 35]. The extraction of grape seeds was carried out with an 65% aqueous solution of ethyl alcohol, considered a harmless solvent for obtaining nutraceuticals [36]. In both dry extracts, GDE and DGSE, the total content of polyphenols (TPC) was measured spectrophotometrically by the methods described in the literature, Table 1 [29, 30]. Hydroalcoholic extracts of native GSE seeds have a lower TPC due to the lipophilic components contained. The results show that after defatting seeds with *n*-hexane, the second extraction, with hydrophilic solvent, solubilizes better the phenolic compounds from the solid matrix of the degreased seeds, and the TPC reaches 978.37 mg GAE/g DW in the DGSE extract.

Table 1

Mass fraction of hydroalcoholic grape seed extracts and their total polyphenol content

Extracts	*GSE*	*DGSE*
The mass fraction of the extract, % DW seeds	7.97±0.68	5.58±0.47
TPC, mg GAE/g DW extract	516.43±11.15	978.37±23.36

Note: GSE - grape seeds extract; DGSE - defatted grape seeds extract; DW - dry weight; TPC - total polyphenol content. Values represent the means of three replicates ± standard deviation.

The physicochemical properties of the refined grape seed oil (GSO) were within the data presented in the bibliography [37, 38]. The refractive index of the oil was determined (1.4794, 25°C) at ABBE Refractometer (ISOLAB Laborgeräte, Germany); the relative density (0.921 kg/dm^3); the acidity index (0.85 mg KOH/g of oil) and the peroxide value (2.44 mEqO_2/kg) were determined according to the known methods [39].

The experimental samples were named as follows: GSO - control sample, refined grape seed oil; GSO+OG - oil fortified with *n*-octyl gallate; GSO+GSE oil fortified with dry grape seed extract; GSO+GSE_{EtOH} - fortified oil with grape seed extract solubilized in ethyl alcohol;

GSO+DGSE - fortified oil with dry extract of defatted grape seeds; GSO+DGSE$_{EtOH}$ - fortified oil with defatted grape seed extract, solubilized in ethyl alcohol. The amount of GSE was calculated to obtain the theoretical concentration of 0.4% polyphenols in oil (or 4 g GAE/kg of oil). The addition of GSE did not change the colour of the oil.

