A QCAR model for predicting antioxidant activity of wild mushrooms

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Wild mushrooms have been described as sources of natural antioxidants, particularly phenolic compounds. However, many other compounds present in wild mushrooms can also act as antioxidants (reducers), so whole extracts from a wide range of species need to be examined. To gain further knowledge in this area, the relationship between the antioxidant potential (scavenging effect and reducing power) and chemical composition of twenty three samples from seventeen Portuguese wild mushroom species was investigated. A wide range of analytical parameters reported by our research group (including ash, carbohydrates, proteins, fat, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, phenolics, flavonoids, ascorbic acid and β-carotene) were studied and the data were analysed by partial least squares (PLS) regression analysis to allow correlation of all the parameters. Antioxidant activity correlated well with phenolic and flavonoid contents. A QCAR (Quantitative Composition-Activity Relationships) model was constructed, using the PLS method, and its robustness and predictability were verified by internal and external cross-validation methods. Finally, this model proved to be a useful tool in the prediction of mushrooms' reducing power.

Keywords: wild mushrooms; chemical composition; antioxidant activity; QCAR; PLS

1. Introduction

Free radicals play important roles in many physiological and pathological conditions [1]. In general, excess of free radicals caused by the imbalance between free radical generation and scavenging may contribute to disease development. Free radicals can damage membranes, proteins, enzymes and DNA, increasing the risk of diseases such as cancer, Alzheimer's, Parkinson's, angiocardiopathy, arthritis, asthma, diabetes, and degenerative eye disease [1,2].

Mushrooms have become attractive as functional foods and as a source of physiologically beneficial compounds including antioxidants [3,4]. Different wild mushroom species were reported to have antioxidant activity, which was mainly related to their phenolic content [5–11]. Nevertheless, none of the available reports present a quantitative study to obtain a predictive model for antioxidant potential. Furthermore, other chemical

substances in mushrooms including proteins, carbohydrates, vitamins and fibres could also contribute to the antioxidant capacity [12].

Our recent investigation provided an insight to the chemical composition, nutritional value and antioxidant properties of several wild mushroom species from the northeast of Portugal, one of the European regions with a higher diversity of wild edible mushrooms, valorising mushrooms as a source of nutrients and nutraceuticals [13–17]. The analysis of nutrients included determination of proteins, fats, ash, and carbohydrates, particularly sugars by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI). The analysis of nutraceuticals included determination of fatty acids by gas-chromatography with a flame ionisation detector (GC-FID), and other phytochemicals such as phenolic compounds by HPLC coupled to a diode array detector and mass spectrometry (HPLC/DAD-ESI/MS), carotenoids and ascorbic acid, by spectrophoto-metric techniques. The antioxidant activity was screened through chemical and biochem-ical assays [15,16,18–20]. Numerous tests have been used for measuring the antioxidant capacity of food and biological samples. However, there is no universal method that can measure the antioxidant capacity of all samples accurately and quantitatively [21]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) and reducing power (RP) assays are two of the most widely used methods for antioxidant activity screening.

The relationship between chemical substances including phenolic compounds and antioxidant properties may be complex, and there are very little data to elucidate the relationship between chemical composition and antioxidant capacity of wild mushrooms. Herein, the antioxidant potential (RSA and RP) and chemical composition of some Portuguese wild mushrooms were evaluated using linear regression analysis (partial least squares, PLS), in order to find possible relationships between those parameters. Furthermore, a quantitative composition-activity relationships (QCAR) model was constructed in order to predict the reducing power of mushrooms.

2. Materials and methods

2.1 *Data set*

A total of 23 samples from 17 Portuguese wild mushroom species were used in this study (Table 1). The samples were selected using the following criteria: wild mushrooms studied by our research group using the same methodologies; results of chemical composition and antioxidant activity available on the same sample. In some species, results considering different stages of fruiting body growth (SI, SII, and SIII) and different conservation conditions (frozen and dehydrated) were available, and therefore used in this study.

