Determination of total antioxidant capacity of 410 red wines

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The antioxidant capacity of 410 red wines was determined to characterise the wines according to grape variety, geographic origin and duration of extraction. The data consisted of 410 red wines and 23 chemical measurements on each wine. The wines represented 65 grape varieties, 22 countries, 72 regions and 11 vintages ranging from 1999 to 2010. The data were analysed statistically. Antioxidant capacity was treated as the dependent variable and the other data as independent variables. Linear regression of bootstrap samples indicated that the most important quantitative independent variables were absorbance at 420 nm, volatile acids, total acids, citric acid, dry extract and duration of extraction. Antioxidant capacity showed strongest association with absorbance at 420 nm and duration of extraction. Of qualitative variables, only country and region yielded significant pairwise differences of interest. On average French wines had statistically significant higher antioxidant capacities than wines from Australia, whereas French and Spanish wines had statistically significantly higher antioxidant capacities than wines from South Africa and the USA on average.
Keywords: wine, antioxidant capacity, TEAC, grape variety, geographic origin

Bestimmung der antioxidativen Kapazität von 410 Rotweinen. *Die antioxidative Kapazität von 410 Rotweinen wurde bestimmt, um die Weine nach Rebsorte, Herkunft und Extraktionsdauer zu charakterisieren. Die Daten umfassten 410 Rotweine und 23 chemische Parameter je Wein. Unter den Weinen fanden sich 65 Sorten, 22 Länder, 72 Regionen und elf Jahrgänge von 1999 bis 2010. Die Daten wurden statistisch analysiert. Die antioxidative Kapazität wurde als abhängige Variable, die anderen Daten als unabhängige Variablen behandelt. Die lineare Regression von Bootstrap-Samples zeigte, dass die wichtigsten quantitativen unabhängigen Variablen die Absorption bei 420 nm, die flüchtigen Säuren, Gesamtsäure, Citronensäure, Extrakt und Extraktionsdauer waren. Die antioxidative Kapazität zeigte die größte Korrelation mit der Absorption bei 420 nm und der Extraktionsdauer. Von den qualitativen Variablen zeigten nur Land und Region signifikante paarweise Unterschiede, die von Interesse waren. Im Schnitt haben französische Weine statistisch signifikant höhere antioxidative Kapazitäten als australische, während französische und spanische Weine im Schnitt statistisch signifikant höhere antioxidative Kapazitäten hatten als Weine aus Südafrika und den USA.*
Schlagwörter: Wein, antioxidative Kapazität, TEAC, Rebsorte, Herkunft

La détermination de la capacité antioxydante de 410 vins rouges. *La capacité antioxydante de 410 vins rouges a été determinée afin de caractériser les vins selon le cépage, l'origine et la durée d'extraction. Les données ont été prélevées sur 410 vins rouges, 23 paramètres chimiques par vin ayant été pris en compte. Parmi les vins, on comptait 65 variétés en provenance de 22 pays et de 72 régions, et onze millésimes de 1999 à 2010. Les données ont été analysées statistiquement, la capacité antioxydante étant traitée comme variable dépendante, les autres données comme variables indépendantes. La régression linéaire des échantillons bootstrap a montré que les principales variables quantita-*

tives indépendantes étaient l'absorption à 420 nm, les acides volatils, l'acidité totale, l'acide citrique, l'extrait et la durée d'extraction. La capacité antioxydante a présenté la plus grande corrélation avec l'absorption à 420 nm et avec la durée d'extraction. Parmi les variables qualitatives, seuls le pays et la région, par paires, présentaient des différences significatives intéressantes. En moyenne, les vins français possèdent des capacités antioxydantes statistiquement significatives plus prononcées que les vins australiens, tandis que les vins français et espagnols possèdent en moyenne des capacités antioxydantes statistiquement significatives plus élevées que les vins en provenance d'Afrique du Sud et des États-Unis.

Mots clés : vin, capacité antioxydante, TEAC, variété, origine

There are several methods for the determination of the antioxidant capacity of various matrices (Brand-Williams et al., 1995; Re et al., 1999; Alho, 1999; Rivero-Perez et al., 2007; Di Pietro and Bamforth, 2011). The determination of antioxidants in wines has become widespread during the past decade (Burns et al., 2000; Cimino et al., 2007; De Beer et al., 2003; Landrault et al., 2001). Most of the methods are based on spectroscopy and differ from one another mainly in the chemical radical used in the determination. We have correlated antioxidant capacity with the geographic origin and grape variety of 104 wines in an earlier publication (Mäntylä et al., 2009). The aim of the present study was to investigate the correlation between various wine characteristics and the antioxidant capacity of wine.