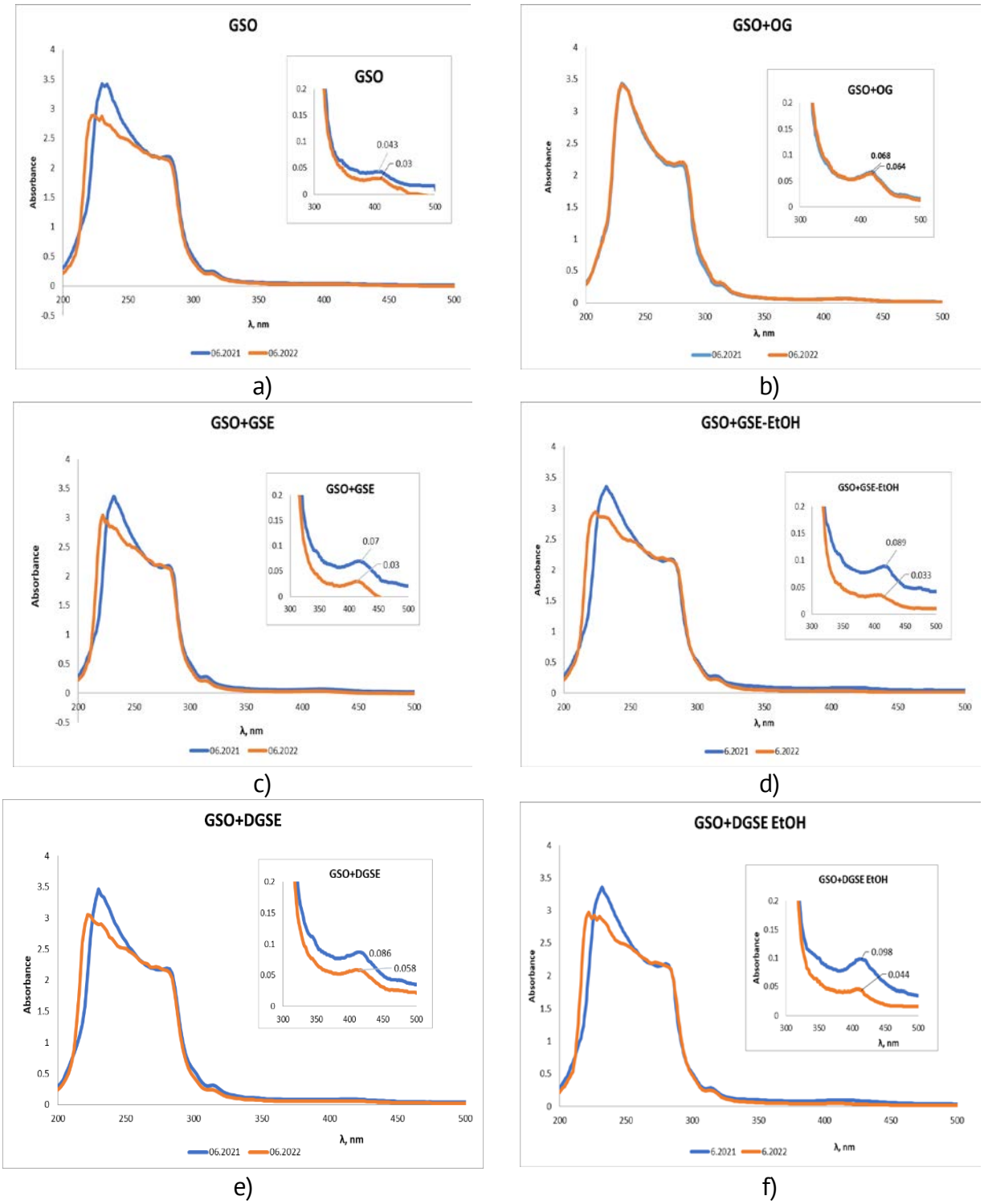

Figure 1. UV-Vis spectra of oil samples:
a) grape seed oil; b) oil fortified with n-octyl gallate; c) oil fortified with dry extract of grape seed oil; d) oil fortified with grape seed extract solubilized in ethyl alcohol; e) oil fortified with dry extract of defatted grape seeds; f) oil fortified with defatted grape seed extract solubilized in ethyl alcohol.

The UV-Vis spectrum was recorded before and after 12 months of storage for all of the fortified oil samples. In the refined GSO control sample, after storage, the absorbances decreased the most in the 200-250 nm wavelength ranges, due to the reduction in the concentration of triglycerides, free fatty acids and the formation of some secondary oxidation products in the oil (Figure 1).

Vegetable oils with a high content of polyunsaturated acids, at the end of one year of storage in dark, environmental conditions, accumulate secondary oxidation products - alkenals with *cis*- or *trans*- configurations at the double bonds, epoxides, ketones, hydrocarbons, dimers and polymers of PUFA, and triglycerides [40, 41].

The best oxidative stabilization and preservation effect of grape seed oil was recorded for synthetic antioxidant *n*-octyl gallate. The UV-Vis absorbances of the GSO+OG sample after 12 months of storage in polystyrene containers, in dark, environmental conditions, remained unchanged (Figure 1, b). The decrease of the UV-Vis absorbance, in the 280-450 nm range, in all samples except for GSO+OG, is a consequence of the decrease in the concentration of biologically active substances, such as phenolic compounds, carotenoids, tocopherols, phytosterols, *etc*.