The samples were collected in Bragança (in the northeast of Portugal), in the autumns of 2005 and 2006. Different chemical parameters (ash, carbohydrates, proteins, fat, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), phenolics, flavonoids, ascorbic acid and β-carotene), radical scavenging activity (RSA) and reducing power (RP) values were obtained from previous reports of our research group [13–20].

Ash was determined by incineration at $600 \pm 15°C$; the proteins content ($N \times 4.38$) was estimated by the macroKjeldahl method; fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; carbohydrates were calculated by difference; saturated fatty acids (SFA),

Table 1. Chemical composition and antioxidant activity (reduction power, RP and radical scavenging activity, RSA) values of Portuguese wild mushrooms.

Species	Ash (g/100g)	Carbohydrates (g/100g)	Crude proteins (g/100g)	Total fat (g/100g)	PUFA (%)	MUFA (%)	SFA (%)	Phenolics (mg/g ext)	Flavonoids (mg/g ext)	Ascorbic acid (mg/g ext)	β-Carotene (µg/g ext)	EC_{50} (RP) (mg/ml)	EC_{50} (RSA) (mg/ml)	References
Agaricus arvensis	3.53	37.45	56.27	2.75	–	–	–	2.72	1.65	0.02	8.52	4.2	15.85	[13,18]
Agaricus bisporus	9.9	8.25	80.93	0.92	76.41	1.52	22.1	4.49	1.73	0.03	1.95	3.63	9.61	[14,18]
*Agaricus romagnesii**	–	–	–	–	–	–	–	6.18	2.87	0.04	1.32	2.23	6.22	[18]
Agaricus silvaticus	16.48	9.49	71.99	2.05	75.23	7.67	17.1	8.95	3.88	0.04	5.42	2.08	5.37	[14,18]
Agaricus silvicola	14.93	12.18	70.47	2.43	76.95	4.25	18.8	6.4	3.4	0.04	3.02	3.24	6.39	[14,18]
Cantharellus cibarius	12.12	14.25	69.14	4.49	54.08	23.29	22.6	0.88	0.67	0.86	13.56	5.89	7.41	[14]
*Hypholoma fasciculare**	–	–	–	–	–	–	–	17.67	5.09	0.09	24.62	0.95	1.13	[15]
Lactarius deliciosus (F)	9.53	57.68	24.33	8.45	21.68	24.69	53.5	2.95	1.91	–	–	4.65	20.54	[16,22]
Lactarius deliciosus (D)	14.28	60.3	17.87	6.47	23.65	21.85	54.4	3.4	2.71	0.24	90.1	4.98	16.31	[16,22]
Lactarius piperatus (SIII)	9.8	42.48	40.79	6.93	8.64	27.33	64	3.09	0.35	0.13	17.22	5.4	20.24	[17,19]
Lactarius piperatus (SII)	8.77	70.06	14.74	6.44	6.94	11.51	81.5	5.76	1.58	0.16	33.78	2.29	5.19	[17,19]
Lactarius piperatus (SI)	6.94	78.18	6.86	8.11	6.74	6.63	86.6	5.52	1.26	0.15	26.08	2.83	12.92	[17,19]
Lepista nuda	18.46	24.88	59.39	1.77	52.1	30.32	17.6	6.31	3.36	0.23	2.52	3.53	4.41	[15]
Lycoperdon molle	20.16	62.33	16.77	0.73	65.92	9.04	25	11.48	2.45	0.34	4.48	2.27	3.23	[15]
Lycoperdon perlatum	31.89	50.57	17.09	0.44	71.52	4.91	23.6	10.57	2.1	0.21	12.5	2.96	3.95	[15]
Macrolepiota mastoidea (D)	7.96	67.6	21.89	2.55	60.76	19.04	19.9	3.08	2.1	–	–	4.35	8.18	[16]
Macrolepiota mastoidea (F)	11.76	60.68	24.51	3.05	60.89	20.27	18.6	2.69	1.56	–	–	4.44	8.49	[16]
Macrolepiota procera (D)	9.86	80.38	7.62	1.45	64.72	10.17	24.6	3.17	0.99	–	–	4.18	5.38	[16]
Macrolepiota procera (F)	916	79.28	9.36	2.18	62.31	15.23	22.3	2.59	0.9	–	–	4.49	6.95	[16]
Ramaria botrytis	8.8	50.05	39.89	1.37	38.91	44.69	16.4	20.32	16.56	0.27	10.41	0.68	0.66	[15]
Sarcodon imbricatus (D)	12.14	54.43	29.98	3.45	28.69	50.46	20.7	3.06	1.52	0.16	2.53	4.41	5.82	[16,20]
Sarcodon imbricatus (F)	8.31	55.98	25.71	8.94	25.37	52.88	21.6	2.22	1.12	–	–	5.94	10.98	[16,20]
*Tricholoma acerbum**	–	–	–	–	–	–	–	5.53	1.87	0.22	75.48	3.27	3.6	[15]

