Application of Central Composite Design (CCD) for the Optimization of Ultrasound-assisted Extraction (UAE) of Phenolics from Corn (*Zea mays*)

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INTRODUCTON

Corn (*Zea mays*) is the third most important crop worldwide and might serves as a staple food, especially in the developing countries.[1] It becomes more important attributable to naturally presented phenolic compounds.[2,3]

Many studies showed that phenolics have some benefits including play a key role in anti-oxidative defences mechanisms,[4] exhibit health-promoting effects,[5] and inhibitory effects on mutagenesis and carcinogenesis.[6]

The development phenolics extraction may complicate due to its structural diversity as having multiple hydroxyl groups.[7] Furthermore, their potent antioxidant activity leads to fast reaction with other components in the matrix.[8] Hence, a new extraction method that effectively liberate phenolics from matrices and rapidly recover these compounds is required.

Ultrasound-assisted extraction (UAE) appears to be the most promising process to extract phenolics from plant samples due to the cavitation effect, thus aids the extraction solvent to penetrate rapidly into the plant cells and prevents degradation of phenolic compounds.

EXPERIMENTAL DESIGN

A central composite design (CCD) has been used to investigate the effects of six independent variables on the extraction yield at three levels. The range of independent factors and their levels is described bellow:

Variable	-1	0	+1	Unit
Solvent (A)	0	25	50	%MeOH
Temperature (B)	10	40	70	°C
Amplitude (C)	30	50	70	%
Cycle (D)	0.2	0.45	0.7	-
pH (E)	3	5	7	-
Ratio sample:solvent (F)	1:10	1:15	1:20	-

CCD consisted of 45 experimental points carried out in random order.

STATGRAPHICS® Centurion XVI (Statpoint Technologies, Inc., USA) has been used to evaluate the effects of operating parameters on the extracted yield. A second-order model is applied in Response Surface Methodology (RSM):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where $x_1, x_2, \ldots, x_k$ are the factors that influence the response y; β_0, β_{ii} (i = 1, 2,..., k), β_{ij} (i = 1, 2, ...,k; j = 1, 2,...,k) are unknown parameters and ε is a random error. The β coefficients are obtained by the least square method.

ANALYTICAL METHODS

The ultrasound-assisted extractions (UAE) were performed using an ultrasonic probe (model UP200S, medium probe, Hielscher Ultrasonics, 200 Watt).

Sample Preparation
Corn grains were grinded using an Ultraturrax homogenizer (IKA® T25 Digital, Germany) and accurately weighted

Ultrasound-assisted Extraction (UAE)
UAE conditions as regard to DOE; 10 minutes

Extract Handling
Solid material was removed by centrifugations. The extract was adjusted to a certain volume and filtered by nylon syringe filters (0.22µm).

The determination of phenolics was carried out on an ACQUITY UPLC® H-Class system coupled with a Photo-diode Array (PDA) detector and controlled by an Empower™ 3 Chromatography Data Software (Waters Corporation, Milford, MA)

Determination System

Flow Rate	: 0.7 mL min^{-1}
Injection Volume	: 10 µL
PDA scan range	: 200-400 nm
Column Temperature	: 47 °C

Phase A : 0.01% Acetic Acid in MilliQH_2O
Phase B : 2% Acetic Acid in Acetonitrile