In the case of the fortified samples, the absorbance values in the 200-500 nm range show that the grape seed extracts have a weak protective effect on the oil during storage under environmental conditions (Figure 1, a, c - f). After 12 months, in all samples of oil fortified with grape seed extracts, the hydrophilic phenolic compounds formed a sediment. Mentioned by other authors as well, it suggests a more complex interaction between the hydrophilic phenolic compounds used for fortification during the storage period. Research has shown that the antioxidant effect of the polyphenols manifests itself at higher temperatures, under the rancimat conditions and while frying oil [26,27].

By the spectrophotometric method, polyphenols (TPC) were dosed in the hydrophilic fractions of the oil samples before and after the 12 months of storage. As seen in Table 2, before the storage, the GSO control sample has an insignificant TPC of 1.57 mg GAE/kg of oil, due to the refining processes applied during production.

The bibliographic study shows that the TPC in unrefined grape seed oil is of 2.9 mg GAE/kg [12]. Other research has shown that TPC in cold-pressed grape seed oil ranged from 48 to 153 mg GAE/kg of oil [15].

Table 2

Total content of polyphenol (TPC) in oil samples

Sample	TPC (06.2021) mg GAE/kg	TPC (06.2022) mg GAE/kg
GSO	1.57 ± 0.12	0.05 ± 0.01
GSO+OG	222.30 ± 3.64	117.94 ± 2.20
GSO+GSE	38.28 ± 0.95	4.10 ± 0.17
GSO+GSE$_{EtOH}$	127.42 ± 0.98	10.32 ± 0.56
GSO+DGSE	56.86 ± 1.35	6.54 ± 0.45
GSO+DGSE$_{EtOH}$	141.34 ± 1.06	19.67 ± 0.85

Note: GSO - grape seed oil; GSO+OG - oil fortified with n-octyl gallate; GSO+GSE - oil fortified with dry extract of grape seed oil; GSO+GSE$_{EtOH}$ - oil fortified with grape seed extract solubilized in ethyl alcohol; GSO+DGSE - oil fortified with dry extract of defatted grape seeds; GSO+DGSE$_{EtOH}$ - oil fortified with defatted grape seed extract solubilized in ethyl alcohol. Values represent the means of three replicates ± standard deviation.

In the fortified oil samples, GSO+GSE and GSO+DGSE, the initial dosed total polyphenol content, is of 38.2 and 56.8 mg GAE/kg of oil. Although the amount of introduced antioxidants was theoretically calculated to obtain a TPC of 4.0 g GAE/kg oil, a TPC almost 100 times lower was dosed spectrophotometrically in the fortified oil (Table 2). Almost 1.5% of the introduced polyphenols were incorporated into the oil, which represents a very small amount.

The synthetic antioxidant *n*-octyl gallate (*n*-octyl 3,4,5-trihydroxybenzoate, E 311) was added to GSO in an amount of 200 mg GAE/kg, as per The European Food Safety Authority's accepted norms (200-300 mg GAE/kg of oil) [42]. By the spectrophotometric method, a TPC of 222.3 mg GAE/kg was dosed in the GSO+OG sample, which, being fat-soluble, was completely incorporated into the oil, unlike the polar polyphenolic compounds from the grape seed extracts (Table 2).

The solubilization of GSE and DGSE extracts in ethyl alcohol aids the better distribution of polar compounds in the lipophilic phase. The samples of GSO+GSE_{EtOH} and GSO+$DGSE_{EtOH}$ have a higher TPC, of 127.4 and 141.3 mg GAE/kg of oil, compared to the GSO+GSE and GSO+DGSE samples (Table 2). Calculations show that 3.5% of phenolic compounds, out of the 4.0 g GAE/kg initially introduced, were incorporated into the GSO+$DGSE_{EtOH}$ oil sample.

The spectrophotometrically dosed TPC [28-30] in the GSO samples, fortified with grape seed extracts, is lower compared to unrefined olive oil, which contains on average from 250 to 574 mg GAE/kg, depending on the method of obtaining [43]. The low values of TPC dosed in the fortified oil are due to the hydrophilic character of GSE and DGSE, which were extracted with hydroalcoholic solution of EtOH (65%) from grape seeds. These extracts contain phenolic compounds with increased polarity, more in the form of glycosides [16], which are delivered in a reduced amount in the hydrophobic oil.

