Antioxidant capacities and phenolic content of different alimentary supplements from grape or wines

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ABSTRACT

Introduction Grape and wine are very rich in polyphenols, so grape and wine extract have become a widespread nutritional supplement with a broad spectrum of pharmacological, anti-inflammatory, antimicrobial, and other antioxidant effects. The aim of this study was evaluate the phenolic content and the antioxidant properties of different commercial food supplements derived from grape or wines.

Methods: 14 commercial food supplements derived from grape or wine were evaluated, they were bought in pharmacies and para-pharmacies of Burgos (Spain). Depending on their origin they were classified into three groups: Group WG (whole grape *Vitis vinifera products*), Group SS (seed or skin *Vitis vinifera* products), Group OB (specific grape compound with other bioactive compounds).
Total polyphenols, anthocyanins and proanthocyanins were evaluated, so as antioxidant capacity by ABTS and DPPH methods.

Results: Results indicate that the antioxidant capacity of products elaborated with grape extract was higher than those of the product obtained from seeds, skins or from specific grape compound added with other bioactive compounds as Q10 or omega-3. Significant positively direct correlation among phenolic compound levels and antioxidant capacity of the supplements were found.

Keywords: Grape extract**,** Food suplements**,** Polyphenols, Antioxidant capacity.

1. INTRODUCTION

Since the middle of last century, a large number of studies focused on the possible beneficial health effects of wine intake have been carried out (Farre, 1995, Fuchs et al. 1995, De la Torre, 1997; Thun et al., 1997, and Giacosa et al., 2012, among others). Some of these studies suggested that dietary intake of antioxidant wine and grapes supplements, so as other antioxidant sources, may be a useful strategy to reduce the incidence of diseases associated with oxidative stress, such as cancer, atherosclerosis and neurodegenerative diseases.

The wine-health relationship has been extended from wine to wine by-products and products derived from both of them. So, in the last years, a lot grapes and wine functional products have been developed, and they have been applied in pharmaceutical, food or cosmetic industries.

The extracts and oils of *Vitis vinifera* grape have become a widespread nutritional supplement due to its antioxidant composition, especially colligated with their levels of polyphenols, which have shown to have a wide spectrum of pharmacological effects such as anti-inflammatory, cardio, hepatic and neuron protective effects (Delgado Adámez *et al*., 2012; Giacosa *et al*., 2012; Bijak *et al.,* 2013; Gessne *et al.,* 2013 y Malinowska *et al.*,2013).

Grapes are very rich in both non- flavonoid and flavonoid polyphenols and all of them contribute to the antioxidant activity of grapes (Roussis *et al*., 2005, Rivero-Perez *et al.,* 2008), although each one have a different antioxidant potential which depends on their chemical structure (Di-Majo *et al.*, 2008). Furthermore, some authors have described synergistic action among them (Pignatelli *et al.*, 2006). Grape phenols have showed a lot of biological effects including free radical stabilization, chelation of transition metals, and modulation of some antioxidant enzymes.

Seeds and grape skins are very rich in polyphenols, although the phenolic composition of each part of the grapes is very different. During wine making a significant quantity of grapes phenols go into wine, however important quantities of phenols are not extracted and then significant quantities of phenols remain in wine by-products.

Considering all this fact, products made from wine extracts or from wine by-products could be found in pharmacies, drugstores and even in supermarket, which are sold in base to their phenolic contents and their healthy effects.

The main aim of this paper was a comparative study of different wine or grape commercial products, found in different drugstores and herbal-stores of Burgos (Spain), bases on their antioxidant properties so as in their phenolic contents. Data were correlated among them so as were investigated from the consumer's point of view.

2. MATERIALS AND METHODS

Chemicals

2,2′-Azino-bis(3-ethybenzothiazoline-6-sulfonic acid) (ABTS), the free 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$) radical 6-hydroxyl-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) from Sigma-Aldrich (GmbH, Steinheim, Germany). Potassium persulphate (K2O8S2), from Panreac (Barcelona, Spain). Methanol (high-performance liquid chromatography grade) came from Lab-Scan (Dublin, Ireland). All other chemicals were of analytical grade and obtained from either Sigma-Aldrich, Fluka, or Merck.

Samples

The experimental work has been carried out with 14 commercial food supplements derived from grape or wine, they were bought in pharmacies, para-pharmacies and herbalists of Burgos (Spain). Depending on their origin they were classified into three groups: Group 1 (WG) Integral extract from whole grapes *Vitis vinifera;* Group 2 (SS) Seed extract or skin extract from *Vitis vinifera;* Group 3 (OB) Wine extracts with other bioactive compounds.