Gradient Program

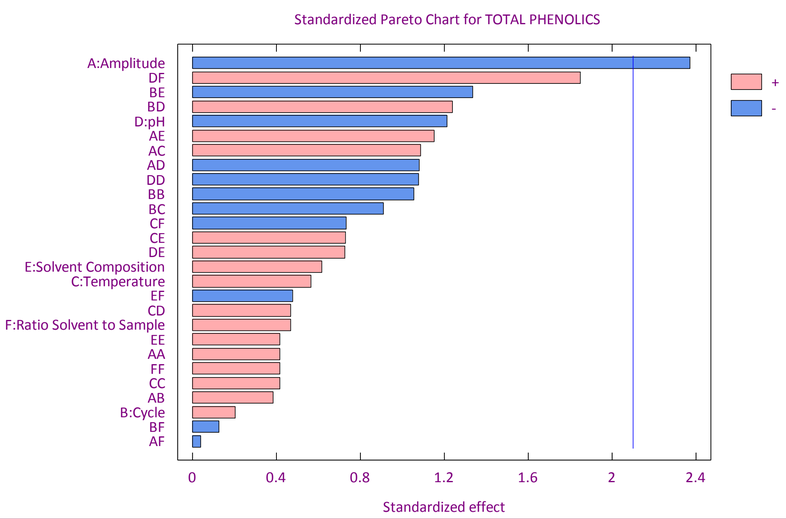
Time (min)	0	1	1.1	2	3	3.5	4	7	8	10
Phase %B	0	10	10	20	20	60	100	100	0	0

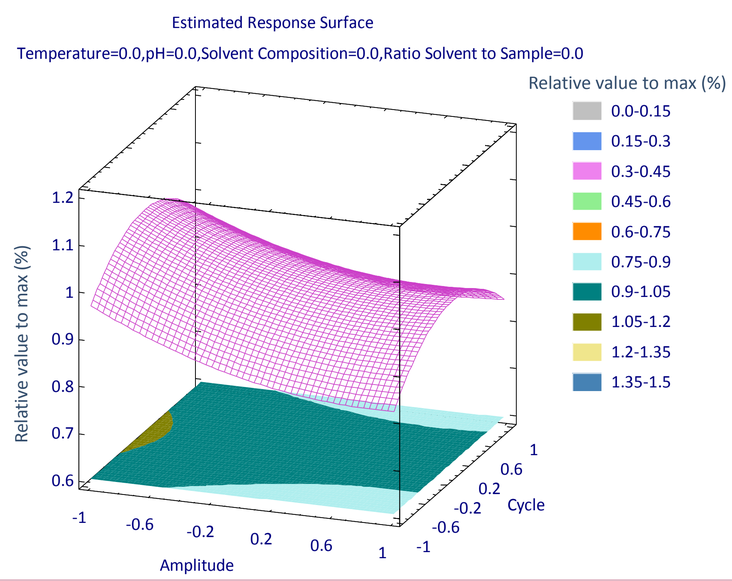
RESULTS

Analytical properties for the UPLC-PDA (Linear Range 0.15-12 mg L^{-1})

Compounds	Linear Equation	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})	Intra-day, CV (%) n=9		Inter-day, CV (%) n=3x3	
					RT	Area	RT	Area
Protocatechuic acid	$y = 54421.70x + 208.24$	0.9997	0.2	0.7	0.21	0.49	0.86	0.79
p-OHBenzoic acid	$y = 116136.01x + 5629.27$	0.9999	0.2	0.5	0.14	0.17	0.76	1.00
Vanillic acid	$y = 95900.55x + 2976.36$	0.9999	0.2	0.7	0.19	0.24	0.64	0.73
Chlorogenic acid	$y = 13703.67x - 68448.81$	0.9999	0.1	0.4	0.17	0.27	0.80	1.23
Caffeic acid	$y = 108664.31x - 34804.94$	0.9999	0.1	0.4	0.25	0.35	1.39	0.62
p-Coumaric acid	$y = 166513.11x - 2772.38$	0.9999	0.1	0.4	0.36	0.36	1.40	0.64
Ferulic acid	$y = 141074.71x - 80282.13$	0.9993	0.4	1.2	0.39	0.20	1.10	0.81

