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Predictive Models for Optimisation of Acetone Mediated Extraction of Polyphenolic Compounds from By-Product of Cider Production

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Abstract

Response surface methodology (RSM) was applied to provide predictive models for optimisation of extraction of selected polyphenolic compounds from cider apple pomace under aqueous acetone. The design of experiment (DoE) was conducted to evaluate the influence of acetone concentration % (v/v), solid-to solvent ratio % (w/v), temperature (˚C) and extraction time (min) and their interaction on phenolic contents, using the Central Composite Rotatable Design (CCRD). The experimental data were analysed to fit statistical models for recovery of phenolic compounds. The selected models were significant ($P < 0.05$) and insignificant lack of fits ($P > 0.05$), except for Chlorogenic acid and Quercetin 3-glucoside which had significant lack of fits ($P < 0.05$). All models had satisfactory level of adequacies with coefficients of regression $R^2 > 0.9000$ and adjusted R^2_{Adj} reasonable agrees with predicted R^2_{Pri}. Coefficient of variation < 5% for each determination at the 95% confidence interval. These models could be relied upon to achieve optimal concentrations of polyphenolic compounds for applications in nutraceutical, pharmaceutical and cosmetic industries.

Keywords

Cider Apple Pomace, Predictive Models, Optimisation, Polyphenolic Compounds

1. Introduction

Mathematical modelling is an indispensable tool in many applications in science

and engineering. It is the art of translating problems from an application area into tractable mathematical formulations whose theoretical and numerical analysis provides deep understanding to answers and guidance that are useful for originating applications [1]. Mathematical concepts and language are employed to facilitate proper explanation of the system and also explain the effects of different factors, and to make predictions of their behaviour [2]. Modelling based on mathematics provides thorough understanding of the system to be modelled and allows different applications of modern computing capabilities [3]. Models serve as tools for the understanding of very important and complex processes or systems [4]. Different types of models have been proposed and applied in chemical process for optimisation and for designing experiments to give better understanding of complex systems. Response surface methodology (RSM) is a multivariate statistical technique that evaluates the interrelationship between process parameters and responses [5] [6] [7]. Response Surface Methodology was set out by Box *et al.*, 1950 [8] and was a collection of mathematical and statistical techniques used to improve the performance of systems for maximum benefits [9]. By fitting a polynomial equation to an observed data from within a designed of experiment (DoE), the technique was able to predict the behaviour of a response based on the set of independent variables [9]. Response surface methodology provides adequate information from a relatively fewer experimental runs compared to one factor at time procedure which involved plenty of time in experimental trials for model generation. The one factor at a time procedure requires more experiments to be able to explain the interaction of the independent variables on overall dependent quantity or response. Response surface methodology utilises three (3) levels of independent factors to produce experimental designs and employ polynomial models for analysis. RSM has important application in process development, formulation and design of contemporary products in addition to established ones. The technique is widely applicable in chemical and biochemical processes for varied objectives [10]. Comprehensive description of design of experiments by response surface methodology can be obtained from [11] [12] [13].

The current research seeks to demonstrate the possibility of developing predictive models that are reliable for optimisation of the recovery of polyphenolic compounds from cider apple pomace using aqueous acetone as a solvent. Apple pomace is the residue of apple juice and cider production and composed between 20% - 35% by weight of the original production feedstock. The amount of the pomace generated and its composition will depend on the variety of the apple and the techniques used in extracting the juice [14]. Apple pomace is a potential source of carbohydrate, fibre, polyphenolics and pectin [15] [16] which find application in the food, feed, pharmaceutical, cosmetics, chemical, and biofuels sectors [17]. The major polyphenolic compounds found in apples include; Epicatechins, Procyanidins, Phloridzin, Quercetin conjugates and Chlorogenic acids.

2. Materials and Methods

2.1. Apple Pomace

The apple pomace sample composed of 7 varieties of cider apples made of Harry Masters Jersey, Yarlinton Mill, Michelin, Dabinett, Brown Snout, Vilberie and Chisel Jersey, and were collected from Universal Beverages Limited (UBL), Ledbury owned by Heineken international. The apple pomace residues were mixed rigorously to obtain mixture characteristic of the original pomace sample and divided into parts and stored in freezer bags at −20˚C till further investigations.

2.2. Chemical Reagents

All chemical standards and solvents employed in this investigation were ordered at the highest grade of purity from suppliers indicated in the methodologies. Acetonenitrile, and glacial acetic acid were obtained from Fisher Scientific (UK).

2.3. Dry Weight Content of Apple Pomace

A bench top laboratory convention oven (103˚C ± 3˚C) from STATUS International, UK was used for dry weight content. The American Oil Chemist Society (AOCS) standard procedure was utilised to determine the dry matter content, and the results were expressed as the percentage of total fresh weight of the apple pomace as received.

2.4. Apple Pomace Sample Preparation

The apple pomace samples were freeze dried using a vacuum freeze dryer EQ03 (Vacuum and Industrial products). The dried pomace samples from the freeze dryer were placed in desiccator for 30 minutes for samples to return to ambient conditions. Freeze dried pomace residue was pulverised using a domestic Moulinex blender 530 (KEMAEU, France). The blending machine was stopped intermittently after every 20 seconds of milling and the pomace powder packed in dark plastic bags and stored in a cool dry place for subsequent use.

