EXTRACTION OF SOME BIOLOGICALLY ACTIVE POLYPHENOLS FROM WALNUT SEED SEPTUM: MATHEMATICAL MODELLING

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Three-factor two-level fractional experimental design, FFE 2^{3-1} was used in order to evaluate the influence of Ethyl Alcohol (X_1), sodium carbonate / citric acid, H_3Cit (X_2) and polygalacturonase enzyme, PGU (X_3) to the extraction of biologically active compounds (BACs) from the septum. Septum samples, 2 g each, were extracted with 50mL of solution, prepared according to Table 1.

Table 1. Extraction conditions planning matrix: encoded and real coordinates

Nexp.	X_1, EtOH, % (v)		X_2, Na_2CO_3 / H_3Cit, % (m)			X_3, PGU, % (m)	
1	-	20	-	H_3Cit	0.05	+	0.01%
2	+	40	-	H_3Cit	0.05	-	0.00%
3	-	20	+	Na_2CO_3	0.05	-	0.00%
4	+	40	+	Na_2CO_3	0.05	+	0.01%

Extracted BAC per 100g of septum, mg/100g, of Gallic Acid (***Gal***), Catechin (***Cat***), Epicatechin (***Epi***), Casuarictin (***Cas***) and Ellagic Acid (***Ell***), were determined by reversed-phase HPLC with Photo Diode Array detection on C_{18}-column. Following regressions were calculated:

$$Gal = 8.4X_0 - 3.0X_1 - 2.8X_2 + 3.6X_3$$

$$Cat = 107X_0 + 17X_1 - 8X_2 + 11X_3$$

$$Epi = 27.5X_0 - 7.5X_1 - 14.0X_2 + 7.8X_3$$

$$Cas = 22.4X_0 + 2.8X_1 + 0.7X_2 - 4.2X_3$$

$$Ell = 32.6X_0 + 2.2X_1 + 4.8X_2 - 6.6X_3$$

The regressions show that the factors under research, have ambiguous effects on the extraction of biologically active compounds (***BACs***) from the septum. Thus, high concentrations of ethyl alcohol reduce the yield of ***Gal***, but increase the extractivity of hexahydroxydiphenic acid derivatives (***Cas*** and ***Ell***). Alkaline medium (Na_2CO_3) reduce the yields of ***Gal***, ***Cat*** and ***Epi***, but increase the yields of ***Cas*** and ***Ell***. Finally, the effects of polygalacturonase are also grouped according to BACs polarity: enzyme facilitating the release of more polar BACs.

The totality of the factors influence can be explained by the interaction of BACs with different polarity with the biopolymer (cellulose-lignin) matrix of the septum. The most acceptable conditions for the extraction of all biologically active substances at once are close to the center of the experiment: ethanol-water mixture 30:70 containing H_3Cit (~ 0.025%) and PGU (~ 0.005%).

Keywords: *Catechin, Casuarictin, Ellagic Acid, Epicatechin, Gallic Acid, HPLC, Regressions*

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