Notes: *More that 50% missing data; F, frozen mushrooms; D, dehydrated mushrooms; SI, immature (cap diameter less than 4.5 cm); SII, mature (cap diameter between 4.5 and 7 cm) with immature spores; SIII, mature (cap diameter higher than 7 cm) with mature spores. All the other samples were lyophilised and in SII.

monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were determined by GC-FID after a trans-esterification procedure. Phenolics, flavonoids, ascorbic acid and β-carotene were determined by spectrophotometer assays. These phytochemicals are frequently analysed in mushrooms and reported in literature.

For antioxidant activity data we used the results of two *in vitro* chemical assays, previously reported by us: reducing power (RP [measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form]) and scavenging activity on DPPH radicals (RSA [measuring the decrease in DPPH radical absorption after exposure to radical scavengers]). These assays are the most commonly used methods for assessment of the antioxidant properties of natural products. Both assays are suitable for solvent extracts and as rapid assays, they can be applied for monitoring the activity of numerous samples over a limited period of time [12,23]. Moreover, they are reproducible and strongly correlated with phenolic compounds [12]. The RSA was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100, \tag{1}$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The RP was obtained by measuring the absorbance of the product obtained by the reduction of the ferric ion to the ferrous form; a higher absorbance indicates higher RP.

To make the RSA and RP data homogenous and directly comparable, all the values were reported as EC_{50} (expressed in mg/mL, concentration required to achieve 50% of RSA or 0.5 of absorbance in RP).

2.2 *Statistical analysis*

The relationships between antioxidant activity (RP and RSA) and the different chemical composition parameters were studied using the PLS [24] method implemented in SIMCA-P v12 statistics software [25], and using NIPALS algorithm for missing data [26]. Because *Agaricus romagnesii*, *Hypholoma fasciculare*, and *Tricholoma acerbum* had more than 50% of missing data, these observations were not used in the models.

The statistical parameters for this study are described in Table 1. The goodness of fit of the model was evaluated using the following statistical parameters: squared correlation coefficient (r^2), standard deviation of regression (s), significance of the model (ρ) and Fisher ratio value (F).

The predictive stability and robustness of the model was first verified by internal cross-validation by calculating the following parameters: Q^2_{LOO} ("Leave-One-Out"; 1-PRESS/TSS were PRESS is the predictive error sum of squares and TSS the total sum of squares) and $RMSE_{(training\ set)}$ (root mean squared errors for the training set) [27,28].

2.3 *QCAR model*

To build the QCAR model the complete data set (Table 1) and the PLS method implemented in SIMCA-P v12 were used. The 23 samples were first divided into two groups: training and test sets. The training set, representing about three-quarters of the total number of samples (17 samples), was used to build the QCAR model. The remaining quarter (6 samples) was assigned to the test set and used to validate the model.

The division was made to cover the entire antioxidant activity scale [29,30] and the samples included in the training set were randomly selected within each group [31].