2.5. Extraction of Polyphenolic Compounds from Freeze-Dried Apple Pomace

Known weight of homogenised freeze dried apple pomace was weighed into 100 ml Duran bottles and acetone was added 1% - 8% (w/v) solid-to-solvent ratio and the bottle tightly covered. Extractions were done in an incubator Max Q 4000 series benchtop shaker (Thermo Scientific). Extraction temperatures and time were set and shaking (150 rpm) and automatically stops when extraction time elapses. Extracts rich in polyphenolic compounds were transferred into 50 ml centrifuge tubes and centrifuged in Juan C4 I at 4000 g for 10 minutes. Supernatant volumes were recorded stored at −20˚C. Extractions at 60˚C and 85˚C were done within Grant OLS200 water bath.

2.6. Experimental Design for Optimization of Acetone Mediated Extraction

The design of the experiments was done similar to the procedure previously described in [18]. The design was composed of one factor at a time (OFAT) experiments and the overall design by response surface methodology (RSM). Solvent concentration (%, (v/v)), solid-to-solvent ratio (1% - 8% (w/v)), temperature and extraction time influenced the recovery of polyphenolic compounds. Stat-Ease Design Expert software 7.0, was employed to set up experiments with varying independent variables, utilising the central composite rotatable design (CCRD). In all, thirty (30) experimental runs consisting of 16 trials for factorial points, 8 runs for axial points and 6 duplicates run around the central point (Table 1).

Table 1. Experimental design by central composite rotatable design using 4 factors.

Run order	A-acetone conc. %(v/v)	B-Temp °C	C-Solid/Solvent ratio. % (w/v)	D-Time, min
1	40.0	60.0	1.0	90.0
2	60.0	35.0	1.0	60.0
3	40.0	10.0	8.0	90.0
4	40.0	10.0	1.0	90.0
5	60.0	35.0	4.5	60.0
6	40.0	60.0	8.0	30.0
7	100.0	35.0	4.5	60.0
8	40.0	10.0	8.0	30.0
9	60.0	35.0	4.5	60.0
10	20.0	35.0	4.5	60.0
11	40.0	10.0	1.0	30.0
12	40.0	60.0	8.0	90.0
13	60.0	10.0	4.5	60.0
14	60.0	35.0	11.5	60.0
15	80.0	60.0	8.0	90.0
16	60.0	35.0	4.5	5.0
17	80.0	10.0	8.0	30.0
18	60.0	35.0	4.5	60.0
19	80.0	10.0	8.0	90.0
20	80.0	60.0	1.0	30.0
21	60.0	35.0	4.5	120.0
22	80.0	60.0	1.0	90.0
23	80.0	60.0	8.0	30.0
24	60.0	35.0	4.5	60.0
25	60.0	35.0	4.5	60.0
26	60.0	85.0	4.5	60.0
27	80.0	10.0	1.0	90.0
28	80.0	10.0	1.0	30.0
29	40.0	60.0	1.0	30.0
30	60.0	35.0	4.5	60.0

2.7. Identification and Quantification of Polyphenolic Compounds by High Performance Liquid Chromatography (HPLC)

High performance liquid chromatographic (HPLC) procedure in a reverse mode, previously published in literature was used to separate phenolic compounds [19]. Polyphenolic compounds in extracts were resolved using an Agilent 1100 series HPLC system with DAD-UV detector linked to a Chemstation software. The column used was Prodigy 5 µm ODS3 100A, C18 (250 × 4.6 mm I.D) from Phenomenex (Torrance, CA, USA) with a guard column operated at 40˚C. Eluent A of the mobile phase was composed of 2% (v/v) of glacial acetic acid in water. Eluent B consisted of 0.5% of acetic acid in 50/50 (v/v) of water and acetonitrile. Pure acetonitrile (100%) was the Eluent C. The injection volume was 10 µl per sample and the solvent gradient systems for the separations was as follows: starting with 10% of B and increasing the gradient to 55% B in 50 minutes. Further increase from 55% B to 100% B was done in 10 minutes and finally decreased from 100% B to the initial 10% B in 5 minutes. Eluent C was used to recondition the column under isocratic flow by pumping 100% acetonitrile for 10 minutes, and 10% B also for 10 minutes. The flow rate was 1 ml/min and polyphenolic compounds were monitored at 280 nm for flavanols, 320 nm for hydrocinnamic acid and 370 nm for flavonols. Retention times and spectra data were collected.

2.8. Preparation of Phenolic Standard

Stock solutions (1 mg/ml) of Chlorogenic acid (≥95%), (-) Epicatechin (≥90%), ± Catechin hydrate, Phloridzin dihydrate (≥99%), Procyanidin B2 (≥90%), Quercetin-3-β-D-glucoside (≥90%), Quercetin-3-D-galactoside (≥97%) Phloretin, in Chromasolv for HPLC (Sigma-Aldrich, UK). The stock solutions were diluted appropriately (0.01 - 1 mg/ml) and injected in triplicates into the HPLC equipment. Calibration curves were constructed and quantification of polyphenolic compounds in samples was derived from the calibration curves of corresponding standards.

