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A Novel Approach to Measure the Total Antioxidant Power of Wines through Near Infrared Spectroscopy and Its Relevance in Human Nutrition

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Abstract: Wine is the product of the alcoholic fermentation of the grape (*Vitis vinifera*). As such, it is a hydroalcoholic solution with a variable content of nutritional and functional (polyphenols) molecules, the latter involved in its antioxidant potential. The organoleptic variables of wine, together with its potential positive effects on health of a light-moderate intake, have always been topics of great interest within the cultures. In the label of wine, alcohol is the only declared variable. On the other hand, there is no information about the content of "positive" molecules, such as those associated with the antioxidant power. This value could be very important to classify the wines, helping oenologist and nutritionist in qualifying them as a component of Mediterranean diet. Moreover, one of the most critical aspects in evaluating the role of wine in human health through epidemiological prospective studies is the quality of the products used and their antioxidant potential. This research aimed to optimize and validate an analytical approach based on a portable device (SCIO®), using NIRS (near-infrared reflectance spectroscopy). It allows the measure of TAP (total antioxidant power) of wine through the glass. Research findings are promising. To the best of our knowledge, this is the first time that an easy-to-use and cheap hand-held scanner is validated to measure the TAP of a beverage.

Key words: TAP of wines, near-infrared spectroscopy of wines, NIRS portable device.

1. Introduction

Many studies have shown the positive effects of light to moderate wine consumption in the reduction of risk factors for chronic diseases and the cardiovascular mortality [1-5]. In fact, the French Paradox suggests that a daily light to moderate wine consumption with meals reduces the risk factors for the cardiovascular diseases and increases life expectancy. Studying the French Paradox, Renaud and de Longeril [6] showed a correlation between a 40% reduction in the incidence of cardiovascular diseases and a platelet anti-aggregation effect of alcohol. Another recent research has shown that red wine consumption is related to an increase in HDL (high density lipoprotein) cholesterol levels [7]. It has been also shown that with a light to moderate red wine consumption there is an improvement in the cognitive abilities and a decrease in the incidence of dementia [8, 9]. The increase of the global population over the age of 65 is associated with a higher incidence of chronic diseases, so that a light to moderate wine consumption could modulate the incidence of cardiovascular diseases and age-associated cognitive impairment [10]. For all the beneficial effects described above are due, at least partially, to several active molecules present in wine, most of them have antioxidant potential. In particular, polyphenols (such as resveratrol), which are more abundant in red wine, are the most frequently cited actors in the protective

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effect of wine [11].

The aim of this research was the development of a simple tool to measure the total antioxidant power (TAP) of beverages and in particular of wine. A novel method based on the near-infrared reflectance spectroscopy (NIRS) is here described and validated comparing results with the most usual test based on radical scavenging activity. The method requires a portable device, which provides information *in situ* on the antioxidant potential of the sample considered. This tool could help researchers in producing new data on the role of light to moderate consumption of wine on the modulation of age-related diseases.

2. Materials and Methods

2.1 Wines

The study includes 40 bottles of commercial wine, 30 of which were red (Malbec, Cabernet Sauvignon) 5 rosé (Malbec, Cabernet Sauvignon) and 5 white (Sauvignon Blanc, Chardonnay). They were from the harvest 2016 Mendoza-Argentina and had a wide price range. Bottles of wine were stored in the same conditions of light and temperature (13 ºC) until the day of analysis.

2.2 Antioxidant Activity

2.2.1 DPPH Assay

The antioxidant activity of wine samples was measured spectrophotometrically, as a measure of radical scavenging activity, using 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) (Sigma Aldrich® Germany) [12, 13]. Red wine was diluted 1:20 (*v*/*v*) in water, while white wine was used as such. A control sample containing a volume of solvent (methanol) equivalent to wine was used to measure the maximum DPPH absorbance. Aliquots of 0.1 mL of 100 µM solution of 5% DPPH in methanol were mixed with 0.1 mL of each sample. Samples, in triplicate, were mixed and incubated at room temperature in the dark for 30 min. The absorbance at 517 nm was recorded to determine the concentration of residual DPPH.

