# Ooptimization of a solid-liquid extraction method for blackberry fruits bioactive compounds using a Box-Behnken design.

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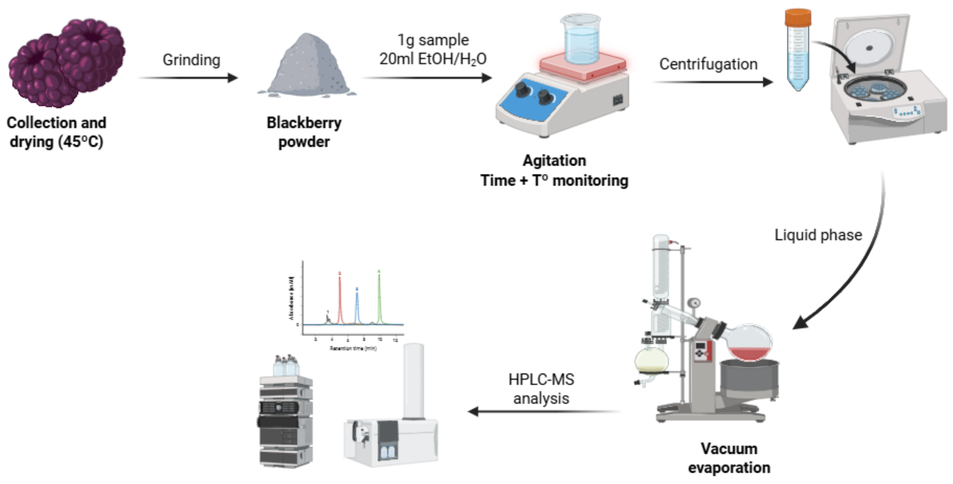
## INTRODUCTION

Blackberries (Rubus fruticosus) are rich in phenolic compounds with notable antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable as functional food ingredients[1,2]. This study optimized the solid-liquid extraction method using GRAS solvents (ethanol and water), with total phenolic content as dependent variable. Response Surface Methodology (RSM) was employed to enhance the efficiency and sustainability of the extraction process for potential food and nutraceutical applications.

## MATERIALS AND METHODS




Equation 1. Second order polynomial equation for RSM.