The fortification of the oil with extracts of phenolic antioxidants has as an immediate effect the significant increase of the DPPH• scavenging effect, compared to the control sample. The highest antioxidant activity was determined for the GSO+OG sample, of 0.698 mmol TE/kg. In case of GSO+$DGSE_{EtOH}$, the DPPH• radical inhibition capacity of 0.580 mmol TE/kg is close to the antioxidant activity of *n*-octyl gallate, Table 3.

Table 3

Antioxidant activity of oil samples

Sample	DPPH• (06.2021)	DPPH• (06.2022)
	Concentration, mmol TE/kg	Concentration, mmol TE/kg
GSO	0.003 ± 0.001	n.d.
GSO+OG	0.698 ± 0.002	0.396 ± 0.003
GSO+GSE	0.078 ± 0.003	0.026 ± 0.001
GSO+GSE_{EtOH}	0.481 ± 0.004	0.047 ± 0.001
GSO+DGSE	0.295 ± 0.004	0.035 ± 0.001
GSO+$DGSE_{EtOH}$	0.580 ± 0.002	0.055 ± 0.001

Note: GSO - grape seed oil; GSO+OG - oil fortified with n-octyl gallate; GSO+GSE - oil fortified with dry extract of grape seed oil; GSO+GSE_{EtOH} - oil fortified with grape seed extract solubilized in ethyl alcohol; GSO+DGSE - oil fortified with dry extract of defatted grape seeds; GSO+$DGSE_{EtOH}$ - oil fortified with defatted grape seed extract solubilized in ethyl alcohol, n.d. - data not determined. Values represent the means of three replicates ± standard deviation.

The bibliographic study shows that the antioxidant capacity of the hydrophilic fractions in vegetable oils is lower compared to that of the lipophilic fractions. For example, the DPPH• antioxidant activity of the hydrophilic fraction of sesame oil is 0.50 mmol TE/kg, and that of the lipophilic fraction is 1.15 mmol TE/kg [44].

Manzano et al. [26] proved that the introduction of high concentrations (0.04%) of polyphenols in soybean oil delayed the release of fatty acids and the formation of peroxides; increased 20-fold the antioxidant activity against the DPPH• radical after roasting.

After 12 months of storage, TPC decreased in all of the fortified oil samples by approximately 10 times, except for GSO+OG, where TPC decreased nearly twice (Table 2).

The same effect, of significant reduction of DPPH• antioxidant activity, is observed in all of the samples (Table 3). The oil treated with OG, after storage under environmental conditions, exhibited a fairly good antioxidant activity (0.396 mmol TE/kg).

4. Conclusions

The effect of grape seed oil fortification with hydroalcoholic extracts of antioxidants from grape seeds (GSE), was studied up to and after 12 months. Research has shown that due to the hydrophilic nature of grape seed extracts, a small number of phenolic compounds have been incorporated into the oil. It was established that oil fortification with GSE has the immediate effect of increasing the total polyphenol content (TPC) in the oil by up to 141.34 mg GAE/kg and increasing the DPPH• antioxidant activity by up to 0.580 mmol TE/kg (in the control sample, grape oil refined, these values were 1.57 mg GAE/kg and 0.003 mmol TE/kg, respectively). After 12 months, TPC and DPPH• antioxidant activity decreased nearly 10-fold in all GSE-fortified oil samples. In the oil treated with *n*-octyl gallate, after 12 months, TPC values and antioxidant activity decreased twice. The difference between the oxidative status of the oil was observed according to the absorbances in UV-Vis; absorbances of the GSO+OG sample, up to and after 12 months, remained unchanged. It was determined that the extract of phenolic antioxidants from grape seeds had a lower protective effect on the oil during storage under ambient conditions compared to the synthetic antioxidant *n*-octyl gallate.

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

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