# METHOD OPTIMIZATION FOR ENHANCED BIOACTIVE COMPOUNDS EXTRACTION FROM HAZELNUT (*Corylus avellana* L.) INVOLUCRE: PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY

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## Introduction

A diet rich in tree nuts, as many studies demonstrated, present benefit for human health. Several of these benefits extend to their by-products. The aim of this research was to assess the phytochemical profile and the *in vitro* antioxidant activity of the acetone extracts of hazelnut involucre (HI) based on an experimental design. The richest polyphenolic extract was further analyzed by HPLC-MS/MS and a number of phenolics and phytosterols were quantified.

## Methods

Experimental plan – factors (Table 1): stirring time, pH, % water in solvent. The extracts analyzed for:

- Total phenolic content (TPC) - gallic acid equivalents per dry weight of HI (mg GAE/g dw) – spectrophotometric assay
- Total flavonoid content (TFC) - quercetin equivalents per dry weight of HI (mg QE/g dw) - $AlCl_3$ solution
- Condensed tannin content (CTC) - catechin equivalents per dry weight of HI (mg CE/g dw) - vanillin assay
- Total antioxidant activity (TAA) - trolox equivalents per dry weight of HI - mg TE/g dw) – TEAC assay
- 18 phenolic standards – 11 phenols quantified – HPLC-MS/MS
- 4 sterol standards – 3 sterols quantified – HPLC-MS/MS

## Results and Discussion

**Table 1.** Matrix of experimental design and experimental results for TPC, TFC, CTC, TAA for hazelnut involucre extracts based on a factorial design.

| Exp No | Stirring time (min) | pH | % water in acetone | TPC | TFC | CTC | TAA |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 3 | 0 | 2,62 | 2,23 | ND | 9,86 |
| 2 | 3 | 3 | 0 | 4,88 | 2,88 | 0,03 | 18,9 |
| 3 | 1 | 7 | 0 | 4,33 | 3,64 | 0,15 | 16,01 |
| 4 | 3 | 7 | 0 | 2,59 | 1,80 | 0,06 | 8,65 |
| 5 | 1 | 3 | 50 | 320,81 | 32,12 | 22,67 | 1049,75 |
| 6 | 3 | 3 | 50 | 377,43 | 43,10 | 28,07 | 1296,51 |
| 7 | 1 | 7 | 50 | 332,78 | 39,82 | 27,43 | 1811,42 |
| 8 | 3 | 7 | 50 | 334,68 | 37,32 | 24,23 | 1706,22 |
| 9 | 1 | 5 | 25 | 292,98 | 34,47 | 24,41 | 1065,73 |
| 10 | 3 | 5 | 25 | 210,67 | 25,04 | 17,48 | 885,31 |
| 11 | 2 | 3 | 25 | 188,82 | 22,01 | 15,02 | 718,15 |
| 12 | 2 | 7 | 25 | 257,22 | 28,06 | 22,83 | 823,02 |
| 13 | 2 | 5 | 0 | 15,50 | 10,56 | 0,22 | 109,8 |
| 14 | 2 | 5 | 50 | 313,21 | 32,56 | 22,57 | 1261,77 |
| 15 | 2 | 5 | 25 | 197,39 | 25,53 | 18,09 | 790,24 |
| 16 | 2 | 5 | 25 | 245,38 | 22,38 | 17,48 | 749,62 |
| 17 | 2 | 5 | 25 | 196,32 | 24,30 | 14,99 | 714,15 |