The percentage of inhibition of the maximal absorbance was calculated according to the equation:

$$\% \text{ inhibition} = [(A_{DPPH} - A_{wine})/A_{DPPH}] \times 100$$

in which *A* is the absorbance of DPPH and wine, respectively. IC50 values correspond to the concentration of the sample, which scavenge 50% of DPPH free radicals.

2.2.2 NIRS

The NIRS device used in this research emits a light beam in the range of the near-infrared between 740 and 1,070 nanometers which, interacts with chemical groups, such as -CH, -NH and -OH contained in the molecules, so that a certain amount of electromagnetic radiation is absorbed [14, 15]. In other words, the NIRS is based on the reflection of molecular absorptions, which are the consequence of modifications of energetic vibrational states due to the interact ion of molecules with the radiation. NIRS analysis was performed with a portable device SCIO ® developed in Herzliya (Israel) (Fig. 1). A model has been developed, building a database of combined readings at DPPH and NIRS. When the necessary number of samples for the expected performance of the model is reached, a statistical study of the Pearson R^2 correlation is performed. The results were expresed as nanometers (nm).

2.3 Statistic Analysis

The statistical analysis used to assess the performance of the model was based on the Karl Pearson's correlation coefficient = R^2 (software SCIO Lab), where R^2 is the correlation between the estimate and true values and RMSE is the root mean square error. A perfect model will have $R^2 = 1$ and RMSE = 0.

3. Results and Discussion

The values of TAP measured by DPPH for different commercial wines are shown in Table 1. The values listed represent the average of TAP of each wine, with their standard deviation. The higher the IC50 the lower the TAP.

Fig. 1 SCIO® molecular sensor measuring the antioxidant activity of wine in a glass.

NIRS curves of the reflectance spectrum of the wines studied are shown in Fig. 2. They are curves without artefacts or confusing effects. Data obtained by the two tests were elaborated in a linear regression as shown in Fig. 3, each dot in the graph is a wine sample, where (*y*) is the NIRS measure and (*x*) the DPPH value. Lines at the upper and lower limit represent a 20% margin error. As may be seen from the data dispersion model in our research, the Pearson R^2 calculations for the 40 samples had a high positive correlation R = 0.762.

After the publication by Gershman in 1954 [16] of the theory of free radicals, oxygen toxicity and radiations, the scientific evidence that links the damage produced by oxidative stress to the ageing process and the development of chronic diseases such as cardiovascular, obesity, diabetes, cancer and neurodegeneration is undeniable. Because natural antioxidant defences start to be insufficient from age 30 [17], the intake of antioxidants from food is paramount to slow or reverse part of the processes mediated by the free radicals. An investigation [18] showed that light to moderate wine consumption, in both an occidental and a Mediterranean diet, could contribute to the increase of plasma antioxidant capacity, the decrease of oxidative DNA damage, and the normalization of endothelial function. These results were more significant in the group consuming an occidental diet. Wine is a universal beverage and one of the main components of a Mediterranean diet [19]. In a prospective study including 1,882 men with a 29-year follow-up (1974-2003) and declaring a specific preference for alcoholic beverages (beer, wine or spirits), the mortality was registered according to the type of beverage consumed. The general mortality was 33.0% of the initial population, but mortality rate was higher among people consuming spirits (36.9%), followed by beer (32.3%), wine drinkers (29.5%) [20]. The positive attribute of wine is related to its moderate alcohol content and its antioxidant properties, the latter varying widely with a lower protective activity of white wines compared to red wines. Polyphenols are the most important wine antioxidants; their natural content varies significantly and depends on the region, grape variety or varietal, soil, climate, irrigation, harvest, performance, winemaking, ripening and storage. Frankel and co-workers [21-23] showed that the TAP of wine could be correlated to the inhibition of LDL cholesterol oxidation. The postprandial period is the phase where reactive oxygen species increase mostly, due to the digestion and metabolism of food [23]. There are data showing that moderate wine consumption with meals could decrease the metabolic risk associated with the absorption of free radicals. It has been shown that the measurement of TAP in wines by DPPH presents a high correlation with the total polyphenol content [24]. In this context, the possibility to measure the antioxidant activity of wine could help oenologists, nutritionists and suitably informed consumers in the choice of wine, that should be always consumed light-moderately. It could be also an important tool for epidemiological prospective

Table 1 The values listed DPPH and NIRS, represent the average of TAP of each wine, with their standard deviation.