3. Results and Discussion

The mean dry matter content of the homogenized cider apple pomace under this investigation was 27.7 ± 0.3 g/100g fresh weight. Dry weight value reported for apple pomace in literature ranges, from 21.8 - 33.6 g/100g [20] [21] [22]. Mean dry matter content of the freeze dried apple pomace was 28.3 ± 0.6 g/100g fresh weight.

3.1. Identification and Quantification of Phenolic Compounds in Extracts

The polyphenolic compounds in the extracts were identified by comparing retention times (t_R) and spectra data at maximum absorbance with known phenolic standards. Chlorogenic acid, Caffeic acid, Epicatechin, Procyanidin B, Quer-

cetin-3-galactoside and Quercetin-3-glucoside and Phloridzin, were found to be present in the extracts. These phenolic compounds were identified in industrial apple pomace and documented in literature [4] [19] [23] [24] [25]. The chromatogram of the aqueous acetone extract of the phenolic compounds at 320 nm is shown (Figure 1).

The calibration equations, derived from the plots of concentrations of phenolic standard versus the chromatographic peak areas are shown in Table 2. Concentrations of phenolic compounds (mg/kg) dry weight of apple pomace of various design combinations were obtained from the regression equations of corresponding standards and reported (Table 3).

3.2. Model Selection

A number of modelling options were explored for possible selection, including two factor interactions, quadratic and cubic models. These were tested to select

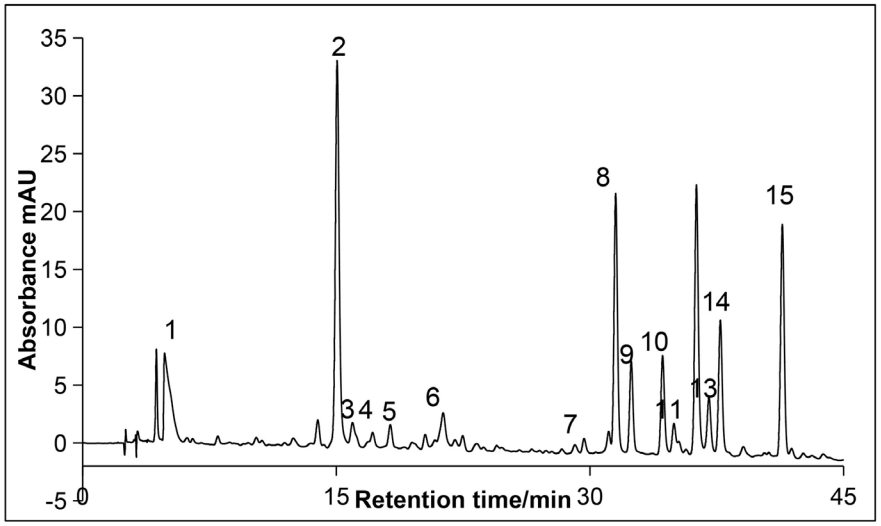

Figure 1. Chromatogram (320 nm) of the extract from the cider apple pomace in aqueous acetone. 1 = solvent peak (acetone), 2 = chlorogenic acid, 3 = procyanidin B2, 4 = caffeic acid, 5 = epicatechin, 7-Ferulic acid, 8 = quercetin-3-galactoside, 9 = quercetin-3-glucoside, 15 = phloridzin.

Table 2. Equations for calibration of standard phenolic compounds from HPLC.

Phenolic Standard	Regression equation	Correlation Coefficient (R^2)
Chlorogenic acid	$y = 25667x$	0.9992
Procyanidin B2	$y = 4.9706x$	0.9833
Quercetin-3-galactoside	$y = 26.232x$	0.9998
Quercetin-3-glucoside	$y = 13829x$	1.0000
Phloridzin	$y = 14704x$	0.9999
Epicatechin	$y = 6210x$	0.9998
Catechin	$y = 5901.3x$	1.0000

Table 3. Concentration of phenolic compounds (mg/kg) dry weight of the cider apple pomace.