The goodness of fit of the models was evaluated using r^2, s, ρ, and F. The predictive stability and robustness of the model was first verified by internal cross-validation calculating the following parameters: Q^2_{LOO}, permutation test of SIMCA-p software and $RMSE_{(training\ set)}$ [27,28,32]. Using the test set, the model was further checked by external cross-validation by calculating parameters: Q^2_{ext} (External, 1-PRESS/SD) and $RMSE_{(test\ set)}$ (root mean squared errors for the test set). PRESS is defined as the sum of the squared difference between the observed value and the predicted value for each compound in the test set, and SD is defined as the sum of the squared deviation between the observed value and the mean measured value of the training test [27].

3. Results and discussion

3.1 *Relationships between antioxidant activity and chemical composition*

The relationships between antioxidant activity (RP and RSA) and several chemical components from various species of Portuguese wild mushrooms were evaluated using PLS regression (Figure 1). This approach has been adopted because PLS can predict possible relationships using the chemical variables as explicative ones. PLS extracts a few linear combinations (PLS factors) of the chemical and antioxidant data that predict as much of the systematic variation in the sample data as possible. Standardized regression coefficients for the relationships between variables revealed by the PLS were estimated by cross-validation and were used to evaluate the importance of each chemical component on antioxidant activity (Figure 1).

This analysis presented good statistical parameters for both RP and RSA, as summarized in Table 2. Information about some chemical compounds was not available (Table 1). To overcome this lack of information, we used a specific statistic algorithm available in SIMCA-P [26]. This algorithm dynamically selects samples with more than 50% of the chemical compound results. From the data set available, four mushroom species did not meet this criterion and were not considered in this initial analysis.

A similar relationship between chemical composition and antioxidant activity was observed for RSA (Figure 1a) and RP (Figure 1b), with the exception of fatty acids and ascorbic acid. A close observation of the standardized coefficients of the analysed chemical parameters shows that antioxidant activity is strongly positively related to phenolics and flavonoids contents (Figure 1). This is in agreement with several manuscripts reporting phenolic compounds as the main antioxidant substances in mushrooms, particularly phenolic acids and flavonoids [33]. Phenolic substances (ArOH) serve as oxidation terminators by scavenging radicals to form resonance stabilized radicals [34], according to:

$$RO_2^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$$
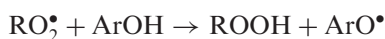
Ascorbic acid is a well-known powerful antioxidant, reported in mushrooms in lower amounts than phenolics [33]. Accordingly, it seems to contribute positively to the RSA but with less significance. Otherwise, it gives a negative contribution to the reducing power of the samples. In fact, the ascorbic acid mechanism of action has been related to free radicals scavenging effects and not to reducing processes with electrons transference (which is present in the RP assay – reducing Fe^{3+} to Fe^{2+}); ascorbic acid protect biomembranes against lipid peroxidation damage by eliminating peroxyl radicals in the
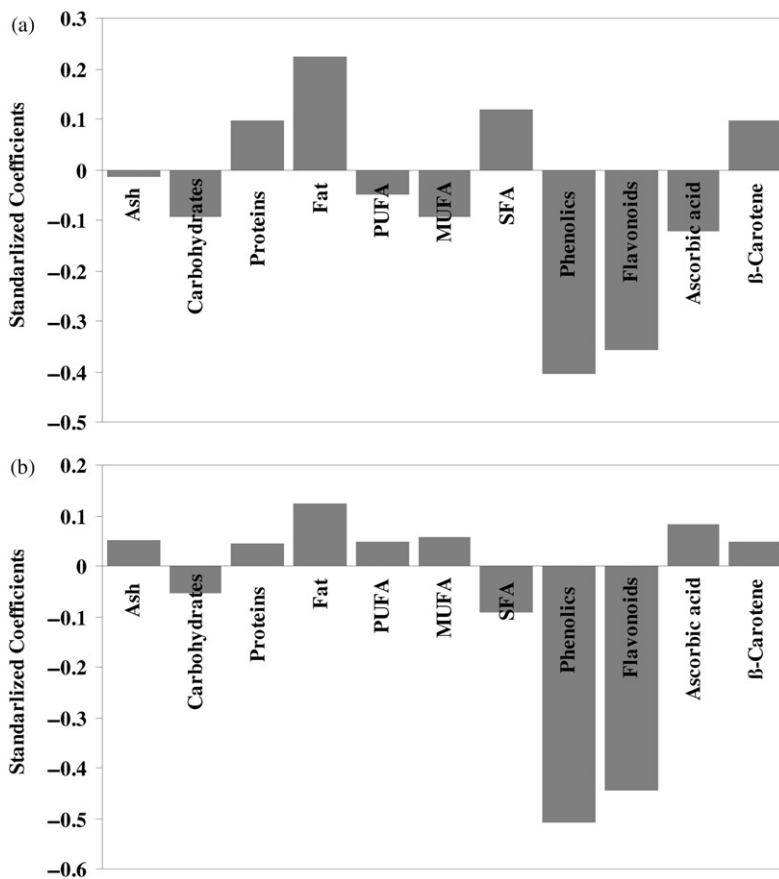
Figure 1. Standardized coefficients of the chemicals compounds, Ash, Carbohydrates, crude protein (Proteins), total fat (Fat), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), total phenols (Phenolics), Flavonoids, Ascorbic acid and β-Carotene, used in the approached model for radical scavenging activity (a) and reducing power (b).