Characterization of samples by direct methods

Total polyphenol content and total antioxidant capacity were directly evaluated using modified methods of Folin-Ciocalteau (Singleton and Rossi., 1965), ABTS (Miller and Rice-Evans, 1997) and DPPH (Brand-Williams et al., 1995) assays respectively, according Del Pino-Garcia et al., (2012). Also, proanthocyanidins content were evaluated by direct method according Porter *et al.*, (1986) with slight modifications. The main difference with standard methods is that a small amount of product is weighted and directly resuspended in the reactives. The results are expressed as total polyphenols (PPT, mg of gallic acid /g of sample), proanthocyanidins (PR, mg/g B1-B2/g of sample) and antioxidant capacity (ABTS and DPPH, µmol of Trolox /g of sample).

Preparation of methanolic extracts of samples

To characterize the samples we also obtained methanolic extracts. The preparation of the extracts was performed using the method described by Izcara and Gonzalez–SanJosé (2001). One gram of each sample was placed in a 25 mL of methanol: HCL (97:3 v/v). Extraction was performed for 48 hours in shaking conditions. After extraction, the samples were filtered. The extraction was repeated in triplicate for each sample.

Phenolic composition of methanolic extracts

In extracts total polyphenols (PPT extract) were quantified by reaction with Folin-Ciocalteu reagent (Singleton & Rossi, 1965), and expressed as mg of gallic acid /g of sample and total anthocyanins (TA extract) were evaluated according to the variation of colour in function of pH (Paronetto, 1977), and were expressed as mg of malvidin-3-glucoside /g of sample.

Statistical analysis

The statistical analysis of the data was carried out by analysis of the variance (ANOVA). A significant level of $p \leq 0.05$ was used. Multivariable regression models were applied to study correlations between the analysed parameters using Statgraphics Plus software.

3. RESULTS AND DISCUSSION

Fourteen different products were found and analyzed. All of them are usually sold as dietary supplements and they are advertised as products with healthy effects due to their high levels of antioxidant. Some of them even advertise about anti-aging effects, anti-cholesterol effects or as enhancer of vitality and immune system.

All studied products were commercialized in capsules of daily intake, although the quantity of products in each capsule was very different among products, so as the levels of antioxidants declared on labels.

According to the information advertised on the packing and leaflets, about the raw material from which products were made, they were classified into three different groups (table 1):

- Products containing or obtained from whole grapes (*Vitis vinifera* one), were grouped in the group labelled as **WG** (whole grapes).
- Products derived from grape seeds and skins, by separated, were considered as a different group, and were labeled as **SS** (seed or skins).
- Products containing some specific grape bioactive compounds usually mixture other ones from other vegetal sources were grouped and labeled as **OB** (other specific bioactive compounds).

The groups were made considering the fact that factors as the type of vegetal raw material and the tissue or part of the vegetal raw material used to make the supplements can influence notably their composition and their antioxidant capacity.

It is important to note that not only products from OB group were formulated as mixture with other antioxidants. So, all the WG supplements were a mixture of whole grape with other vegetables extracts, as pomegranate and *Polygonum*, and in general with other antioxidant as Selenium. Two products from SS group were also a mixture with Polygonum, however in general the dietary complements of this group were not supplemented with other antioxidants.

Table 1 shows some examples of the large offer of this type of products, and the high variability in vegetal components. According with this fact, labels showed important differences among the levels of bioactive compounds of each supplement. However, any reference, about how the levels of the bioactive compounds were determined, was not found in the labels or in the prospects of each pack.

The quantitative results obtained after analyzing all studied products (table 2) showed strong discrepancies among the levels of global polyphenols, anthocyanins and proanthocyanins determined by us and those declared in the labels. This fact could be due to the use of different analytical protocols; and it is evidence and highlights the importance of indicating, together with the values of the bioactive compound levels, the sample treatment protocols and analytical methodologies applied to their quantification.

An example of this fact, it also note when data from PPT direct and PPT extracts were compared.

Table 1. Information declared on the labels of studied commercial products.

WG	WG1	465	Grape 133		0,7/15		8	Pomegranate 125	Se 0,05 mg
	WG2	490	Grape 192				9,6	Polygonum 4,8 Allium cepa 30	
	WG3	506	Grape 110				50	Polygonum 110	Colourin g Se 0,05 mg
	WG4	475	Grape 135	124	3/20,3		9,5	Polygonum 10	Se 0,055 mg
	WG5	466	Grape 175				23,3	Pomegranate 12,5 Brown seaweed150	Colourin g
SS	SS1	600	Seed + Skin	136	74	37,5	0,202		
	SS2	800	Seed + Skin	134	72	35	9		
	SS3	600	Seed + Skin	136	74	37,5	0,202		
	SS4	416	Seed + Skin	200			10	Polygonum 18	Colourin g
	SS5	576	Seed + Skin	50	7,5		95	Polygonum 190 Licopene 0,33	Q10, 100 mg
OB	OB1	469	Grape 40					Onagra oil 73	

			Seed Oil 120					Borage oil 73	
	OB2	1278	Resv.+Vit + Min				5		Vit. C,E,B
	OB3	715	Resv.+ Fish oil+ Q10				5	EPA 125 SDA 125 Omega3 50	Q10 10mg Vit.E
	OB4	846	Resv.+Fis h oil+Vit.E				2,5	EPA 300 DHA 75	Vit.E 12 mg

PPT= total polyphenols; AN = total anthocyanins; PR = total proanthocyanins; TAN = total tannins; RESV = resveratrol; Antiox = antioxidant.