Method	Red (n = 30)	Rosé (n = 5)	White (n = 5)
	Malbec Cabernet Sauvignon Harvest 2016 Mendoza	Malbec Cabernet Sauvignon Harvest 2016 Mendoza	Sauvignon Blanc Chardonnay Harvest 2016 Mendoza
DPPH, IC50 (mL)	0.08 ± 0.03	1.43 ± 0.59	2.60 ± 0.19
NIRS (nm)	0.28 ± 0.48	1.79 ± 0.80	2.24 ± 0.28

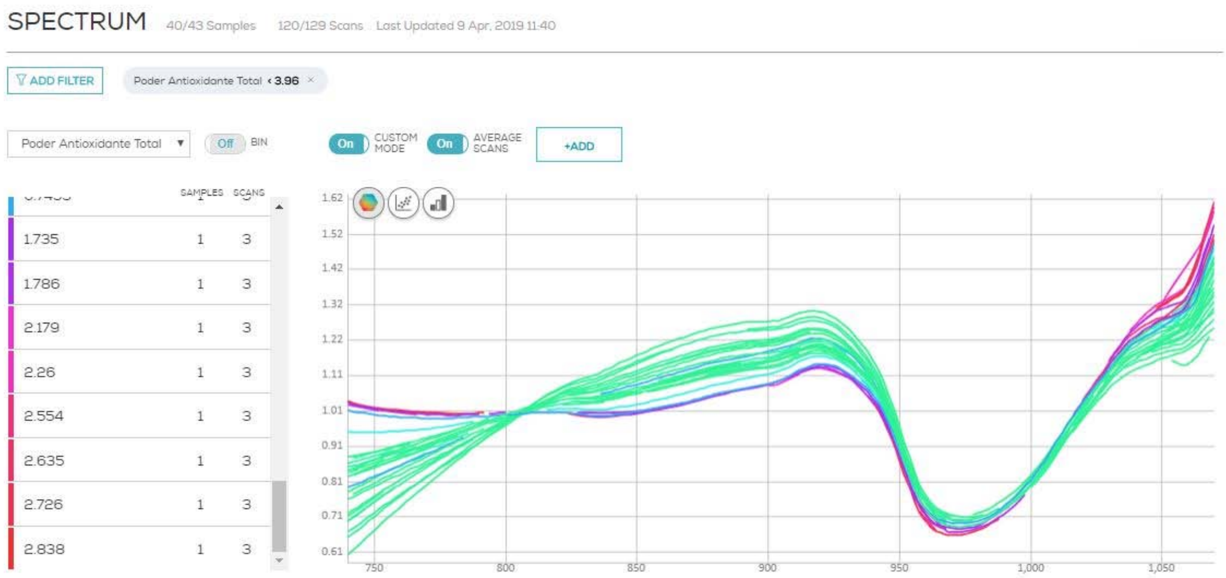

Fig. 2 Spectrum of the 40 wine samples studied by NIRS.