Table 2. Statistical parameters of the models, radical scavenging activity (RSA) and reducing power (RP), using the PLS method.

Parameter	RSA	RP
n	20	20
s	2.985	0.488
r^2	0.84	0.92
ρ	0.011	0.023
F	4.74	3.87
Q^2_{LOO}	0.59	0.70
RMSE	3.1942	0.5162

Notes: n, number of samples; s, standard deviation; r^2, squared correlation coefficient; ρ, significance; F, Fisher ratio; Q^2_{LOO}, "Leave-One-Out" correlation coefficient; RMSE, root mean squared errors for the training set.

aqueous phase before the latter can initiate lipid peroxidation [35]. Carbohydrates gave a small positive contribution possibly due to the presence of mannitol (reducing sugar), a very abundant sugar in mushrooms which functions to provide support and expansion of the fruit body [13–17]. Unsaturated fatty acids (MUFA and PUFA) also show a small positive contribution to RSA but a negative contribution to RP; the presence of double bonds make them susceptible to oxidation – they can react with free radicals and become radicals themselves. Therefore, they act mostly as free radical scavengers. Particularly, oleic (C18:1) and linoleic acid (C18:2), abundant in mushrooms [13–17], proved to have more than 80% of RSA [36]. Nevertheless, it should be noted that fatty acids exist in mushrooms at very low concentrations.

Surprisingly, β-carotene gave a small negative contribution to the antioxidant activity. This can be explained by the fact that this compound is present in mushrooms only in vestigial amounts [33]. The negative correlation between the fat and the antioxidant properties was expected since total fat obtained by soxhlet extraction includes linked compounds without reducing antioxidant properties and no free fatty acids. To obtain fatty acids, a derivatization process should be done. Saturated fatty acids (SFA) are not antioxidants and therefore, seem not to contribute to the scavenging effects. Stearic acid (C18:0), a fatty acid abundant in mushrooms, proved to have less than 20% of RSA [36]. Surprisingly, SFA show a slightly positive contribution to the RP of samples, probably through the reducing properties of the carboxylic moiety. The biosynthesis of phenolic compounds is derived from some amino acids, including tyrosine and tryptophan, in the shikimic acid pathway. A possible explanation for the negative contribution of proteins to the antioxidant properties is that they might be used as a source of amino acids to obtain phenolics, decreasing proteins content. The negative correlation between the ash and the reducing properties can also be explained as ash contains minerals and heavy metals (including iron) which can act as pro-oxidants [12].

