

Optimization of combined microwave- and enzyme-assisted green extraction aiming to produce polyphenols-rich extracts from olive pomace

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1. Supplementary material

1.1. Tables

Table S1. Statistical analysis of the results of the 2⁴⁻¹ FFD used to determine the significant parameters for MEAE-Tan.

| | Effect | Std. error | P-value |
|-------------------------|--------|------------|----------|
| Mean/Intercept | 308.77 | 15.14 | 0.000001 |
| X1 (Temperature) | 7.48 | 30.38 | 0.400612 |
| X2 (Ramp and hold time) | 15.81 | 30.38 | 0.621496 |
| X3 Pomace:water ratio | -37.73 | 30.38 | 0.260623 |
| Enzyme concentration | 215.93 | 30.38 | 0.000390 |

Table S2. Statistical analysis of the results of the 2⁴⁻¹ FFD used to determine the significant parameters for MEAE-Tan-Cel-Pec.

| | Effect | Std. error | P-value |
|-------------------------|--------|------------|-----------|
| Mean/Intercept | 112.95 | 3.12 | <0.000001 |
| X1 (Temperature) | 32.56 | 6.25 | 0.002001 |
| X2 (Ramp and hold time) | -10.93 | 6.25 | 0.130885 |
| X3 Pomace:water ratio | 14.56 | 6.25 | 0.058748 |
| Enzyme concentration | 47.06 | 6.25 | 0.000285 |

Table S3. Analysis of variance (ANOVA) of MEAE-Tan results in 15 min of reaction.

| Source of variation | SS | DF ^a | MS | Fcal | Ftab | F Test ^b |
|---------------------|------------|-----------------|-------------|-------|-------|---------------------|
| Regression | 21179609.4 | 5 | 4235921.886 | 16.43 | 5.05 | 3.254 |
| Residue | 1288846.3 | 5 | 257769.2686 | | | |
| Lack of fit | 1225659.3 | 3 | 408553.0871 | 12.93 | 19.16 | 0.675 |
| Pure error | 63187.1 | 2 | 31593.54093 | | | |
| Total | 22468455.8 | 10 | | | | |

^aDegree of freedom. ^bF (0.95; 5.5) = 5.05; F (0.95; 3.2) = 19.16. R² = 0.94264.

Table S4. Role of enzyme concentration and pomace/water ratio on the extractability of OP phenolics using MEAE-Tan in 15 min of reaction (2² CCRD results at 15 min and validation of the predictive model).

| Run | Variables | | Total phenolic content (mg GAE/kg pomace) | | |
|-------------------|---|-----------------------------------|---|--------------------------------|-----------------|
| | X1 Enzyme concentration (%, w/w) | X2 Pomace:water ratio (w/w) | Y _{exp} ^a | Y _{pred} ^b | ΔY ^c |
| 1 | (-1) 0.9 | (-1) 1:11 | 3611.3 | 3835.3 | 224.0 |
| 2 | (1) 2.1 | (-1) 1:11 | 5053.86 | 5661.6 | 607.8 |
| 3 | (-1) 0.9 | (1) 1:4.7 | 1768.19 | 1333.7 | -434.5 |
| 4 | (1) 2.1 | (1) 1:4.7 | 3440.32 | 3389.6 | -50.7 |
| 5 | (-1.41) 0.66 | (0) 1:6.6 | 1397.06 | 1582.1 | 185.1 |
| 6 | (+1.41) 2.34 | (0) 1:6.6 | 4678.48 | 4319.2 | -359.3 |
| 7 | (0) 1.5 | (-1.41) 1:15 | 6388.8 | 5834.7 | -554.1 |
| 8 | (0) 1.5 | (+1.41) 1:4 | 2089.38 | 2469.3 | 379.9 |
| 9 | (0) 1.5 | (0) 1:6.6 | 2723.54 | 2929.1 | 205.6 |
| 10 | (0) 1.5 | (0) 1:6.6 | 3020.08 | 2929.1 | -91.0 |
| 11 | (0) 1.5 | (0) 1:6.6 | 3041.6 | 2929.1 | -112.5 |
| Validation | (2.34) 1.41 | (1:15) -1.41 | 7583.11 | 7110.6 | -472.5 |

^aExperimental value. ^bValue predicted by the quadratic model. ^cDifference between predicted and experimental values.