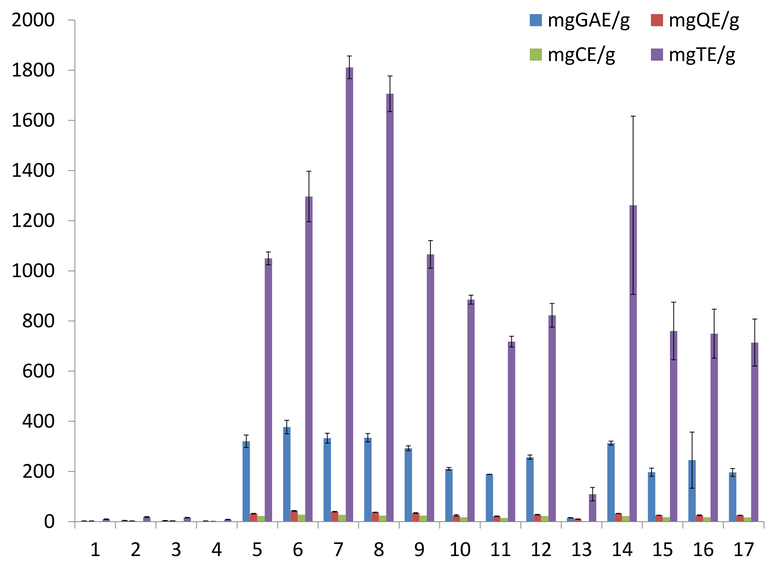


**Figure 1.** TPC, TFC, CTC, TAA for analyzed hazelnut involucre extracts.

- The best results for TPC, TFC, CTC, TAA obtained using binary-solvent systems compared to mono-solvent systems (Table 1).
- Positive relationship between TPC, TFC, CTC and TAA (Figure 1).
- The best results for TPC, TFC, CTC were obtained for 50% water in acetone and pH 7 (Figure 2, Figure 3, Figure 4).
- The best results for individual polyphenols were obtained for equal mixture (1:1) acetone and water as extraction solvent (Table 2).
- The best results for individual phytosterols were obtained for 100% and 75% acetone as extraction solvent (Table 2).