3.2 QCAR model

Based on the preliminary analysis we then set out to develop a QCAR model that could be used for predictive purposes. As phenolic and flavonoid contents were the parameters that better correlated to antioxidant activity they were chosen to build the model. Also RP analysis consistently gave better statistical parameters when compared to RSA analysis (Table 2) and thus we selected RP values to build the predictive QCAR model (Table 3).

Figure 2 shows the number of samples assigned to the three groups of antioxidant activity: 0–3, 3–4.5 and 4.5–6 according to EC_{50} values.

The QCAR model equation obtained and the statistical parameters was the following:

$$EC_{50} RP = 10^{(-0.0239517 \times \text{Phenolics (mg/g ext)} - 0.027891 \times \text{Flavonoids (mg/g ext)} + 0.740363)} \qquad (2)$$

$n = 17$, $r^2 = 0.84$, $s = 0.8114$, $F = 37.53$, $\rho = 2.37 \times 10^6$, $Q^2_{LOO} = 0.83$, $RMSE_{\text{(training set)}} = 0.7507$, $Q^2_{ext} = 0.95$, $RMSE_{\text{(test set)}} = 0.4979$

where n is the number of samples used, r^2 is the squared correlation coefficient, ρ is the significance parameter of the model, F is the Fisher ratio, Q^2_{LOO} is the "Leave-One-Out" correlation coefficient and $RMSE_{\text{(training set)}}$ and $RMSE_{\text{(test set)}}$ are root mean squared errors for the training and test sets, respectively.

The model was evaluated for its robustness and predictive power by internal leave-one-out (LOO) validation, as demonstrated by r^2 and Q^2 values, and by external validation
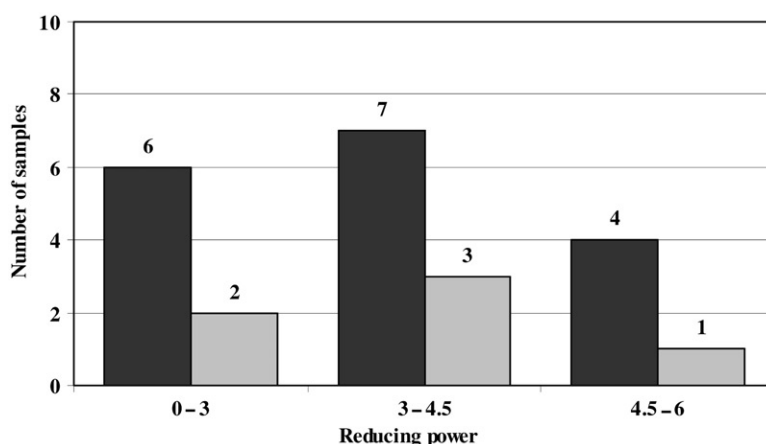
Figure 2. Distribution of reducing power (EC$_{50}$ RP) versus number of samples for the training set (black) and test set (grey) of the QCAR model.

Table 3. Phenolics, flavonoids, experimental and predicted EC$_{50}$ reducing power values of Portuguese wild mushrooms.