Std Order	CGA	PHL	Q-3-gal	Q-3-glu	E-CAT	Pr-B2
1	183.17 ± 4.7	686.35±21.5	159.99 ± 2.4	111.14 ± 2.6	ND	ND
15	170.85 ± 4.0	545.01 ± 10.6	128.88 ± 1.9	89.45 ± 1.7	142.50 ± 9.4	216.85 ± 11.8
19	187.78 ± 7.1	677.32 ± 17.1	171.33 ± 0.5	115.22 ± 3.0	130.30 ± 10.8	201.55 ± 19.8
22	160.99 ± 5.7	562.13 ± 18.9	142.92 ± 4.7	98.83 ± 3.7	132.9 ± 9.7	208.33 ± 1.1
16	201.80 ± 8.7	722.53 ± 25.3	175.09 ± 8.2	117.74 ± 4.7	193.6 ± 33.1	165.80 ± 26.0
23	191.88 ± 5.9	634.49 ± 15.9	174.67 ± 0.4	117.84 ± 2.5	141.5 ± 8.0	227.81 ± 4.4
6	177.39 ± 12.2	641.86 ± 37.7	174.10 ± 12.6	116.91 ± 7.6	173.2 ± 37.9	140.35 ± 28.3
26	190.15 ± 6.8	693.36 ± 20.8	177.78 ± 0.3	118.84 ± 3.3	140.7 ± 10.7	451.69 ± 22.4
14	175.36 ± 7.6	636.60 ± 23.2	173.16 ± 8.1	116.47 ± 4.7	167.4 ± 32.8	150.40 ± 23.3
20	157.05 ± 22.0	776.84 ± 52.9	176.32 ± 18.2	134.37 ± 0.0	ND	ND
11	221.58 ± 9.0	785.27 ± 30.8	186.58 ± 6.1	128.20 ± 2.5	ND	ND
21	184.12 ± 10.9	813.70 ± 31.9	187.83 ± 7.1	131.44 ± 6.7	ND	ND
13	146.69 ± 6.2	484.60 ± 20.6	133.68 ± 5.3	92.88 ± 3.8	132.2 ± 9.1	210.25 ± 5.7
9	167.89 ± 8.7	723.64 ± 36.7	162.88 ± 7.8	114.46 ± 5.9	ND	ND
28	191.38 ± 8.4	713.80 ± 5.7	181.05 ± 1.3	121.81 ± 4.4	152.5 ± 20.0	224.06 ± 3.1
7	162.72 ± 10.6	588.53 ± 6.9	135.03 ± 1.4	93.17 ± 1.2	141.7 ± 5.9	216.38 ± 12.8
18	34.49 ± 4.2	273.55 ± 33.1	30.34 ± 6.5	29.22 ± 0.0	ND	ND
5	156.00 ± 3.5	516.75 ± 12.2	136.02 ± 2.7	94.57 ± 2.1	133.1 ± 6.9	218.26 ± 9.0
25	191.19 ± 5.5	727.07 ± 12.3	176.54 ± 3.3	122.26 ± 2.7	132.5 ± 4.0	217.18 ± 5.4
17	140.67 ± 0.9	314.72 ± 4.2	147.77 ± 0.8	103.45 ± 1.0	109.9 ± 1.6	207.83 ± 10.1
24	191.99 ± 2.9	707.97 ± 18.1	178.16 ± 1.9	119.19 ± 2.2	157.82 ± 10.7	222.60 ± 2.1
12	168.67 ± 14.8	894.62 ± 62.4	172.08 ± 15.4	119.92 ± 0.0	ND	ND
8	190.21 ± 11.3	717.27 ± 28.7	173.37 ± 8.1	116.41 ± 4.3	193.27 ± 19.3	167.11 ± 28.0
30	200.41 ± 3.1	705.85 ± 14.4	182.74 ± 1.6	125.56 ± 1.4	148.98 ± 0.2	225.72 ± 6.0
29	193.29 ± 2.5	709.51 ± 13.0	184.98 ± 1.9	120.09 ± 1.1	142.30 ± 1.8	212.80 ± 0.6
20	248.06 ± 4.7	784.18 ± 20.2	180.15 ± 1.7	120.74 ± 2.1	264.15 ± 30.0	339.78 ± 18.7
10	143.60 ± 13.7	845.59 ± 67.5	164.39 ± 14.0	ND	ND	ND
2	124.51 ± 11.6	847.53 ± 64.9	173.26 ± 11.8	ND	ND	ND
3	189.90 ± 0.9	674.83 ± 44.2	149.79 ± 0.9	104.02 ± 1.4	ND	ND
27	192.57 ± 3.0	682.51 ± 15.8	175.66 ± 1.7	121.14 ± 1.6	138.97 ± 5.2	216.97 ± 3.1

Std = standard, CGA-Chlorogenic acid; PHL-Phloridzin; Q-3-gal-Quercetin-3-galatoside; Q-3-glu-Quercetin-3-glucoside; E-CAT-Epicatechin; Pr-B2-Procyanidin B2; ND not detected.

suitable model that best fits, and capable of depicting the real time response of the surface. For a given model to be appropriate, then it should be significant ($P < 0.05$) and an insignificant lack of fit ($P > 0.05$). Analysis of variance (ANOVA)

at 95% confidence interval were performed utilising Stat-Ease software on the data shown in Table 3, to study the influence of the solvent concentration, solid to solvent ratio, temperature and extraction time on overall recovery of the responses. The results obtained were fitted into a generalised second order polynomial model as in (1):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{4} \beta_{ij} x_i x_j \quad (1)$$

where Y is the measured response, β_0 β_i β_{ii} and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms respectively and x_i and x_j are coded design variables. Selected models for phenolic compounds were significant ($P < 0.05$) and insignificant lack of fit ($P > 0.05$), except for Chlorogenic acid Quercetin 3-glucoside which had significant lack of fit ($P < 0.05$). All models had satisfactory level of adequacies with coefficients of regression $R^2 > 0.9000$, meaning more than 90% of the data generated can be explained by the predictive models. Adjusted correlation coefficients R_{Adj}^2 reasonable agrees with predicted correlation coefficient R_{Pri}^2. Coefficients of variation were < 5% for each determination at the 95% confidence interval. The yields of polyphenolic compounds were significantly affected by acetone concentration, solid-to-solvent ratio, temperature in addition to their interactions. Summary of the analysis of variance (ANOVA) of quantified phenolic compounds is shown in Table 4.