Material and Methods

410 wines from 65 grape varieties were chosen for this study. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as an antioxidant standard. The analytical procedure is described in Mäntylä et al. (2009).
The same wine samples were analysed for ethanol, total acids, volatile acids, glucose and fructose concentration as well as density, pH-value and dry extract using a FOSS WineScan FT120 instrument (FOSS, Hillerød, Denmark). Samples were diluted 1:20 with distilled water and their absorbance at 420 nm was determined using a Thermo Scientific Arena 20XT automated photometric analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Free and total sulphur dioxide were determined with a FOSS FIAstar system (FOSS, Hillerød, Denmark).

Analytical software

All statistical analyses were performed using SAS® Enterprise Guide® 4.3.

Results and Discussion

Method

The method was validated earlier (Mäntylä et al., 2009). The validation yielded a relative standard uncertainty of measurement of 5.1 % for antioxidant activity. Compared with non-automated methods, automation brought a major improvement in the number of samples that can be analysed at the same time. The new method allows up to 80 samples to be simultaneously analysed in an overnight run, for instance.

Statistical analysis

In the analysis, sample descriptors treated as qualitative were grape variety, country and region. Effect of vintage was not analyzed. In blends of grape variety the variety was set as a value to an effect called "Grape max 70 %" when the defined blend contained at least 70 % of the variety (n = 350) and otherwise the value was set as missing, resp. no grape variety was mentioned (n = 60). Categories with fewer than five samples and cases with a missing category value were combined under the label "Other" for all qualitative variables. Other measurements were treated as quantitative and their values were standardized to have mean 0 and standard deviation 1. Additionally one quantitative random variable was generated to the data. In order to compare the effect of continuous independent variables to an effect of an random variable, one was generated to the data.
Effect of country was tested by dividing data at first half and half to a test and a validation dataset by subsetting each category separately with a 50/50 rule. A general linear model was fitted to both sets. All pair wise comparisons were tested using *Tukey-Kramer* method as the multiple comparison adjustment method. Significance level of 95 % was applied. Effects of grape variety and region were tested similarly, except that the data was not divided to test and validation sets.

Figures 1a and 1b show that antioxidant capacity (TEAC) values are quite widely distributed within most countries and grape varieties, respectively, and that median values between groups are quite close together. Regarding differences between countries, the greatest median values shown in figure 1a are for France, Portugal and Spain whereas the lowest values are for Australia, South Africa and USA.

Results of tests on test set (Table 1), which agreed with results on validation set, for effect of country showed that French wines have on average statistically significantly greater values of antioxidant capacity compared to wines from Australia, South Africa and USA. Spanish wines had on average statistically significantly greater values of antioxidant capacity compared to wines from South Africa and USA. Other comparisons did not show significant differences between groups. R-square of the test set model, as well as validation set model, was 0.20.

'Syrah' and 'Zinfandel'. However, test results (Table 3) on grape variety showed that the only significant pair wise comparison was between groups "Other" and 'Syrah'. R-square of the model was 0.06.

For quantitative variables, at first, Spearman correlations were calculated between them and antioxidant capacity. Secondly a variable selection method as a form of linear model was applied in order to study the importance of each quantitative variable. In order to stabilize model selection and to reduce effect of outliers, 300 bootstrap samples were created. The sampling was performed twice, since "duration of extraction" was measured only with 77 cases, whereas others with over 406 cases. The first sample was taken without the parameter "duration of extraction" from all cases and the second sample with it from 77 cases where "duration of extraction" was included. Both sample sets were subject to linear regression analysis with forward selection method. P-value 0.1 was selected as level of entry in the model. Variables included in the model were recorded. Thirdly, in order to study the effects of the most frequently selected variables together with variable region more closely, a general linear model was fitted to all data.