Table S5. Role of temperature, enzyme concentration, and pomace/water ratio on the extractability of OP phenolics using MEAE-Tan-Cel-Pec in 15 min of reaction (2³ CCRD results at 15 min and validation of the predictive model).

| Run | Variables | | | Total phenolic content (mg GAE/kg pomace) | | |
|-------------------|------------------------|---|-----------------------------------|---|-------------------------------|-----------------|
| | X1 Temperature (°C) | X2 Enzyme concentration (%, w/w) | X3 Pomace:water ratio (w/w) | Y _{pred} ^a | Y _{exp} ^b | ΔY ^c |
| 1 | (-1) 55 | (-1) 0.9 | (-1) 1:11 | 1403.45 | 1201.58 | 201.9 |
| 2 | (1) 65 | (-1) 0.9 | (-1) 1:11 | 1226.62 | 1037.43 | 189.2 |
| 3 | (-1) 55 | (1) 2.1 | (-1) 1:11 | 1944.94 | 1874.3 | 70.6 |
| 4 | (1) 65 | (1) 2.1 | (-1) 1:11 | 1768.32 | 1591.75 | 176.6 |
| 5 | (-1) 55 | (-1) 0.9 | (1) 1:4.7 | 657.74 | 720.3 | -62.6 |
| 6 | (1) 65 | (-1) 0.9 | (1) 1:4.7 | 610.59 | 567.22 | 43.4 |
| 7 | (-1) 55 | (1) 2.1 | (1) 1:4.7 | 728.51 | 803.69 | -75.2 |
| 8 | (1) 65 | (1) 2.1 | (1) 1:4.7 | 681.56 | 769.42 | -87.9 |
| 9 | (-1.68) 46.5 | (0) 1.5 | (0) 1:6.6 | 995.61 | 1020.9 | -25.3 |
| 10 | (+1.68) 73.5 | (0) 1.5 | (0) 1:6.6 | 807.63 | 943.92 | -136.3 |
| 11 | (0) 60 | (-1.68) 0.5 | (0) 1:6.6 | 527.30 | 693.72 | -166.4 |
| 12 | (0) 60 | (+1.68) 2.0 | (0) 1:6.6 | 1041.77 | 1036.94 | 4.8 |
| 13 | (0) 60 | (0) 1.5 | (-1.68) 1:20 | 2390.02 | 2715.01 | -325.0 |
| 14 | (0) 60 | (0) 1.5 | (+1.68) 1:4 | 850.75 | 687.34 | 163.4 |
| 15 | (0) 60 | (0) 1.5 | (0) 1:6.6 | 696.33 | 629.56 | 66.8 |
| 16 | (0) 60 | (0) 1.5 | (0) 1:6.6 | 696.33 | 735.42 | -39.1 |
| 17 | (0) 60 | (0) 1.5 | (0) 1:6.6 | 696.33 | 695.32 | 1.0 |
| Validation | (-1.68) 46.5 | (1.68) 2.0 | (-1.68) 1:20 | 2793.00 | 2938.25 | -145.25 |

^aTotal phenolic content predicted by the quadratic model. ^bExperimental total phenolic content.

^cDifference between predicted and experimental values.

Table S6. Analysis of variance (ANOVA) of MEAE-Tan-Cel-Pec results in 15 min of reaction.

| Source of variation | SS | DF ^a | MS | Fcal | Ftab | F-Test ^b |
|---------------------|------------|-----------------|-------------|-------|------|---------------------|
| Regression | 4603728.97 | 9 | 511525.4409 | 11.29 | 3.68 | 3.07 |
| Residue | 317119.5 | 7 | 45302.79014 | | | |
| Lack of fit | 311406.6 | 5 | 62281.32438 | 21.80 | 19.3 | 1.13 |
| Pure error | 5712.9 | 2 | 2856.454533 | | | |
| Total | 4920848.5 | 16 | | | | |

^aDegree of freedom. ^bF (0.95; 9,7) = 3.68; F (0.95; 5,2) = 19. R² = 0.93.