3.2.1. Predictive Model for Extraction of Chlorogenic Acid

Concentration of Chlorogenic acid in extracts varied from 124.5 to 221.58 mg/kg dry weight of the apple pomace with mean concentration of 176.24 mg/kg. The predictive model in terms of actual factors is shown in Equation (2).

$$\begin{aligned} \text{Chlorogenic Acid} = {} & +100.52918 + 3.21207A + 0.29177B - 6.15519C \\ & + 3.24996 \times 10^{-3} D + 0.24195AC - 0.038655BC + 5.36020 \quad (2) \\ & \times 10^{-3} BD - 0.026760CD - 0.037565A^2 - 0.56300C^2 \end{aligned}$$

Table 4. Significance of design factors and interaction terms on responses.

Response	Significance level ($p < 0.05$)											
	A	*B*	*C*	*D*	*AC*	*AD*	*BC*	*BD*	*CD*	A^2	B^2	C^2
CGA	✓	✓			✓			✓		✓		✓
PHL	✓	✓	✓	✓			✓	✓	✓	✓		✓
Q-gal	✓		✓		✓	✓		✓			✓	
Q-glu	✓	✓	✓		✓					✓		✓
Pr-B2	✓	✓	✓		✓	✓				✓		✓
E-CAT	✓	✓	✓									✓
TPC-HPLC		✓	✓		✓				✓	✓		✓

CGA-Chlorogenic acid; PHL-Phloridzin; Q-gal-Quercetin-galactoside; Q-glu-Quercetin glucoside; Pr-B2-Procyanidin B2; E-CAT-epicatechin; TPC-HPLC-total phenolic content (HPLC); ✓-significant. A-acetone concntration; B-Temperature; C-Solid-to-solvent ratio; and D-extraction time.

Chlorogenic acid is polar within polyphenolic compounds and recovery from plant sources require a certain reasonable level of polarity of the solvent. An increase in temperature from 10˚C to 60˚C for 1% solid-solvent ratio for acetone concentration of 40% (v/v), caused yield of Chlorogenic acid to increase by 14%, but decreases by approximately 20% as concentration of acetone approaches 80% (v/v). concentration of acetone 52% (v/v) at 40˚C was reported as good for recovering Chlorogenic acid from apple pomace [26]. The current investigation revealed 46% (v/v) of acetone at 60˚C as good for extracting Chlorogenic acid from the cider apple pomace. Therefore, decreasing the concentration of acetone and increasing temperature favours yield of Chlorogenic acid. Optimal concentration (206.3 mg/kg dry weight of apple pomace) of Chlorogenic acid was recovered and was within the range (30 - 1766 mg/kg) reported for selected cider apples [23]. The variation of design parameters and Chlorogenic acid is shown in **Figure 2**.

3.2.2. Predictive Model for Extraction of Phloridzin

The concentration of Phloridzin in extracts ranged from 314.7 mg/kg to 894.6 mg/kg. The results were consistent with what has been published previously (25 mg/kg to 1061 mg/kg) of cider apples [23]. The model equation based on the regression analysis in terms of actual factors is shown in Equation (3).

$$\begin{aligned}\text{Phloridzin} = {} & +106.89513 + 21.36305A - 1.01061B - 59.51068C \\ & + 0.81180D + 0.18934BC + 0.016002BD - 0.20193CD \\ & - 0.14966A^2 + 4.52601C^2\end{aligned} \quad (3)$$

Phloridzin concentration increased by 16% at 1% solid –solvent ratio as the acetone concentration was increased to 80% (v/v), and decreased by 24% as solid-solvent ratio approaches 8%. Temperature had minimal effect on the recovery

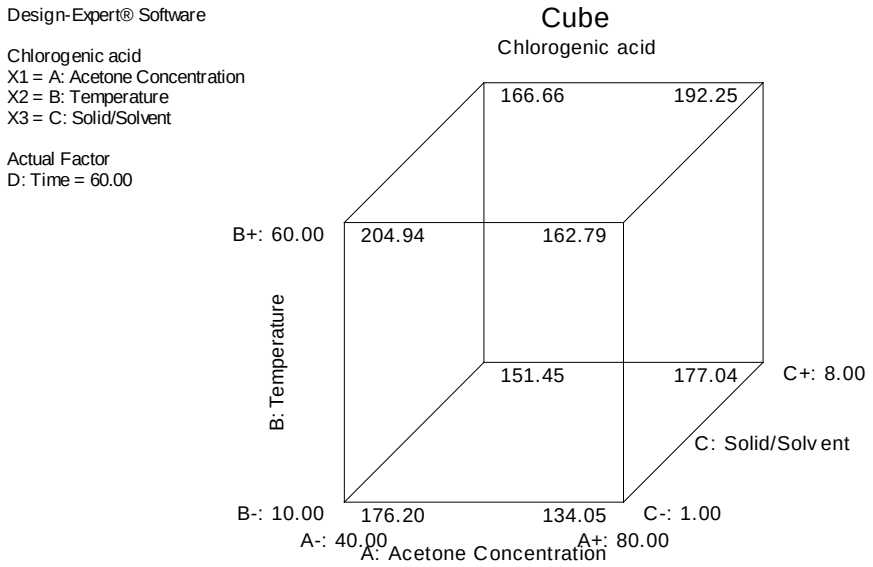

Figure 2. Effects of acetone concentration (% v/v), temperature (˚C) and solid-to-solvent ratio (% w/v) of cider apple pomace on the concntration of Chlorogenic acid (mg/kg) for 60 minute extraction time.