Table 1: Group sizes in the original dataset, least-square means of antioxidant capacity, their confidence limits in the test set by country and statistically significant pairwise differences

Id	Country	N	TEAC LSMean	95 % Confidence limits		Sig. diff. from id
1	Argentina	39	24.34	22.37	26.31	
2	Australia	48	20.82	19.16	22.57	4
3	Chile	66	21.73	20.24	23.23	
4	France	57	24.84	23.24	26.43	2,8,10
5	Italy	56	21.70	20.07	23.32	
6	Other	19	25.96	23.24	28.68	
7	Portugal	9	24.88	21.04	28.72	
8	South Africa	39	19.26	17.29	21.23	4,9
9	Spain	56	23.53	21.90	25.15	8,10
10	USA	21	17.90	15.18	20.62	4,9

On regional level, variations within groups are lower compared to country level, but there are some outliers in several groups, as can be seen from Figure 2. Test results (Table 2) show that wines from Bordeaux have on average higher capacity values compared to wines from Australia SE, California, Cape Colony, Central Valley, Coastal Region, Mendoza, "Other", Rapel, Rioja, Riverland and Veneto. Wines from Castile and León have higher values compared to wines from California and Cape Colony. Wines from Languedoc-Roussillon, Mendoza and Tuscany have higher values compared to wines from California. R-square of the model was 0.19.

The highest median values of grape varieties are shown in Figure 1b and are for 'Cabernet Sauvignon', 'Carmenere', 'Malbec', "Other" and 'Tempranillo', whereas the lowest values are for 'Grenache', 'Merlot',

Correlation table (Table 4) shows moderate correlation between antioxidant capacity and duration of extraction and absorbance at 420 nm, whereas other pairs show rather weak correlation.

Model selection on bootstrap samples without the parameter "duration of extraction" showed that the most important explanatory variables seemed to be absorbance 420 nm, volatile acids, total acids, citric acid and dry extract. All of them were selected to the model over half of the time (Table 5) and they all appeared in the most frequently selected model as well. Same procedure with data including "duration of

Table 2: Group sizes, least-square means of antioxidant capacity, their confidence levels by region and statistically significant pairwise differences

Id	Region	Country	N	TEAC LSMean	95 % Confidence limits		Significantly differs from id
1	Apulia	Italy	7	21.94	18.86	25.03	
2	Australia South	Australia	9	22.29	19.57	25.01	4
3	Australia South-East	Australia	8	21.44	18.55	24.32	
4	Bordeaux	France	18	28.35	26.43	30.27	3,6,7,10,11,15-17,19,20,25
5	Bourgogne	France	11	23.14	20.68	25.60	
6	California	USA	17	18.31	16.33	20.29	4,13,15, 23
7	Cape Colony	South Africa	11	19.37	16.91	21.83	4
8	Castile and León	Spain	8	27.01	24.13	29.90	
9	Catalonia	Spain	8	23.75	20.87	26.63	
10	Central Valley	Chile	11	21.86	19.40	24.32	4
11	Coastal Region	South Africa	10	21.80	19.22	24.38	4
12	La Mancha	Spain	5	24.90	21.25	28.55	
13	Languedoc-Roussillon	France	10	24.65	22.07	27.23	6
14	Maipo	Chile	10	22.81	20.23	25.39	
15	Mendoza	Argentina	31	23.24	21.78	24.70	4,6
16	Other		142	21.76	21.07	22.44	4
17	Rapel	Chile	15	22.41	20.31	24.52	4
18	Rhône	France	12	23.56	21.20	25.91	
19	Rioja	Spain	12	22.19	19.84	24.55	4
20	Riverland	Australia	5	20.06	16.41	23.71	4
21	San Juan	Argentina	5	22.58	18.93	26.23	
22	Sicily	Italy	6	23.97	20.64	27.30	
23	Tuscany	Italy	16	24.09	22.05	26.13	6
24	Valdepenas	Spain	8	23.21	20.33	26.10	
25	Veneto	Italy	15	20.67	18.56	22.77	4

Table 3: Group sizes, least-square means of antioxidant capacity, their confidence levels by grape variety and statistically significant pairwise differences

