Exploring the Relationship between HPLC/DAD/MS and Folin Ciocalteau (FC) Method for Minor Polar Compounds Analysis in Extra Virgin Olive Oil

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1 ABSTRACT

Minor Polar Compounds (MPC) of 88 extra virgin olive oils were analysed by HPLC/DAD/MS and by Folin Ciocalteau (FC) spectrophotometric method which quantifies total polyphenols as gallic acid equivalents. The aim of this work was to validate and evaluate the linear association between FC and HPLC data. Data were analysed by Principal Component Analysis (PCA). The eigenvalues indicate that the first three principal components provide a good summary of the data, accounting for about 81.1%.
The first component shows approximately equal loadings on almost all variables, with two remarkable exceptions: Lignans and Apigenin. Notably, the highest positive loadings involve FC, Deacetoxy-oleuropein Aglycone and Oleuropein Aglycone. The second eigenvector, on the contrary has the highest loadings on the variables Lignans (positive) and Flavonoids (negative). Here FC and diphenols (Deacetoxy-oleuropein Aglycone and Oleuropein Aglycone) shows extremely low loadings. It was concluded that FC can reliably estimate total diphenols but cannot give information about valuable compounds such as lignans and flavonoids.

2 INTRODUCTION

Currently, the two most commonly used methods to evaluate polyphenolic content of olive oil are the well-known Folin-Ciocalteu (FC) colorimetric assay [1, 2] and high-performance liquid chromatography (HPLC). FC is a simple and highly efficient procedure, but it is limited by a low specificity. HPLC is sensitive and specific but it is very time consuming and requires special expertise and laboratory [3], [4], [5]. Consistent information about FC validation by reliable analytical protocols is currently lacking for olive oil quality assessment. The purpose of this work is to explore the relationship of FC's results with the results by HPLC/DAD/MS in olive oil analysis, focusing also on the role of the single molecules or classes of minor polar compounds quantified by HPLC, and the corresponding FC results.

Table of the Eigenvectors

	Prin1	Prin2	Prin3	Prin4	Prin5
FC	0.377908	0.060257	-.097702	-.048492	0.175224
Lignans	-.033733	0.595712	0.605264	0.040033	-.016278
Apigenin	0.115629	-.619152	0.526068	0.017164	0.077797
Luteolin Aglycone	0.278545	-.369038	0.340743	-.081912	0.063569
Elenolic Acid Deriv.	0.281991	0.172359	0.249899	-.375483	-.642241
Elenolic Acid	0.290918	0.261645	0.143286	-.281026	0.656556
Tyrosol	0.275888	0.136391	0.104335	0.759859	0.096240
5-hydroxytyrosol	0.345924	-.018633	-.075394	0.386478	-.297333
Deacetoxy-oleurop.	0.371676	0.029584	-.184013	-.117001	-.078041
Oleuropein Ag.	0.380166	0.049035	-.112277	-.036448	-.070261
Secoiridoid Deriv.	0.349988	-.039524	-.291959	-.165260	0.081867

3 MATERIALS AND METHODS

Eighty-eight commercial extra virgin olive oils were selected and analyzed. They were crushed during the period 2002-08 and derived from the Italian cultivars or homogeneous mixtures of cultivars Carboncella, Frantoio, Leccino, Leccio del Corno, Madonna dell'Impruneta, Mignolo, Moraiolo, Pendolino, Seggianese, Taggiasca, mixture of Frantoio, Moraiolo, Leccino and Pendolino, mixture of Frantoio, Moraiolo, Leccino, and Correggiolo, mixture of Frantoio, Moraiolo and Leccino.

The HPLC method adopted was reported elsewhere [6]. It allowes the determination at different wavelengths (280, 350 and 240 nm) of single molecules belonging to different subclasses. The compounds evaluated by HPLC/DAD/MS were 5-hydroxytyrosol, tyrosol, deacetoxy-oleuropein aglycone, lignans as acetoxypinoresinol, oleuropein aglycone, a group of other secoiridoidic derivatives, luteolin, apigenin and elenolic acid.

The total phenolic content by the FC method was determined according to the analytical protocol described by Singleton et al. [9]. The method was adapted for oils as described elsewhere [9]. Data were explored by Principal Component Analysis (PCA).

Table of the Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	6.42583856	5.07681623	0.5842	0.5842
2	1.34902233	0.20375469	0.1226	0.7068
3	1.14526764	0.43290408	0.1041	0.8109
4	0.71236356	0.2574601	0.0648	0.8757
5	0.45490340		0.0414	0.9170

4 RESULTS, DISCUSSION AND CONCLUSIONS

The first principal component explains about 58.4% of the total variance, the second principal component explains about 12.3%, and the third principal component explains about 10.4%. The eigenvalues indicate that three out of five components provide a good summary of the data, with three components accounting for about 81.1% of the total variance (**Table of the Eigenvalues of the Correlation Matrix**).