1.2. Figures

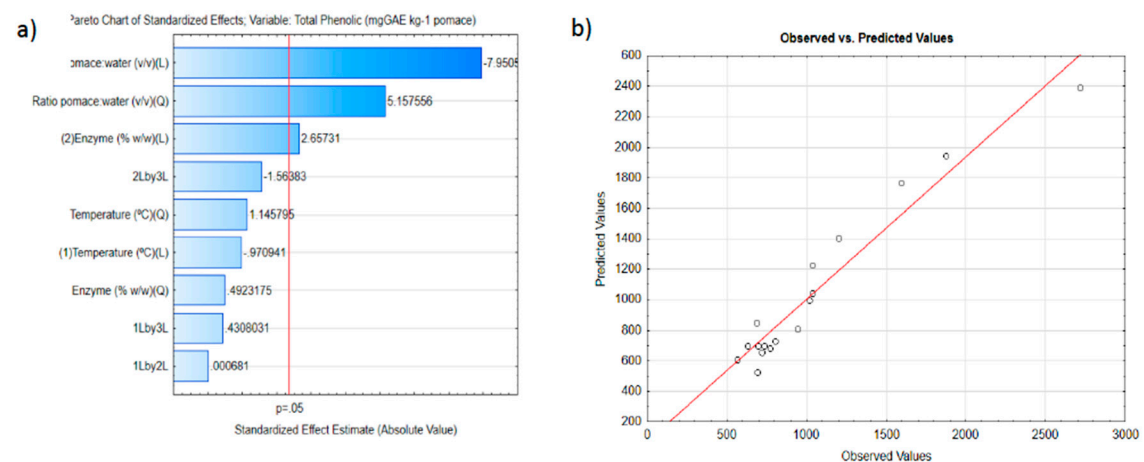
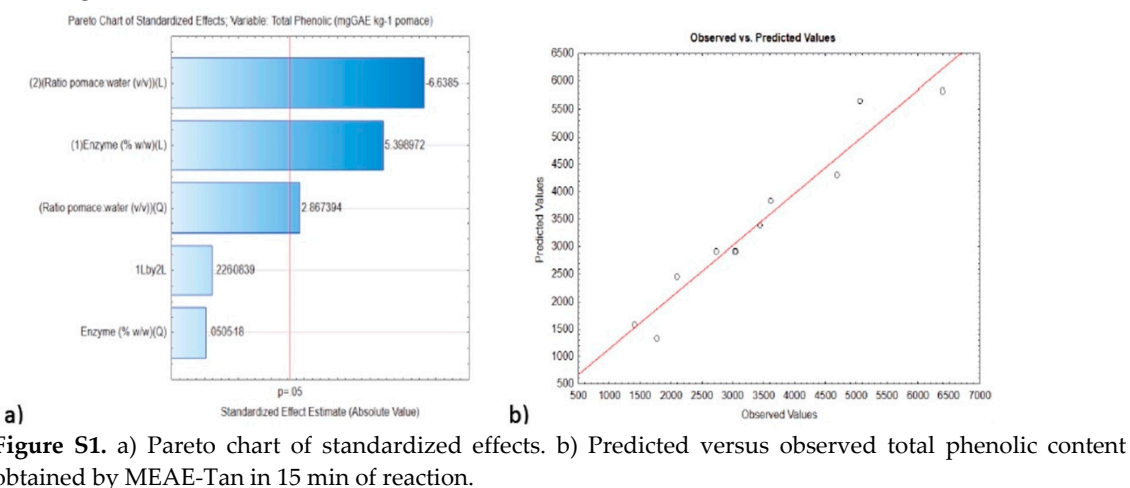


Figure S2. a) Pareto chart of standardized effects. b) Predicted versus observed total phenolic content obtained by MEAE-Tan-Cel-Pec in 15 min of reaction.