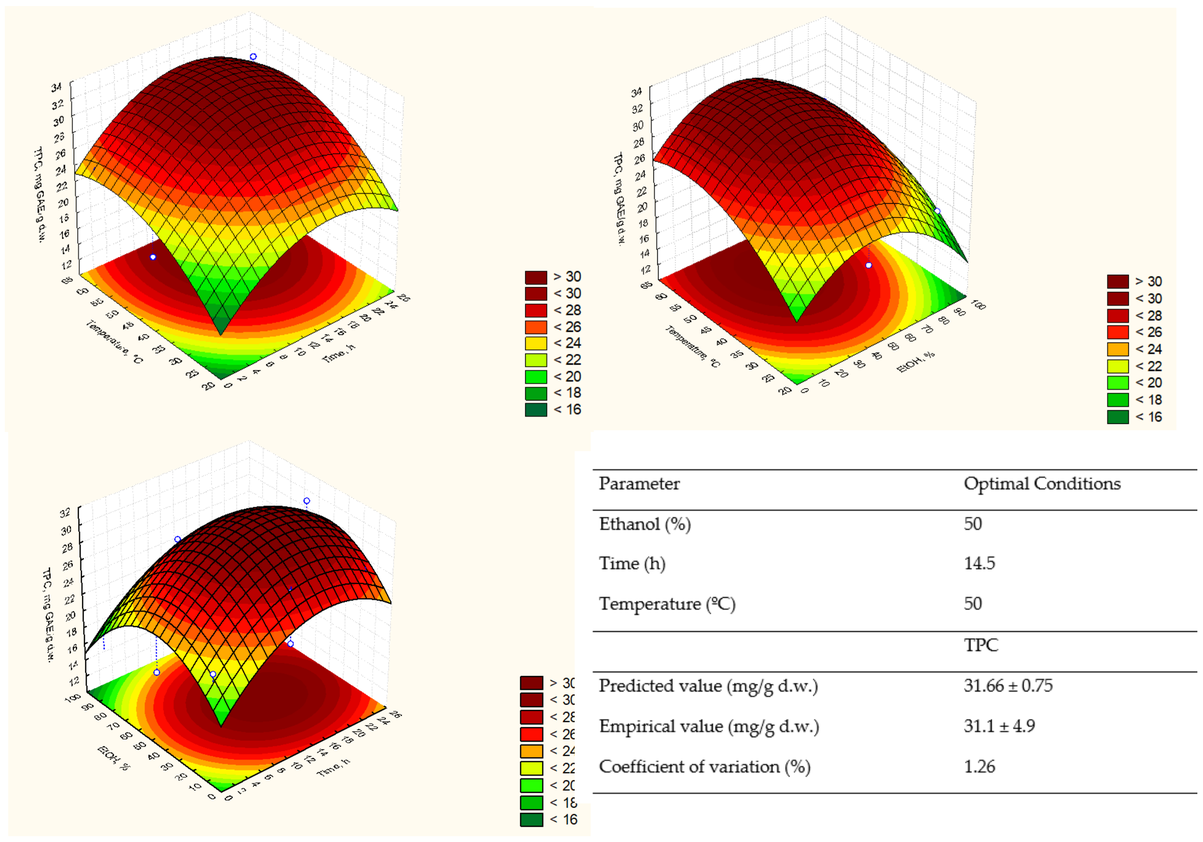
$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^{\ 2} + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ii} X_i X_j$$

## RESULTS

The Box-Behnken design revealed that ethanol concentration (β1), extraction time (β2), and temperature (β3) significantly affected total phenolic content (TPC). The response surface model showed a high predictive accuracy ($R^2$ = 0.9981) with no significant lack of fit (p > 0.05). The strongest effects on TPC were observed for ethanol concentration, followed by time and temperature, with significant linear and quadratic terms. Optimal extraction conditions were determined as 50% ethanol, 14.5 h, and 50 °C, yielding 31.1 ± 4.9 mg gallic acid equivalents (GAE)/g dry weight (predicted value of 31.66 ± 0.75 mg GAE/g d.w). A total of 34 phenolic compounds were characterized by UPLC-QTOF-MS, including phenolic acids, flavonoids, ellagitannins, lignans, and anthocyanins, with cyanidin-3-O-glucoside (1635.15 µg/g d.w.), cyanidin-3-O-rutinoside (505.34 µg/g d.w.), and sanguiin H6 isomer (35.09 µg/g d.w.) being the most abundant.

## CONCLUSIONS

Response Surface Methodology successfully optimized the extraction of antioxidant phenolic compounds from blackberry fruits. The model demonstrated excellent predictive accuracy and robustness, defining optimal conditions (50% ethanol, 14.5 h, 50 °C) that maximize extraction efficiency while maintaining sustainability. Additionally, UPLC-QTOF-MS characterization revealed a rich phenolic profile, with cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and sanguiin H6 isomer identified as the major compounds. These findings support the development of high-value blackberry extracts for functional food and nutraceutical applications.




| Parameter | Optimal Conditions |
|---|---|
| Ethanol (%) | 50 |
| Time (h) | 14.5 |
| Temperature (ºC) | 50 |
| | TPC |
| Predicted value (mg/g d.w.) | 31.66 ± 0.75 |
| Empirical value (mg/g d.w.) | 31.1 ± 4.9 |
| Coefficient of variation (%) | 1.26 |

Figure 1. Response surface plots showing combined effects of process variables for TPC (mg GAE/g d.w.): temperature (ºC)—time (h) (a), temperature (ºC)—% EtOH (b) and % EtOH—time (h) (c). Optimal conditions of extraction and predicted and empirical values of the model (n=3)

