Article

Combining pressurized liquid extraction and enzymatic-assisted extraction to obtain bioactive non-extractable poly-phenols from sweet cherry (Prunus avium L.) pomace

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**Table S1.** Experimental design obtained by Box-Behnken and experimental results obtained under the designed conditions by PLE combined with EAE with Promod enzyme from extraction residue of conventional extraction of sweet cherry pomace using Folin-Ciocalteu (mg GAE/100 g sample), DMAC (mg epicatechin/100 g sample), vanillin (mg epicatechin/100 g sample), butanol/HCl (mg epicatechin/100 g sample), TEAC (µmol Trolox/g sample) and the capacity to inhibit the formation of hydroxyl radical (% of hydroxyl radical inhibition) assays response factors.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | | Extraction conditions | | | | | Analytical results | | | | | |
| Exp No | **Run order** | | **Time (min)** | **Temperature (ºC)** | **pH** | **Folin-Ciocalteu (mg GAE/100 g sample)** | | **DMAC (mg epicat/100 g sample)** | **Vanillin (mg epicat/100 g sample)** | **Butanol/HCl (mg epicat/100 g sample)** | **TEAC (µmol Trolox/g sample)** | **Hydroxyl radical assay (% inhibition hydroxyl radical)** |
| 1 | 15 | | 5 | 60 | 8 | 52.01 | | 0.43 | 60.14 | 7.57 | 0.007 | 7.62 |
| 2 | 4 | | 40 | 60 | 8 | 70.54 | | 0.84 | 112.25 | 0.86 | 0.012 | 14.99 |
| 3 | 6 | | 5 | 80 | 8 | 50.51 | | 0.14 | 47.94 | 1.00 | 0.010 | 17.52 |
| 4 | 10 | | 40 | 80 | 8 | 62.73 | | 0.42 | 52.25 | 0.51 | 0.010 | 18.87 |
| 5 | 9 | | 5 | 70 | 6 | 58.40 | | 0.38 | 58.70 | 30.70 | 0.007 | 37.10 |
| 6 | 17 | | 40 | 70 | 6 | 52.50 | | 0.70 | 65.20 | 15.36 | 0.004 | 39.77 |
| 7 | 5 | | 5 | 70 | 10 | 58.48 | | 0.51 | 58.04 | 1.22 | 0.007 | 21.37 |
| 8 | 16 | | 40 | 70 | 10 | 73.21 | | 0.54 | 57.65 | 12.61 | 0.007 | 7.42 |
| 9 | 11 | | 22.5 | 60 | 6 | 53.51 | | 0.50 | 85.95 | 14.83 | 0.004 | 41.07 |
| 10 | 13 | | 22.5 | 80 | 6 | 54.13 | | 0.58 | 61.81 | 12.62 | 0.006 | 60.68 |
| 11 | 1 | | 22.5 | 60 | 10 | 68.51 | | 1.10 | 67.30 | 15.05 | 0.006 | 24.43 |
| 12 | 8 | | 22.5 | 80 | 10 | 70.41 | | 0.70 | 74.22 | 36.26 | 0.014 | 35.34 |
| 13 | 3 | | 22.5 | 70 | 8 | 82.60 | | 0.74 | 53.56 | 3.98 | 0.006 | 13.23 |
| 14 | 12 | | 22.5 | 70 | 8 | 69.20 | | 0.42 | 51.43 | 13.40 | 0.006 | 14.02 |
| 15 | 2 | | 22.5 | 70 | 8 | 57.41 | | 0.83 | 55.60 | 0.48 | 0.009 | 4.22 |
| 16 | 7 | | 22.5 | 70 | 8 | 50.13 | | 0.49 | 43.35 | 0.61 | 0.007 | 19.67 |
| 17 | 14 | | 22.5 | 70 | 8 | 77.92 | | 0.57 | 91.92 | 12.95 | 0.006 | 11.46 |

**Table S2.** Coefficients of the multiple linear regression model from the experimental design for PLE combined with EAE with Promod enzyme that the best fitted responses (Folin-Ciocalteu, DMAC, vanillin, butanol/HCl, TEAC, and inhibition of hydroxyl radical assays) with the extraction parameters (t: time, T: temperature and p: pH) and the analysis of variance (ANOVA).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | Folin-Ciocalteu | *p-*value | DMAC | *p*-value | Vanillin | *p*-value | Butanol/HCl | *p*-value | TEAC | *p*-value | Hydroxyl radical | *p*-value |
| Constant | 76.5687 |  | 0.714975 |  | 50.4326 |  | 2.31323 |  | 0.00631004 |  | 12.5415 |  |
| t | 3.74087 | 0.0690312 | 0.0988549 | 0.0757302 | 1.48121 | 0.274243 | -1.55757 | 0.259711 | -0.00057974 | 0.023165 | -0.226677 | 0.874268 |
| T | -0.642173 | 0.708234 | -0.0983441 | 0.0768368 | -2.6666 | 0.0781056 | -1.7044 | 0.244879 | 0.0008498 | 0.0122791 | 3.93067 | 0.0248276 |
| p | 4.92316 | 0.0288109 | 0.0657091 | 0.197594 | -0.972796 | 0.432818 | -5.1847 | 0.0143429 | 0.00068073 | 0.0136543 | -7.98952 | 0.00067382 |
| t2 | -5.30567 | 0.0321596 | -0.12659 | 0.0499341 | -1.84528 | 0.176053 | 1.47379 | 0.383005 | 0.00066431 | 0.0270325 | -2.92402 | 0.0664017 |
| T2 | -4.74775 | 0.0463558 | -0.020252 | 0.697806 | 4.16827 | 0.0162499 | -0.925015 | 0.517185 | 0.00037737 | 0.0519364 | 4.04103 | 0.019886 |
| p2 | -3.79391 | 0.0892811 | 0.0230183 | 0.659731 | 7.39256 | 0.00278374 | 5.73637 | 0.0116925 | -0.00072299 | 0.0206362 | 9.85783 | 0.00015956 |
| t\*T | -0.902904 | 0.624473 | -0.0188704 | 0.70636 | 0.305061 | 0.84502 | 0.874684 | 0.510832 | 9.47E-05 | 0.653919 | -0.755402 | 0.601397 |
| t\*p | 2.94867 | 0.149462 | -0.0406045 | 0.429809 | -0.903755 | 0.458469 | 3.93605 | 0.0315481 | 0.00025249 | 0.140774 | -2.08563 | 0.174755 |
| T\*p | 0.182355 | 0.920256 | -0.0668348 | 0.216721 | 4.09835 | 0.0148862 | -1.64187 | 0.361947 | -8.93E-05 | 0.672088 | -1.09081 | 0.455551 |
| R2 | 0.876 | | 0.816 | | 0.943 | | 0.931 | | 0.971 | | 0.941 | |
| RSD | 6.063 | | 0.1655 | | 4.232 | | 4.237 | | 0.000517 | | 5.524 | |
| *p*-value (test of regression) | 0.073 | | 0.167 | | 0.012 | | 0.05 | | 0.009 | | 0.002 | |
| *p*-value (lack of fit) | 0.65 | | 0.346 | | 0.946 | | 0.109 | | 0.213 | | 0.492 | |