Species	Phenolics (mg/g ext)	Flavonoids (mg/g ext)	Experimental EC$_{50}$ RP	Predicted EC$_{50}$ RP	Residues
Agaricus arvensis	2.72	1.65	4.20	4.26	−0.06
Agaricus bisporus	4.49	1.73	3.63	3.84	−0.21
Agaricus romagnesii	6.18	2.87	2.23	3.25	−1.02
Agaricus silvaticus	8.95	3.88	2.08	2.62	−0.54
*Agaricus silvicola**	6.40	3.40	3.24	3.11	0.13
Cantharellus cibarius	0.88	0.67	5.89	5.02	0.87
*Hypholoma fasciculare**	17.67	5.09	0.95	1.50	−0.55
Lactarius deliciosus F	2.95	1.91	4.65	3.83	0.82
Lactarius deliciosus D	3.40	2.71	4.98	4.13	0.85
Lactarius piperatus (SIII)*	3.09	0.35	5.40	3.74	1.66
Lactarius piperatus (SII)	5.76	1.58	2.29	3.62	−1.33
Lactarius piperatus (SI)	5.52	1.26	2.83	4.54	−1.71
Lepista nuda	6.31	3.36	3.53	3.13	0.40
Lycoperdon molle	11.48	2.45	2.27	2.49	−0.22
Lycoperdon perlatum	10.57	2.10	2.96	2.68	0.28
Macrolepiota mastoidea D*	3.08	2.10	4.35	4.06	0.29
Macrolepiota mastoidea F*	2.69	1.56	4.44	4.29	0.15
Macrolepiota procera D	3.17	0.99	4.18	4.33	−0.15
Macrolepiota procera F	2.59	0.90	4.49	4.50	−0.01
Ramaria botrytis	20.32	16.56	0.68	0.62	0.06
Sarcodon imbricatus D	3.06	1.52	4.41	4.21	0.20
Sarcodon imbricatus F	2.22	1.12	5.94	4.53	1.41
*Tricholoma acerbum**	5.53	1.87	3.27	3.60	−0.33

Notes: *Test set observations; F, frozen mushrooms; D, dehydrated mushrooms; SI, immature (cap diameter less than 4.5 cm); SII, mature (cap diameter between 4.5 and 7 cm) with immature spores; SIII, mature (cap diameter higher than 7 cm) with mature spores. All the other samples were lyophilised and in SII.
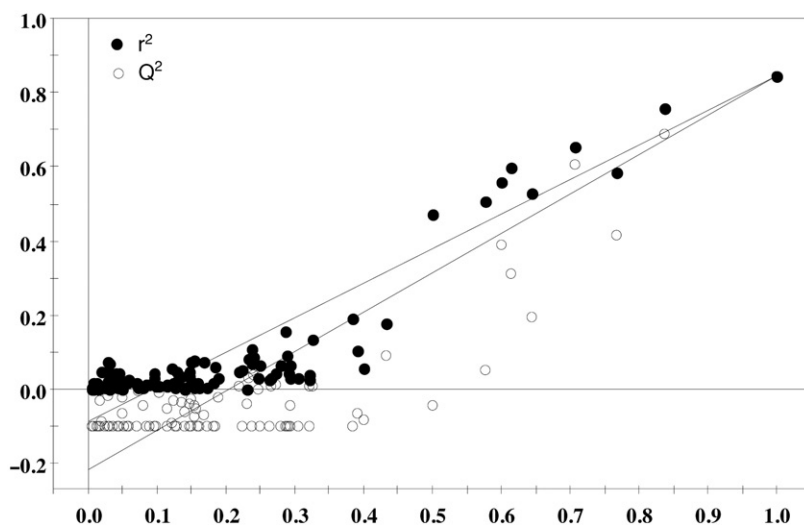
Figure 3. Results of the permutation test. The r^2 and Q^2 values were obtained from 100 permutations for the four developed PLS models. Intercepts: (r^2) $r^2_{\text{permutation test}} = 0, -0.0818$; ($Q^2$) $Q^2_{\text{permutation test}} = 0, -0.244$.

as demonstrated by the Q^2_{ext} value. Also RMSE values, for both the training and test sets, validate the model by presenting low and similar values. External validation is acknowledged to be the best method to validate a model as it is usually immune to over-fitness and over-prediction.

To validate the model even further, a permutation test was performed [27]. In this test the model is recalculated for randomly reordered response data (EC_{50} RP) and these permuted EC_{50} RP values are related to intact predictor data by refitting the model and including cross-validation. When $r^2_{\text{permutation test}}$ and $Q^2_{\text{permutation test}}$ are plotted as a function of the correlation coefficient between the original values and the predicted values, the intercept with the Y axis expresses to which degree these values rely on chance. Figure 3 shows the results obtained from 100 permutations for each of the samples under study. The intercepts of the two regression lines (for $r^2_{\text{permutation test}}$ and $Q^2_{\text{permutation test}}$) indicate the degree of overfitting and overprediction. Intercepts for $r^2_{\text{permutation test}}$ and $Q^2_{\text{permutation test}}$ below 0.30 indicate a valid model, as is the case.

