Chemometrical Optimization and Fast Determination of Debittering of Table Olives by Means of Capillary Electrophoresis

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Abstract

Table olives require a thorough process of debittering in order to achieve the organoleptic quality of olives before consumption. Several chromatographic analytical methods have been used in order to ascertain the debittering process. Capillary electrophoresis (CE) offers several advantages to the determination of the bitter responsible compound oleuropein in table olives, such as speed, reduced amount of solvents and reagents (green chemistry), small amount of sample, high efficiency and lower costs. Despite the evident advantages of CE, this technique has not been used so far for the verification of the debittering of table olives.

Objective

To extract oleuropein from table olives and optimize the analysis via CE using a design of experiments (DoE).

Material & Methods

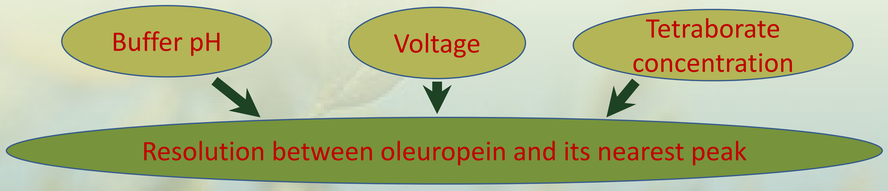
Soluble biophenols, oleuropein among them, were extracted using sequential liquid partition methods with methanol:acetone and hexane from table olives as described before (1). Finally, the samples in methanol were transferred to vials.

CE was run in a P/ACE System 2200 (Beckman Coulter Inc.) using an uncoated fused-silica capillary (50 µm ID x 375 µm OD, 50 cm length). UV detection was performed at 214 nm in cationic mode with pressure injection for 7s.

Method optimization

A preliminary screening experiment fixed the parameters for further optimization.

A **central composite** design was used considering the following factors:



Conclusions

- The obtained model explained **97.0%** of the data variance (R^2).
- A good separation was achieved and the method partially validated (repeatability, reproducibility, linear range).
- **The best separation was achieved at 21ºC; voltage 10 kV; and 20 mM tetraborate in 20 mM phosphate buffer at pH 10.0**.

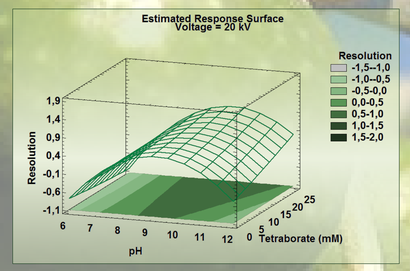
Reference

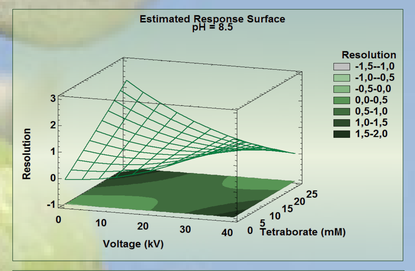
(1) Bianco A and Uccella N. (2000). Biophenolic compounds of olives. *Food Res. Int. 33,* 415-485.

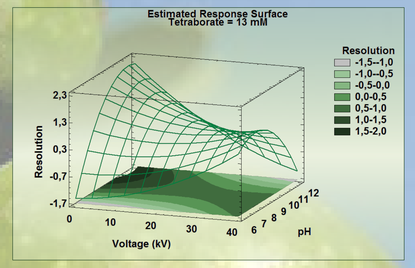
Results

Factor	Level				
	Lower	Central	Upper	Axial (+α)	Axial (-α)
Buffer pH	7	8.5	10	11.02	5.98
Voltage (kV)	10	20	30	36.82	3.18
Tetraborate (mM)	6	13	20	24.77	1.23