of the dihydrochalcone as it only increased by about 1% when temperature was increased from 10˚C to 60˚C as shown in **Figure 3**.

Optimal concentration of Phloridzin (858.92 mg/kg) was achieved using 73% (v/v) acetone at 60˚C for 60 minutes as against 75% (v/v) at 40˚C for 60 minutes reported earlier [26].

3.2.3. Predictive Model for Extraction of Quercetin Glycosides

Quercetin-3-galactoside dominates among other quercetin glycosides in apple peels [27] and ranged in the extracts from 133.7 - 187.8 mg/kg dry weight of apple pomace with mean concentration of 168.6 mg/kg. Quercetin-3-glucoside ranged in extracts from 60 - 128.2 mg/kg. Both results agreed with previous reports (50 - 520 mg/kg) for Quercetin-3-galactoside, and (9 - 152 mg/kg) of quercetin-3-glucoside in cider apples [23]. Transformed quadratic models excluding outliers were appropriate and described the behaviour of the Quercetin glycosides when the design factors were varied. The model equations are shown in Equation (4) and Equation (5).

$$\begin{aligned}\frac{1.0}{\mathrm{Sqrt}\left(Q-\mathrm{gal}\right)} &= +0.086163-1.15229\times10^{-4}A-3.72357\times10^{-4}B+2.1303710^{-3}C\\ &\quad -6.64449\times10^{-5}D-2.7551\times10^{-5}AC+1.52022\times10^{-6}AD\\ &\quad -1.13192\times10^{-6}BD+6.07617\times10^{-6}B^{2}\end{aligned} \tag{4}$$

$$\begin{aligned}\frac{1.0}{\mathrm{Sqrt}\left(Q-\mathrm{glu}\right)} &= +0.099931-4.32652\times10^{-4}A+1.64815\times10^{-3}C-1.0606\\ &\quad \times10^{-4}D-1.5\times4954610^{-4}AC+1.76547\times10^{-6}AD\\ &\quad +1.06599\times10^{-5}A^{2}+6.69141\times10^{-4}C^{2}\end{aligned} \tag{5}$$

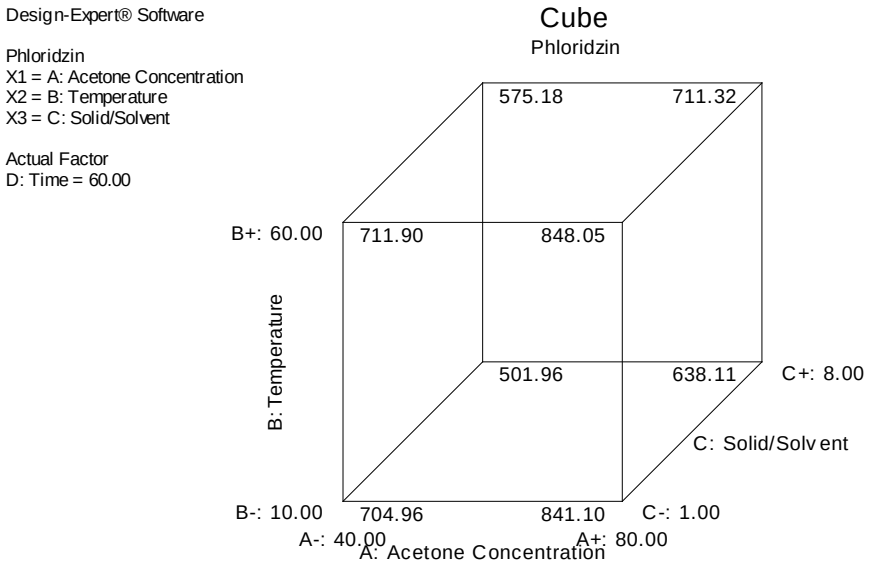

Figure 3. Effects of acetone concentration % (v/v), temperature (˚C) and solid-to-solvent ratio (% w/v) of the cider apple pomace on the concentration of Phloridzin (mg/kg) for 60 minutes extraction time.