A plot of predicted EC_{50} RP versus experimental EC_{50} RP values, for both the training and test sets, is shown in Figure 4. The agreement observed between the predicted and experimental values confirms the predictability of this QCAR model. A plot of the residuals (predicted EC_{50} RP–experimental EC_{50} RP) versus experimental EC_{50} RP, for both the training and test sets is shown in Figure 5. A random distribution of the residuals about zero was observed for both sets. Because of this, no sample was considered a possible outlier as residuals were all within the standard deviation interval ($-3s$ to $3s$) usually considered the limit line for spotting outliers. The relation between RP and phenolic and flavonoids content was not linear, but rather exponential. This is an indication that higher contents of these compounds will elevate by order of magnitudes RP (lower RP values means better antioxidant activity). Overall, as far as we know, this is the
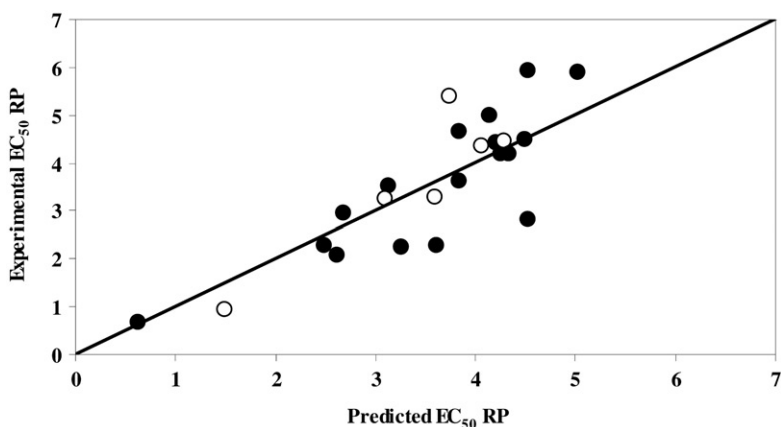
Figure 4. Predicted versus experimental EC_{50} RP for the training (●) and test sets (○).
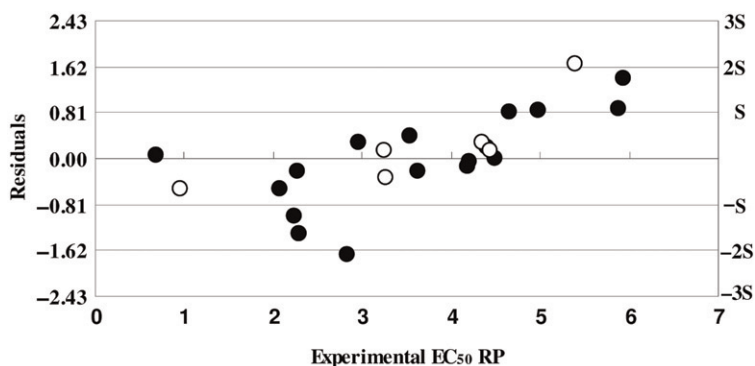

Figure 5. Residual versus experimental log EC_{50} RP for the training (●) and test sets (○).

first report of a QCAR model to predict reducing power using the chemical composition of mushrooms.

4. Conclusion

Several relationships were established between antioxidant activity and chemical composition that provide a better understanding of the complex mechanisms of antioxidant activity of wild mushrooms. The positive effect of flavonoids and phenolic compounds was clearly established and other possible correlations with different composition parameters were discussed. This information allowed the development of a predictive QCAR model using mushroom flavonoid and phenolic composition. Several internal and external validation protocols were used and confirm the predictive power of the QCAR model. External validation is acknowledged to be the best rational protocol to avoid over-prediction and over-fitting. This model will be a useful tool in the study and prediction of antioxidant activity of more mushroom species.

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