**Table 4.** Identification and quantification of phenolic compounds present in blackberry extract.

| Peak No. | Retention Time (Min) | m/z Exp. | m/z Calc. | Error (ppm) | Molecular Formula | Score | Proposed Compound | Quantification (µg/g d.w.) |
|---|---|---|---|---|---|---|---|---|
| Phenolic acids and derivatives | | | | | | | | |
| 3 | 0.433 | 333.057 | 333.061 | −4.8 | $C_{16}H_{14}O_8$ | 93.9 | Jaboticabin | 27.68 ± 0.26 |
| 7 | 4.979 | 223.06 | 223.0606 | −3.6 | $C_{11}H_{12}O_5$ | 97.4 | Sinapic acid | 17.83 ± 0.12 |
| 9 | 5.476 | 385.111 | 385.1135 | −7.3 | $C_{17}H_{22}O_{10}$ | 92.7 | Sinapic acid hexoside | 13.35 ± 0.03 |
| 16 | 6.639 | 183.025 | 183.0293 | −2.4 | $C_8H_8O_5$ | 99.2 | Methylgallic acid | 19.18 ± 0.07 |
| 20 | 7.707 | 433.041 | 433.0407 | −0.2 | $C_{19}H_{14}O_{12}$ | 84.5 | Ellagic acid-pentoside | 27.98 ± 0.02 |
| 21 | 7.888 | 433.041 | 433.0407 | 2.7 | $C_{19}H_{14}O_{12}$ | 92.9 | Ellagic acid-pentoside isomer | 25.62 ± 0.16 |
| 24 | 8.726 | 300.999 | 300.9984 | 3.3 | $C_{14}H_6O_8$ | 100 | Ellagic acid | 19.46 ± 0.13 |
| 29 | 9.276 | 447.056 | 447.0564 | −0.9 | $C_{20}H_{16}O_{12}$ | 94 | Ellagic acid 2-rhamnoside | 20.89 ± 0.10 |
| 32 | 9.917 | 315.012 | 315.0141 | −7.0 | $C_{15}H_8O_8$ | 86 | 3-*O*-Methylellagic acid | 10.41 ± 0.05 |
| Flavonoids and derivatives | | | | | | | | |
| 6 | 4.367 | 463.085 | 463.0877 | −5.8 | $C_{21}H_{20}O_{12}$ | 95.6 | Quercetin-*O*-hexoside | 3.37 ± 0.09 |
| 10 | 5.487 | 577.14 | 577.1405 | −1.6 | $C_{30}H_{26}O_{12}$ | 85.4 | B-type procyanidin dimer | 7.57 ± 0.12 |
| 11 | 5.716 | 315.123 | 315.1232 | −1.9 | $C_{18}H_{20}O_5$ | 85.8 | 4-hydroxy-5,7,4'-trimethoxyflavan | 5.89 ± 0.01 |
| 14 | 6.335 | 289.071 | 289.0712 | 0.7 | $C_{15}H_{14}O_6$ | 87.9 | Epicatechin | 7.90 ± 0.03 |
| 15 | 6.517 | 577.136 | 577.1346 | 3.1 | $C_{30}H_{26}O_{12}$ | 86.5 | B-type procyanidin dimer isomer | 7.94 ± 0.05 |
| 25 | 8.801 | 609.148 | 609.1456 | 3.1 | $C_{27}H_{30}O_{16}$ | 99.8 | Rutin | 8.46 ± 0.15 |
| 26 | 9.046 | 463.089 | 463.0877 | 1.9 | $C_{21}H_{20}O_{12}$ | 99.6 | Quercetin 3-galactoside | 5.63 ± 0.09 |