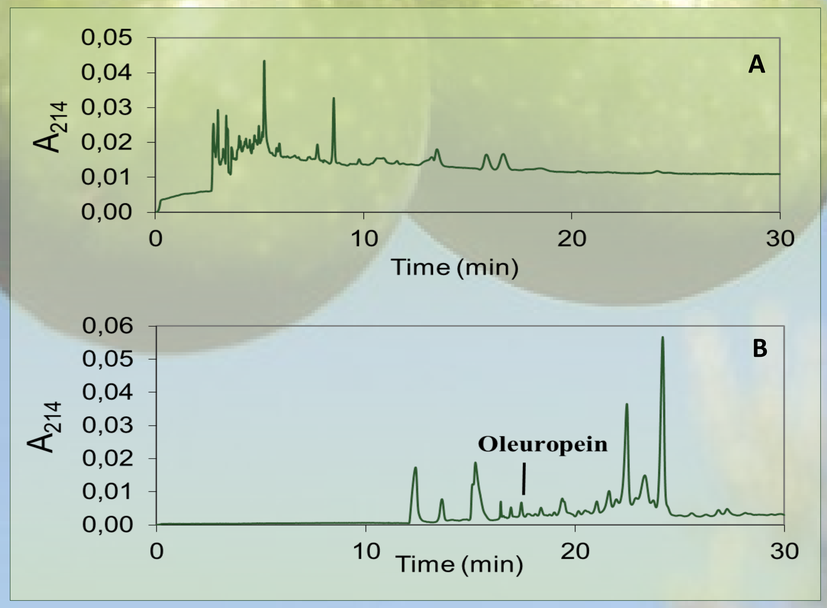
Table. Experimental factors and numeric values for the levels analyzed in the central composite design. Lower, central, upper and axial points are included.



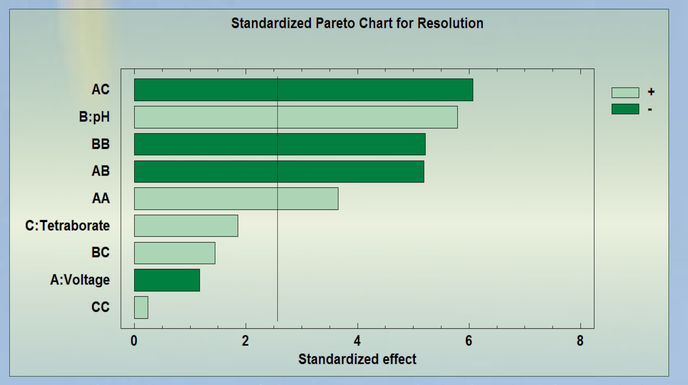




Response Surface Plots. Response surface plots calculated with the obtained model for the response comparing the three experimental factors two at a time while keeping constant the third one at its central value. Trend towards the optimal conditions can be observed for each plot (higher resolution value).



Electropherograms. (A) Electrophoretic separation of phenolic extract of olives without optimization of separation conditions. (Na_2HPO_4 20 mM, $Na_2B_4O_7 \cdot 10\ H_2O$ 20 mM, pH 10 and 30 kV). **(B)** Optimized conditions for oleuropein separation: **Na_2HPO_4 20 mM, $Na_2B_4O_7 \cdot 10\ H_2O$ 20 mM, pH 10 and 10 kV).**



Pareto Chart. Summary of the ANOVA analysis showing the statistical significance of the individual effects and their interactions. Five effects have p-values less than 0.05 (bars go beyond the vertical line), indicating that they are significantly different from zero at the 95% confidence level. The + or – indicate if the effect has a positive or negative effect in the response.

Run	Ret. Time	Area	Run	Ret. Time	Area	Run	Ret. Time	Area
#	(min)	(AU)	#	(min)	(AU)	#	(min)	(AU)
1	16.34	0.215	9	15.82	0.243	17	16.25	0.235
2	16.17	0.237	10	15.73	0.307	18	16.28	0.231
3	15.91	0.221	11	15.58	0.292	19	15.95	0.237
4	15.82	0.251	12	15.49	0.219	20	15.72	0.232
5	15.69	0.257	13	15.44	0.214	21	15.54	0.195
6	15.67	0.221	14	15.34	0.254	22	15.45	0.244
7	16.00	0.191	15	15.34	0.180	23	15.35	0.207
8	15.89	0.187	16	15.28	0.181	24	15.23	0.179
Mean	**15.93**	**0.222**	**Mean**	**15.50**	**0.236**	**Mean**	**15.72**	**0.220**
S.D.	**0.23**	**0.03**	**S.D.**	**0.19**	**0.05**	**S.D.**	**0.40**	**0.02**
C.V.	**1.44**	**11.42**	**C.V.**	**1.26**	**20.02**	**C.V.**	**2.56**	**10.64**

Repeatability table. Summary of the repeatability study, as part of the validation of the method, in three different days for samples of phenolic extracts of olive. Mean values are shown for the retention time and area of the oleuropein peak, along with the standard deviation (S.D.) and Coefficient of variation (C.V.).

Linearity of the method was studied with standards of oleuropein from 0 to 100 ppm at 10 ppm intervals with satisfactory values. R^2 >0.95.