Quercetin-3-galactoside concentration increased by 5.24% when concentration of acetone was raised to 80% (v/v) and slightly when temperature increases from 10˚C - 60˚C with increasing solid-to-solvent ratio as reflected in **Figure 4**. Interaction between acetone concentration and solid-to solvent ratio (AC) was more significant than between temperature and time (BD) as revealed by their negative coefficient values which was higher in AC (2.7551×10^{-5}) than BD (1.13192×10^{-6}). The negative coefficient values of temperature and time as well as their interaction suggested overtime with increasing temperature, less recovery of the glycoside could be recovered as shown in **Figure 4**. Decrease in concentration of the glycoside may be due to degradation or hydrolysis of the sugar moieties attached to the quercetin aglycone. Similar results were reported during ultra-sonication procedure of solvent extraction of Quercetin glycosides from "Idared" apple peels [28].

Optimal acetone concentration of 76% (v/v) with 6% solid-to-solvent ratio was good for extracting quercetin-3-galactoside at 41˚C for 58 minutes extraction time. A predicted concentration of 189 mg/kg of quercetin-3-galactoside was suggested for best desirability at the optimal conditions.

Quercentin-3-glucoside showed different behaviour with extraction parameters (**Figure 5**) compared to Quercetin-3-galactoside (**Figure 4**) although both are classified as quercetin glycosides. Both glycosides interacted differently with experimental factors. Solid-to-solvent ratio term influenced positively the yield of quercetin-3-glucoside whereas temperature controlled the elution of quercetin-3-galoctoside in extracts.

The optimal conditions for extracting Quercetin-3-glucoside using aqueous acetone from the apple pomace were 40% (v/v) acetone, 3.5% solid-to solvent

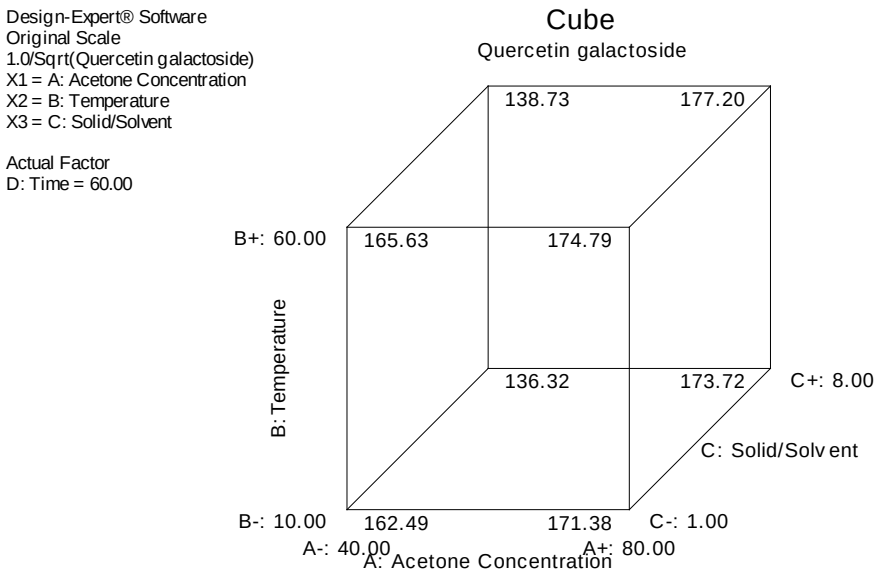

Figure 4. The effects of temperature (˚C), acetone concentration % (v/v) and solid-to-solvent ratio % (w/v) on the concentration of quercetin-3-galactoside (mg/kg) for 60 minutes extraction time.

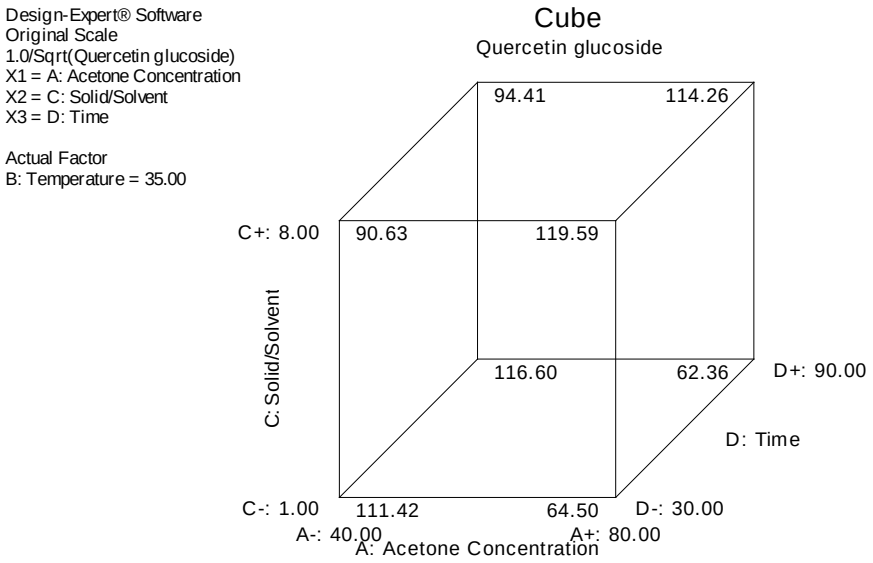

Figure 5. Effects of acetone concentration % (v/v), time (minutes) and solid-to solvent ratio % (w/v) on the concentration of quercetin-3-glucooside at temperature 35˚C.

ratio for 31 minutes at 23˚C extraction temperature. These optimal conditions were different for predicted conditions for recovering quercetin-3-galactoside from the apple peels. It is very important to emphasise that there are no data available in literature to the best of our knowledge as regards good extraction parameters for extracting Quercetin glycosides from cider apple pomace using acetone as an extraction solvent.