| 27 | 9.051 | 609.146 | 609.1456 | 0.7 | $C_{27}H_{30}O_{16}$ | 99.6 | Rutin isomer | 5.50 ± 0.18 |
| 28 | 9.213 | 463.085 | 463.0877 | −5.0 | $C_{21}H_{20}O_{12}$ | 97.5 | Quercetin-*O*-glucoside | 3.96 ± 0.02 |
| 30 | 9.577 | 477.066 | 477.0669 | −2.7 | $C_{21}H_{18}O_{13}$ | 98.2 | Quercetin 3-glucuronide | 4.01 ± 0.01 |
| 34 | 10.199 | 505.1 | 505.0982 | 3.6 | $C_{23}H_{22}O_{13}$ | 99.5 | Quercetin-*O*-acetylhexoside | 3.24 ± 0.11 |
| Ellagitannins | | | | | | | | |
| 19 | 7.519 | 935.079 | 935.0791 | −0.1 | $C_{41}H_{28}O_{26}$ | 100 | Casuarictin | 21.02 ± 0.23 |
| 22 | 8.29 | 934.075 | 934.0712 | 2 | $C_{41}H_{28}O_{26}$ (X2) | 83.6 | Sanguiin H6 | 25.99 ± 0.18 |
| 23 | 8.381 | 934.076 | 934.0712 | 1.5 | $C_{41}H_{28}O_{26}$ (X2) | 89.5 | Sanguiin H6 isomer | 35.09 ± 0.27 |
| Lignans | | | | | | | | |
| 33 | 9.984 | 571.218 | 571.2179 | −0.7 | $C_{30}H_{36}O_{11}$ | 89.6 | Kadsurarin | 28.17 ± 0.14 |
| 35 | 10.509 | 571.213 | 571.2179 | −7.9 | $C_{30}H_{36}O_{11}$ | 85.7 | Kadsurarin isomer | 12.37 ± 0.06 |
| 36 | 10.74 | 341.137 | 341.1389 | −5.6 | $C_{20}H_{22}O_5$ | 99.9 | Kadsurenin B | 8.85 ± 0.10 |
| Anthocyanins (MS+) | | | | | | | | |
| 12 | 5.859 | 449.107 | 449.1084 | −2.2 | $C_{21}H_{21}O_{11}$ | 99.9 | Cyanidin-3-*O*-glucoside | 1635.15 ± 13.24 |
| 17 | 6.691 | 595.165 | 595.1663 | −2.9 | $C_{27}H_{31}O_{15}$ | 92.3 | Cyanidin-3-*O*-rutinoside | 505.34 ± 5.37 |
| 18 | 7.268 | 419.098 | 419.0919 | 0.7 | $C_{20}H_{19}O_{10}$ | 98.6 | Cyanidin-3-*O*-arabinoside | 145.05 ± 1.63 |
| 38 | 11.499 | 465.104 | 465.1033 | 1.1 | $C_{21}H_{21}O_{12}$ | 96.3 | Delphinidin-3-*O*-galactoside | 32.46 ± 0.97 |
| 39 | 11.501 | 611.16 | 611.1612 | −2.3 | $C_{27}H_{31}O_{16}$ | 92.9 | Cyanidin-3,5-*O*-diglucoside | 132.11 ± 2.08 |
| 40 | 11.672 | 465.104 | 465.1033 | 1.9 | $C_{21}H_{21}O_{12}$ | 99.4 | Delphinidin-3-*O*-glucoside | 63.56 ± 1.05 |
| 41 | 11.83 | 611.159 | 611.1612 | 2.1 | $C_{27}H_{31}O_{16}$ | 91.6 | Cyanidin-3-*O*-sophoroside | 94.96 ± 1.58 |
| 43 | 13.572 | 465.106 | 465.1033 | 5.4 | $C_{21}H_{21}O_{12}$ | 82.4 | Delphinidin-3-*O*-galactoside | 11.38 ± 0.46 |

## REFERENCES

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