Id	Grape max. 70 %	N	TEAC LSMean	95 % Confidence limits		Significantly differs from id
1	Cabernet Sauvignon	48	22.73	21.48	23.97	
2	Carmenere	8	22.53	19.47	25.58	
3	Corvina	5	21.84	17.98	25.70	
4	Grenache	7	19.09	15.82	22.35	
5	Malbec	11	23.99	21.39	26.60	
6	Merlot	31	21.58	20.03	23.13	
7	Other	173	23.02	22.36	23.68	10
8	Pinot noir	21	22.90	21.01	24.79	
9	Sangiovese	17	23.43	21.33	25.53	
10	Syrah	51	20.45	19.24	21.66	7
11	Tempranillo	31	23.27	21.72	24.82	
12	Zinfandel	7	19.90	16.63	23.17	

extraction" showed that it also seemed to be an important explanatory variable, although the model selection procedure was far more instable compared to analysis without that variable. With it the generated random variable was chosen to the model almost every fourth time, compared to every tenth time when duraion of extraction was not present in the model.

A model with the most frequently selected variables and without duration of extraction was fitted to all data (n = 407). The model had an R-square value of 0.29. Estimates and their standard errors are presented in Table 6. When the parameter "region" was added to the model (Table 7), R-square value rose to 0.39 and effects of citric and total acids and dry extract became

Table 4: Spearman correlations between quantitative variables and antioxidant capacity

Variable	Correlation coefficient
Duration of extraction	0.457
Absorbance 420 nm	0.446
Volatile acids	0.257
Effective alcohol (%mas.)	0.217
pH-value	0.217
Effective alcohol (%vol.)	0.212
Extract without sugars	0.126
Citric acid	-0.058
Total acids	-0.088
Dry extract	-0.138
SO_2 free	-0.174
SO_2 total	-0.205
Relative density	-0.232
Specific gravity	-0.232
Fructose	-0.249
Glucose and fructose	-0.306
Glucose	-0.354

non-significant. It should be noted, that there were several outlying values amongst residuals of this model.

Almost 40 % of the variation in antioxidant capacity values could be explained by main effects of the dependent variables at hand. Yet several outlying antioxidant values could not be explained by this data. Geographic origin, absorbance at 420 nm and volatile acids proved to be the most important explanatory factors for variance in antioxidant values.

Acknowledgement

The skilful technical assistance of Ms. *Kristina Lindholm* is gratefully acknowledged.

Table 5: Number of times each quantitative variable was selected in a linear model of a bootstrap sample

Order	Model without duration of extraction	Appears in number of models	Model with duration of extraction	Appears in number of models
1	Absorbance 420 nm	300	Citric acid	191
2	Volatile acids	297	pH-value	152
3	Total acids	228	Duration of extraction	147
4	Citric acid	211	Glucose	133
5	Dry extract	177	Specific gravity	107
6	Glucose	121	Absorbance 420 nm	106
7	Effective alcohol (%mas.)	94	Alcohol (%mas.)	88
8	pH-value	82	Total acids	84
9	SO_2 total	66	Volatile acids	75
10	Specific gravity	47	**Random variable**	**74**
11	SO_2 free	45	Density	59
12	Effective alcohol (%vol.)	38	SO_2 free	54
13	Relative density	34	SO_2 total	38
14	Glucose and fructose	34	Alcohol (%vol.)	29
15	**Random variable**	**31**	Extract without sugars	24
16	Fructose	26	Fructose	21
17	Extract without sugars	8	Dry extract	19

Table 6: Parameter estimates with all the most frequently selected quantitative variables

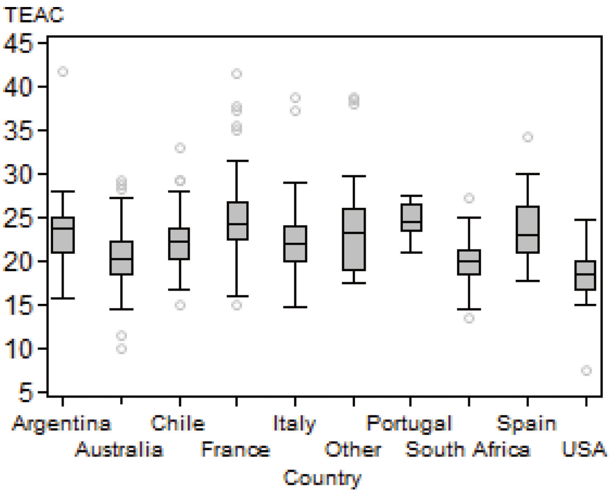
Variable	DF	Parameter estimate	Standard error	t-value	Pr > |t|	95 % Confidence limits	
Intercept	1	21.12	2.44	8.63	<.0001	16.31	25.93
Volatile acids	1	8.22	1.58	5.19	<.0001	5.10	11.33
Total acids	1	-1.08	0.41	-2.63	0.0089	-1.89	-0.27
Citric acid	1	4.80	2.32	2.15	0.0326	0.42	9.54
Dry extract	1	-0.19	0.04	-4.99	<.0001	-0.29	-0.12
Absorbance 420 nm	1	2.21	0.21	10.42	<.0001	1.79	2.63