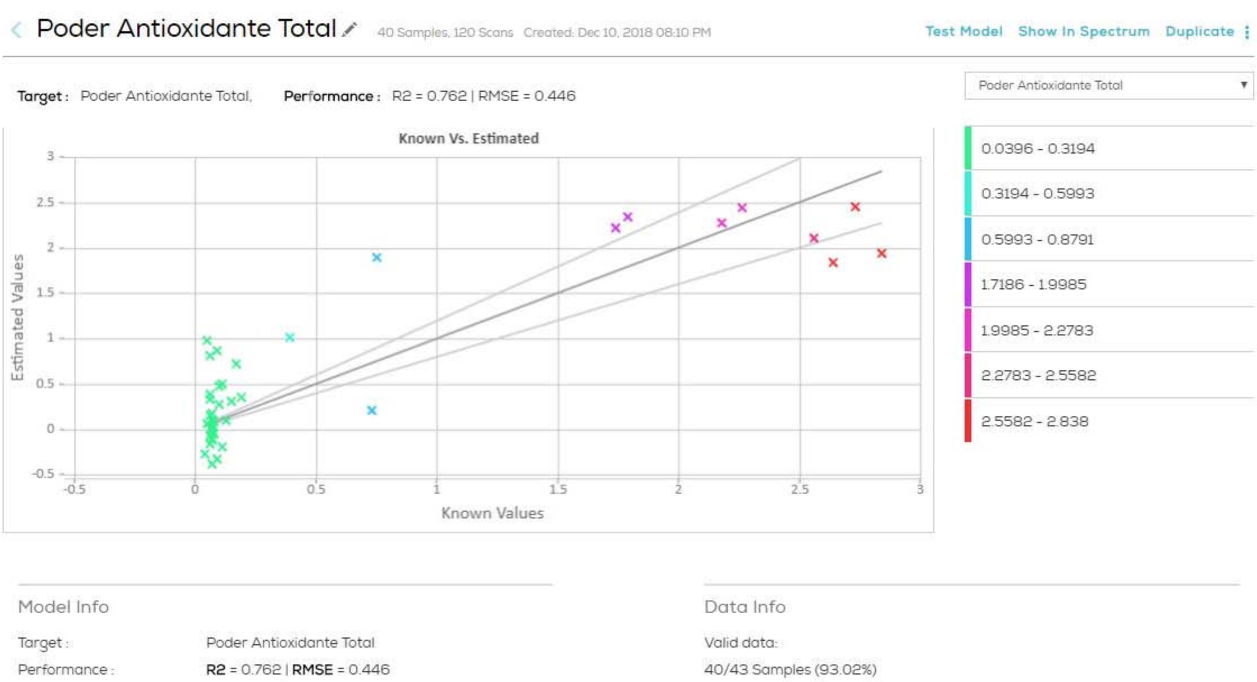

Fig. 3 Reading dispersion model, to calculate the Pearson correlation (R^2).

studies, aimed to confirm that the moderate consumption of wine is associated with the reduction of risk factors for different chronic diseases.

4. Conclusion

The objective of this research was to validate a novel application to measure the TAP in wines through a portable device capable of working through glass. This device is relatively cheap and easy to use; it can inform in a few seconds the TAP of the wine, as an estimate of its healthy, non-destructive quality and *in situ* through the glass. The results of the validation of NIRS with DPPH were promising. And these are important to promote future research on healthy nutrition with NIRS. As far as we know, it is the first time that a validation of near infrared spectroscopy has been carried out to measure the TAP of wines *in situ*. We believe that near-infrared spectroscopy could be a very important tool for winemakers, researchers and consumers interested in healthy nutrition.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] Di Castelnuovo, A., Rotondo, S., Iacoviello, L., Donati, M. B., and de Gaetano, G. 2002. "Meta-analysis of Wine and Beer Consumption in Relation to Vascular Risk." *Circulation* 105: 2836-44.

[2] Rimm, E. B., and Stampfer, M. J. 2002. "Wine, Beer, and Spirits. Are They Really Horses of a Different Color?" *Circulation* 105: 2806-7.

[3] Klatsky, A. L., Friedman, G. D., Armstrong, M. A., and Kipp, H. 2003. "Wine, Liquor, Beer, and Mortality." *Am J Epidemiol* 158: 585-95.

[4] Mukamal, K. J., Conigrave, K. M., Mittleman, M. A., Camargo, C. A., Stampfer, M. J., Willett, W. C., and Rimm, E. B. 2003. "Roles of Drinking Pattern and Type of Alcohol Consumed in Coronary Heart Disease in Men." *N Engl J Med*. 348: 109-18.

[5] Streppel, M. T., Ocké, M. C., Boshuizen, H. C., Kok, F. J., and Kromhout, D. 2009. "Long-Term Wine Consumption Is Related to Cardiovascular Mortality and Life Expectancy Independently of Moderate Alcohol Intake: The Zutphen Study." *J Epidemiol Community Health* 63: 534-40.

[6] Renaud, S., and De Lorgeril, M. 1992. "Wine, Alcohol, Platelets, and the French Paradox for Coronary Heart Disease." *Lancet* 339: 1523-6.