Table 2. Levels of total polyphenols (PPT), anthocyanins and proanthocyanins measured on the different commercial products studied. The total polyphenol content (PPT) was assessed using direct method (PPT Direct) and on a methanolic extract (Extract PPT).

[redacted]									
WG	WG1	61,9 ±	1,72 ^{f}	115 ±	0,164 ^{i}	5,509 ±	0,021^{f}	0,179 ±	0,003 ^{f}
	WG2	76,4 ±	1,73 ^{g}	116 ±	0,409 ^{j}	0,467 ±	0,021^{a}	0,251 ±	0,001 ^{i}
	WG3	85,6 ±	1,91^{h}	117 ±	0,164 ^{k}	0,980 ±	0,207^{b}	0,320 ±	0,001 ^{j}
	WG4	49,6 ±	1,88 ^{e}	111 ±	0,409 ^{h}	0,819 ±	0,228^{b}	0,162 ±	0,007 ^{e}
	WG5	131 ±	6,86 ^{i}	142 ±	0,246 ^{l}	3,824 ±	0,041^{d}	0,244 ±	0,001 ^{h}
SS	SS1	41,2 ±	1,15 ^{d}	79,2 ±	0,246 ^{f}	12,192 ±	0,062^{g}	0,053 ±	0,001 ^{c}
	SS2	52,3 ±	1,04 ^{e}	105 ±	0,246 ^{g}	5,025 ±	0,083^{e}	0,043 ±	0,006 ^{b}
	SS3	37,7 ±	0,630 ^{d}	71,4 ±	0,327 ^{d}	12,676 ±	0,083^{h}	0,044 ±	0,006 ^{b}
	SS4	63,1 ±	0,402 ^{f}	115 ±	0,655 ^{i}	2,622 ±	0,124^{c}	0,214 ±	0,001 ^{g}
	SS5	77,1 ±	1,83 ^{g}	117 ±	0,491 ^{j,k}	Nd*	0,053 ±	0,003 ^{c}	

OB	**OB1**	41,4 ±	1,75 [d]	74,5 ±	0,737 [e]	5,582 ±	0,041[f]	0,115 ±	0,001 [d]
	OB2	29,3 ±	0,779 [c]	59,9 ±	0,164 [c]	Nd*	0,052 ±	0,003 [c]	
	OB3	14,8 ±	0,715 [b]	19,1 ±	0,000 [a]	Nd*	0,018 ±	0,002 [a]	
	OB4	10,9 ±	0,670 [a]	25,3 ±	0,327 [b]	Nd*	0,022 ±	0,002 [a]	

Values show mean values ± SD n = 3. Different letters for the same column indicate significant differences (p≤0.05) among dietary supplements. (*) Nd = Not detected.* gal = galic acid; glc = glucose; mvl = malvidine; pro = proanthocyanin

Considering the high variability observed among the levels of bioactive compounds of the fourteen products analyzed, a high variability on their antioxidant capacities was also expected. So, the obtained ABTS and DPPH results were not surprised and agreed perfectly with the phenolic composition evaluated (figure 1). ABTS and DPPH values were highest in WG products. This fact agrees with the high levels of proanthocyanins. The lowest levels of total polyphenols of the products of OB group agree with their lowest antioxidant capacities.

SS4 and SS5 products showed high values of ABTS which were similar to those of WG group. Although no clear conclusion can be drawn, it seems interesting to note that these two products are those advised with the higher content of resveratrol (according to its label) and furthermore, in both case it is declared the presence of *Polygonum, a* constituent of many of the products of the WG group.

Figure 1: Total antioxidant capacity (TAC) of studied dietary food supplements measured by the $ABTS^{\cdot+}$ and DPPH. Columns show average values and bars showed the corresponding SD (n=3*). Different letters on the column indicate significant differences (p≤0.05) among mean values (LSD test).*

4. REFERENCES

1. **Bijak M., Saluk J., Bladel M., Ponczek M.B., Nowak P. (2013).** «Antithrombin Effect of Polyphenol-Rich Extracts from Black Chokeberry and Grape Seeds ». *Phytotherapy research- Phytotherapy*, 27, 71–76 .