Table 7: Sum of squares table for the most frequently selected quantitative variables and region

Source	DF	Type III SS	Mean square	F-value	Pr > F
Volatile acids	1	190.13	190.13	14.68	0.0001
Absorbance*	1	1396.04	1396.04	107.78	<.0001
Region	21	860.93	40.70	3.16	<.0001
Citric acid	1	28.19	28.19	2.18	0.1410
Total acids	1	31.36	31.36	2.42	0.1206
Dry extract	1	46.49	46.49	3.59	0.0589

*420 nm

Fig. 1: Box plot of antioxidant capacity values by country (left) and grape variety (right)



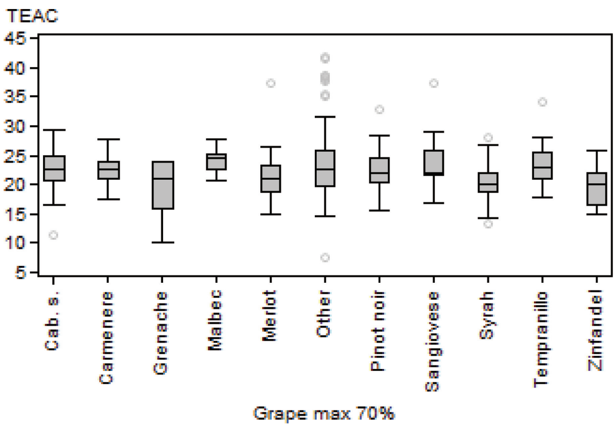
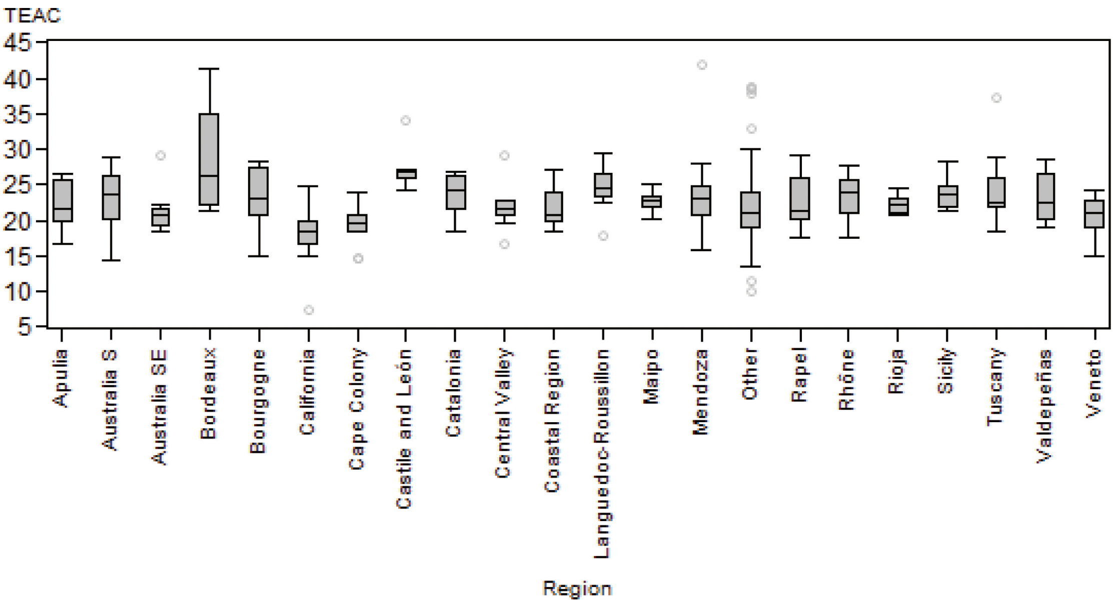


Fig. 2: Box plot of antioxidant capacity values by region



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Received July 7, 2012