The first component reflects overall performance of almost all variables since the first eigenvector shows approximately equal loadings on almost all of them, with two remarkable exceptions: Lignans and Apigenin (**Table of the Eigenvectors** and **Graph of the variables under study**). Notably, the highest positive loadings involve FC, Deacetoxy-oleuropein Aglycone and Oleuropein Aglycone. The second eigenvector on the contrary has the highest loadings on the variables Lignans (positive) and Flavonoids (negative). Here FC and diphenols (Deacetoxy-oleuropein Aglycone and Oleuropein Aglycone) shows extremely low loadings. The third eigenvector has a very high positive loading on Lignans and flavonoids, but low and negative loadings on FC and diphenols. In the first three components, the secoiridoidic derivatives basically follow diphenols.

This suggests that the FC and HPLC estimations of total diphenols content are reliably correlated, but the fact that lignans and flavonoids do not show any linear relationship with FC should be considered and suggests caution about interpretation of FC results for olive oils characterized by very different phenolic profiles.

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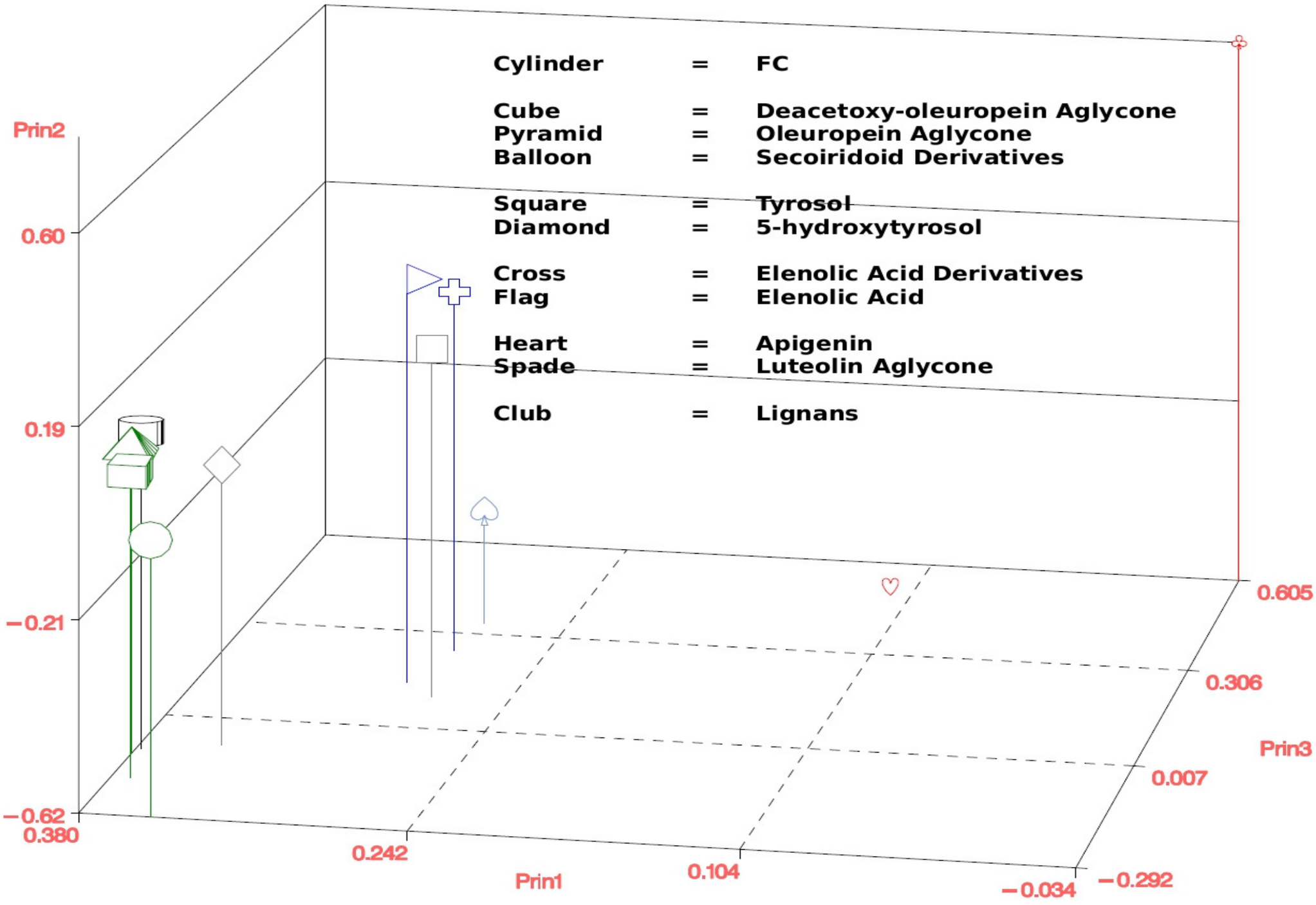

Graph of the variables under study

The variables are represented in the space of the first three principal components (Prin1, Prin2 and Prin3)