2. **Brand-Williams W., Cuvelier M.E., Berset C. (1995).** «Use of a free radical method to evaluate antioxidant activity». *Lebensmittel-Wissenschaft & Technologie*, 28, 25-30.
3. **De la Torre M.C. (1997).** «Ideas actuales sobre la composición del vino. Situación que apoya la hipótesis “Vino y salud”». *Distribución y Consumo,* (febrero-marzo), 67.
4. **Del Pino-García R., García-Lomillo J., Rivero-Pérez M.D., Muñiz P., González-SanJosé M.L. (2012)**. «Direct methods for determination of total polyphenols and antioxidant activity in powder products obtained from vinification by-products». In Proceedings of 6th ISANH Congress on Polyphenols Applications, pp 56.
5. **Delgado Adámez J., Gamero Samino E., Valdés Sánchez E., González-Gómez D. (2012)** «In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (*Vitis vinifera L.*) ». *Food Control*, *24, 136-141.*
6. **Di-Majo D, La-Guardia M, Giammanco S, La-Neve L, Giammanco M. (2008).** «The antioxidant capacity of red wine in relation-ship with its polyphenolic constituents». *Food Chemistry, 111, 45-49.*
7. **Farre, R. (1995).** «El vino como componente de la dieta». *Distribución y consumo,* (diciembre-enero), *126-127.*
8. **Fuchs C. S., Stampfer M.J., Colditz, G.A., Giovannucci E.L., Manson J.E., Kawachi I., Hunter D.J., Hankinson S.E., Hennekens C.H., Rosner B., Speizer F.E., Willett W.C. (1995).** «Alcohol Consumption and Mortality among Women». *The New England Journal of Medicine,* 19, *1245-1250.*
9. **Gessne D.K., Fiesel A., Most E., Dinges J., Wen G., Ringseis R., Eder K. (2013).** «Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF- B and Nrf2 in the duodenal mucosa of pigs». *Acta Veterinaria Scandinavica,* 55, 18.
10. **Giacosa, A. , Adam-Blondon, A.F., Baer-Sinnott, S., Barale, R., Bavaresco, L., Di Gaspero, G., Dugo, L., Curtis Ellison, R., Gerbi, V., Gifford, D., Janssens, J., La Vecchia, C., Negri, E., Pezzotti, M., Santi, L., Santi, L., Rondanelli, M. (2012)** « Alcohol and wine in relation to cancer and other diseases». *European Journal of Cancer Prevention ,21,103-108.*
11. **Izcara, E., Gonzalez-San José, M.L. (2001).** «Análisis de los métodos rápidos de

extracción para seguir la maduración fenólica de la uva *». Enólogos*, 3, 15-18, 28.

12. **Malinowska J., Oleszek W., Stochmal A., Olas B. (2013).** «The polyphenol-rich extracts from black chokeberry and grape seeds impair changes in the platelet adhesion and aggregation induced by a model of hyperhomocysteinemia». *European Journal of Nutrition*, 52, 1049–1057.
13. **Miller N.J., Rice-Evans C.A. (1997).** « Factors influencing the antioxidant activity determined by ABTS radical cation assay». Free Radical Research, 26, 195-199.
14. **Paronetto, L. (1977).** Polifenoli e tecnica enologica. Selepress, Milan, Italy.
15. **Pignatelli P, Ghiselli A, Buchetti B, Carnevale R, Natella F, et al. (2006).** « Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine *». Atherosclerosis, 188,* 77-83.
16. **Porter L.J., Hrstich L.N., Chan B.G. (1986).** « The conversión of procyanidin and prodelphinidins to cyanidin and delphinidin ». *Phytochemistry*, 25, 223-230.
17. **Rivero-Pérez MD, Muñiz P, González-Sanjosé ML. (2008).** « Contribution of anthocyanin fraction to the antioxidant properties of wine *». Food Chemistry and Toxicology 46,* 2815-2822.
18. **Roussis IG, Lambropoulo I, Soulti K. (2005).** « Scavenging capacities of some wines and wine phenolic extracts *». Food Technological and Biotechnology, 43,* 351-358.
19. **Singleton, VL., & Rossi, JA.** (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *American Journal of Enology and Viticulture 16,* 144-158.
20. **Thun, M.D.; Richard Peto, F.R.S.; Alan, D.; Lopez, Ph.D.; Jane, H.; Monaco, M.S.; S. Jane Henley, B.A.; Clark, W.; Heath, M.D. y Richard Doll, F.R.S. (1997).** «Alcohol Consumption and Mortality among Middle-Aged and Elderly U.S. Adults». *The New England Journal of Medicine,* 24, 1705-1714.