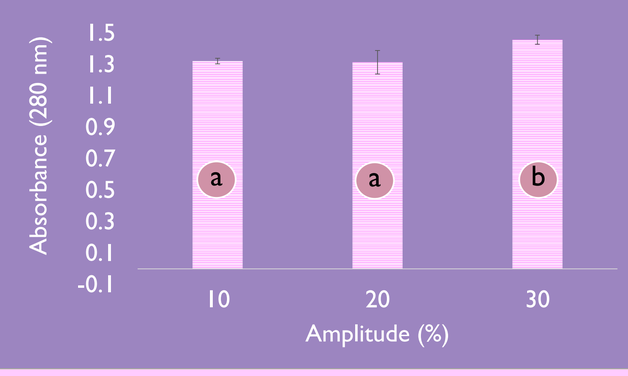
UAE OPTIMIZATION





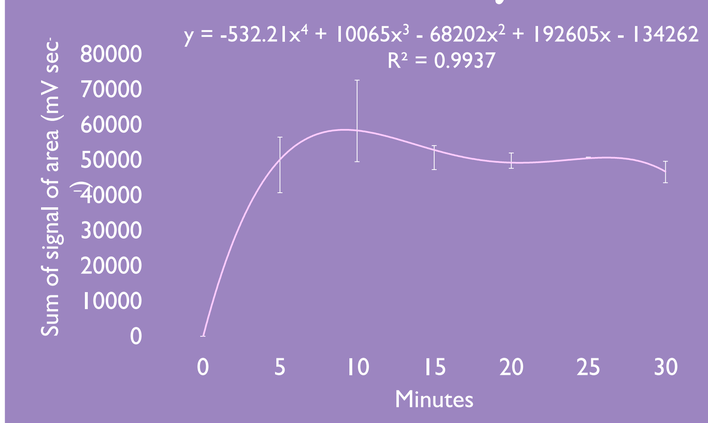
Amplitude (A) significantly affects the recovery; ↑ Amplitude ≡ ↑ ↑ ↑ Recovery
Since the coordinates of the highest point of response was in the corner of the design region, further amplitude optimization should be tested.

Additional optimization: Amplitude



Single-factor ANOVA indicates that amplitude has a significant effect as $F_{calculated}$ (170.6) >> $F_{critical}$ (5.14) on the extraction yield. Hence, 30% was defined as the optimum amplitude.

Kinetic Study



Phenolics can be completely extracted from corn sample within 10 minutes of extraction time

Optimized conditions by RSM

Factors	Level	Optimum
MeOH	0.98	75%
Temp.	1.00	50°C
Amplitude	-1.00	30%
Cycle	-0.34	0.4
pH	-0.17	4.0
Ratio	-0.96	1:2.5

PERFORMANCE OF THE METHOD

Compounds	Recovery (%)	Precision CV (%)	
		Intra-day	Inter-day
Caffeic acid	71.51	1.02	2.13
Ferulic acid	110.70	2.63	3.18
p-Coumaric acid	97.22	2.73	2.12
p-OHBenzoic acid	73.50	1.05	1.14
Protocatechuic acid	84.33	3.80	10.57
Sinapic acid	78.94	1.48	2.98
Vanillic acid	72.19	9.81	12.01

The developed and validated method was then successfully applied to the analysis of phenolics content in four corn cultivars including red, yellow, black and white

REAL SAMPLE APPLICATION

Compounds	Red	White	Black	Yellow
Protocatechuic acid	TR	ND	0.684±0.004	ND
p-OHBenzoic acid	ND	ND	ND	ND
Vanillic acid	0.701±0.023	ND	ND	TR
Chlorogenic acid	1.501±0.065	TR	0.588±0.196	TR
Caffeic acid	1.137±0.125	0.511±0.044	TR	0.623±0.011
p-Coumaric acid	1.190±0.047	0.784±0.053	1.558±0.002	0.446±0.049
Ferulic acid	1.619±0.192	1.534±0.060	TR	TR

Note: Unit in mg Kg^{-1} sample
ND, not detected due to less than LOD.
TR, trace due to the concentration less than LOQ but higher than LOD.

The major phenolic compounds in corn grain were **ferulic** and ***p*-coumaric acid**. The grain from red corn cultivar consists the highest amount of phenolics while the yellow cultivar posses the lowest level of phenolic compounds.

ACKNOWLEDGEMENT

W. Setyaningsih thanks the CIMB Foundation for a Ph.D. studentship through the CIMB Regional Scholarships

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