[7] Nova, E., San Mauro-Martìn, I., and Diaz-Prìeto, L. E. 2019. "Wine and Beer within a Moderate Alcohol Intake Is Associated with Higher Levels of HDL-c and Adiponectin." *Nutrition Research* 63: 42-50.

[8] Letenneur, L., Larrieu, S., and Barberger-Gateau, P. 2004. "Alcohol and Tobacco Consumption as Risk Factors of Dementia: A Review of Epidemiological Studies." *Biomed Pharmacother* 58: 95-9.

[9] Mukamal, K. J., Kuller, L. H., Fitzpatrick, A. L., Longstreth, W. T. Jr, Mittleman, M. A., and Siscovick, D. S. 2003. "Prospective Study of Alcohol Consumption and Risk of Dementia in Older Adults." *JAMA* 289: 1405-13.

[10] De Lange, D. W., and van de Wiel, A. 2004. "Drink to Prevent; Review on the Cardioprotective Mechanisms of Alcohol and Red Wine Polyphenols." *Semin Vasc Med.* 4: 173-86.

[11] Catalgol, B., Batirel, S., Taga, Y., and Ozer, N. 2012. "Resveratrol: French Paradox Revisited." *Front. Pharmacol.* 3: 141. https://doi.org/10.3389/fphar.2012.00141.

[12] Brand-Williams, W., Cuvelier, M. E., and Berset, C. 1995. "Use of Free Radical Method to Evaluate Antioxidant Activity." *LWT-Food Science and Technology* 28: 25-30.

[13] Leong, I. P., and Shui, G. 2002. "An Investigation of Antioxidant Capacity of Fruits in Singapore Markets." *Food Chem* 76: 69-75.

[14] Shenk, J. S., Workman, J., and Westerhaus, M. 2001. "Application of NIR Spectroscopy to Agricultural Products." In *Handbook of Near Infrared Analysis*, 2nd ed., edited by D. A. Burns, and E. W. Ciurczac. CRC Press. Boca Raton, Florida: Taylor & Francis Group, pp. 419-74.

[15] Murray, I., and Cowe, I. 2004. "Sample Preparation." In *Near Infrared Spectroscopy in Agriculture*, edited by C. A. Roberts, J. Workman, and J. B. Reeves. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, pp. 75-115.

[16] Gershman, R. 1954. "Oxygen Poisoning and X-Irradiation: A Mechanism in Common." *Science* 119: 623-6.

[17] Enden-Inal, M., Sumal, E., and Kanbak, G. 2002. "Age-Related Changes in the Glutathione Redox System." *Cell Biochemistry and Function* 20: 61-6.

[18] Urquiaga, I., Strobel, P., Perez, D., Martinez, C., Cuevas, A., Castillo, O., Marshall, G., Rozowski, J., and Leighton, F. 2010. "Mediterranean Diet and Red Wine Protect against Oxidative Damage in Young Volunteers."

Atherosclerosis 211: 694-9.

[19] Rifler, J.-P. 2018. "Is a Meal without Wine Good for Health?" *Diseases* 6: 105.

[20] Strandberg, T. E., Strandberg, A. Y., Salomaa, V. V., Pitkälä, K., Tilvis, R. S., and Miettinen, T. A. 2007. "Alcoholic Beverage Preference, 29-Year Mortality, and Quality of Life in Men in Old Age." *Journal of Gerontology, Series A: Biological Sciences & Medical Sciences* 62: 2138.

[21] Frankel, E. N., Waterhouse, A. L., and Kinsella, J. E. 1993. "Inhibition of Human LDL Oxidation by Resveratrol." *Lancet* 341: 1103-4.

[22] Frankel, E. N., Waterhouse, A. L., and Teissedre, P.-L. 1995. "Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins." *J. Agric Food Chem*. 43: 890-4.

[23] Pastor, R. F., Restani, P., Di Lorenzo, C., Orgiu, F., Teissedre, P. L., Stockley, C., et al. 2017. "Resveratrol, Human Health and Winemaking Perspectives." *Crit Rev Food Sci Nutr*. 5: 1-19.

[24] Paixão, N., Perestrelo, R., Marques, J. C., Câmara, J. S. 2017. "Relationship between Antioxidant Capacity and Total Phenolic Content of Red, Rosé and White Wines." *Food Chemistry* 105: 204-14.