**Table 2.** Matrix of experimental design for bioactive compounds recovery from hazelnut involucre extracts.

| Exp No | Factorial Design with Coded Values | | | Determination (Experimental Results) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X1 | X2 | X3 | Y1 | Y2 | Y3 | Y4 | Y5 | Y6 | Y7 | Y8 | Y9 | Y10 | Y11 | Y12 | Y13 | Y14 |
| 1 | 1 | 3 | 0 | ND | ND | ND | ND | ND | ND | 0.37 | 0.23 | 1.23 | 0.43 | 3.78 | 26.18 | 3.45 | 916.69 |
| 2 | 3 | 3 | 0 | 0.16 | 13.62 | 0.54 | 5.29 | 21.73 | 3.10 | 0.94 | 0.54 | 2.69 | 1.13 | 8.36 | 61.06 | 5.75 | 2444.0 |
| 3 | 1 | 7 | 0 | 0.11 | 10.28 | 0.41 | 3.78 | 14.55 | 2.44 | 0.70 | 0.44 | 3.09 | 1.01 | 7.31 | 30.05 | 2.32 | 1442.9 |
| 4 | 3 | 7 | 0 | ND | ND | ND | ND | ND | ND | 0.80 | 0.49 | 2.04 | 0.79 | 6.51 | 36.03 | 1442.87 | 1408.8 |
| 5 | 1 | 3 | 50 | 1.48 | 155.08 | 2.65 | 55.39 | 103.98 | 15.78 | ND | ND | 31.61 | 9.21 | 76.21 | ND | ND | ND |
| 6 | 3 | 3 | 50 | 3.61 | 201.95 | 2.58 | 63.59 | 131.15 | 16.61 | ND | ND | 51.72 | 17.74 | 114.26 | ND | ND | ND |
| 7 | 1 | 7 | 50 | 3.18 | 158.06 | 2.77 | 53.14 | 124.58 | 21.10 | ND | 3.23 | 43.90 | 10.25 | 97.15 | ND | ND | ND |
| 8 | 3 | 7 | 50 | 2.05 | 161.14 | ND | 42.87 | 103.52 | 12.29 | ND | ND | 42.26 | 13.29 | 94.52 | ND | ND | ND |
| 9 | 1 | 5 | 25 | 3.48 | 216.97 | 3.33 | 69.07 | 140.89 | 18.71 | 6.58 | 3.97 | 50.99 | 15.71 | 112.05 | ND | 77.68 | 3145.78 |
| 10 | 3 | 5 | 25 | 1.62 | 108.01 | 2.01 | 37.15 | 70.58 | 9.83 | 3.19 | ND | 30.93 | 9.1 | 71.32 | 195.28 | 45.04 | 5166.14 |
| 11 | 2 | 3 | 25 | 1.65 | 150.77 | 2.63 | 53.22 | 97.26 | 14.51 | 2.74 | 2.49 | 31.04 | 8.64 | 71.33 | 185.63 | 31.38 | 3792.66 |
| 12 | 2 | 7 | 25 | 3.73 | 243.02 | 5.53 | 91.93 | 227.37 | 25.41 | 4.40 | 3.30 | 36.23 | 10.32 | 85.39 | 145.24 | 24.79 | 3480.22 |
| 13 | 2 | 5 | 0 | 0.27 | 19.98 | 0.87 | 11.83 | 33.32 | 4.71 | 2.14 | 1.50 | 5.89 | ND | 17.99 | 77.68 | 12.66 | 2843.16 |
| 14 | 2 | 5 | 50 | ND | 78.04 | 2.41 | 44.25 | 100.40 | 13.59 | ND | ND | 29.52 | 8.59 | 67.21 | ND | ND | ND |
| 15 | 2 | 5 | 25 | 2.41 | 172.79 | 3.39 | 56.21 | 117.09 | 14.81 | 3.14 | 2.55 | 36.43 | 10.57 | 85.62 | 141.49 | 28.96 | 3821.43 |
| 16 | 2 | 5 | 25 | 1.89 | 169.99 | 2.65 | 59.39 | 111.95 | 12.48 | 3.90 | 2.89 | 36.71 | 10.86 | 83.39 | 197.31 | 31.49 | 5305.01 |
| 17 | 2 | 5 | 25 | 1.89 | 176.77 | 3.78 | 57.64 | 116.71 | 15.31 | 3.61 | 2.65 | 37.23 | 10.98 | 86.73 | 178.96 | 22.76 | 3213.27 |

**X1, stirring time (min); X2, pH; X3, water in acetone (%, v/v). Y1—Epicatechin; Y2—Catechin; Y3—Syringic acid; Y4—Gallic acid; Y5—Protocatechuic acid; Y6—Vanillic acid; Y7—p-Coumaric acid; Y8- Ferulic acid; Y9- Hyperoside; Y10—Isoquercitrin; Y11—Quercitrin; Y12—Stigmasterol; Y13-Campesterol; Y14—Beta-sitosterol. All responses are expressed as µg bioactive compound per gram of dry weight hazelnut involucre. ND—not determined.**

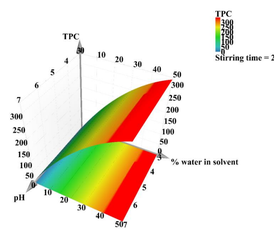


**Figure 2.** Response surface for predicting TPC recovery

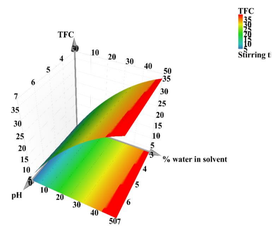


**Figure 3.** Response surface for predicting TFC recovery

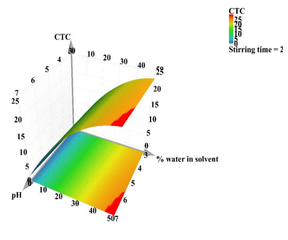


**Figure 4.** Response surface for predicting CTC recovery

## Conclusion

The phytochemical profile and the antioxidant activity of the analyzed extracts prove that this plant matrix can be a valuable source of bioactive compounds for the food and/or pharmaceutical industry.

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