3.2.4. Predictive Model for Extraction of Epicatechin

Epicatechin, is a major flava-3-ol, in selected cider apples with concentration in extract ranging from 0 - 193 mg/kg. Similarly, 46 mg/kg to 2225 mg/kg had been reported in fresh cider apples [23]. The regression analysis predicted model equation as shown in Equation (6) and the variation of design parameters with epicatechin concentration shown in **Figure 6**.

$$\begin{aligned}\text{Epicatechin} = &-53.92179 - 0.12460A - 0.037316B + 56.08802C \\ &+ 0.16379AC + 0.051178BC - 5.00284C^2\end{aligned} \quad (6)$$

3.2.5. Predictive Model for Extraction of Procyanidin B2 under Acetone

Molecular and structural differences within Proanthocyannidins make their extraction and quantification very challenging. Their complexation with other non-soluble polymers underestimates their quantification due to incomplete extraction [29]. About 50% - 93% of apple Procyanidins may be retained within cell wall material during processing of apple juice [30]. Procyanidin B2, is a major representative of the various groups of the proanthocyanidins in apple peels [19] and varied in the extract from 0 (not detectable) to 227.8 mg/kg with mean concentration of 137.68 mg/kg. Result was consistent with previous reports (56 mg/kg to 1362 mg/kg) of selected British cider apples [23]. Predicted model equation in terms of actual factors of Procyanidin B2 is shown in Equation (7).

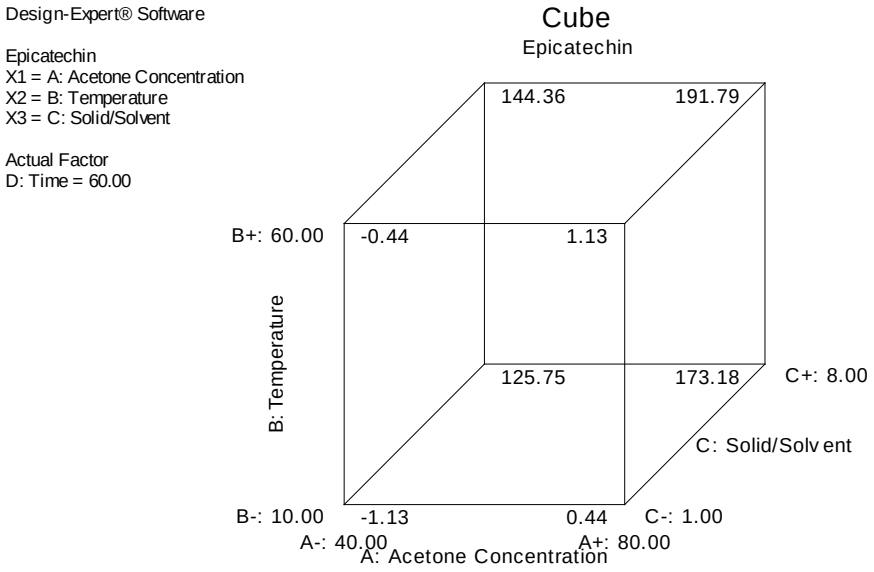

Figure 6. Effects of acetone concentration % (v/v), temperature (˚C) and solid-to-solvent ratio % (w/v) on concntration of epicatechin (mg/kg) dry weight cidr apple pomace for 60 minutes extraction time.

$$\begin{aligned}\text{Procyanidin B2} = &-182.02469 + 2.96978A - 0.011758B + 124.61390C \\ &- 0.35523D - 0.21364AC + 0.034340BC - 0.022831A^2 \\ &- 9.59429C^2 + 2.80355\times 10^{-3}B^2\end{aligned} \quad (7)$$

The variation Procyanidin B2 with experimental factors is shown in **Figure 7**.

The concentration of Procyanidin B2 increases as solid-solvent ratio, temperature and acetone concentration increases and decreases significantly for further increase in these parameters. Optimal solvent concentration and solid-solvent ratio for extracting Procyanidin B2 from the apple pomace at 25˚C for 40 minutes were 54% (v/v) and 6% respectively.

3.3. Effect of Design Variables on Total Phenolic Content by the HPLC Method

Acetone concentration and solid-to solvent ratio and their interaction was the most significant factors in the recovery of the polyphenolic compounds. The model predicted total phenolic content in terms of actual design factors as in Equation (8)

$$\begin{aligned}\text{TPC} = &+320.47139 + 27.34556A - 0.23484B + 42.73751C + 0.66181D \\ &+ 0.67946AC + 0.031083BD - 0.29702CD - 5.91447A^2\end{aligned} \quad (8)$$

The case statistics report showing actual values versus those predicted using the model equation is shown in **Table 5**

The contour plot of the total phenolic content (mg/kg) quantified by HPLC method is shown in **Figure 8**.

Acetone concentration and solid-to-solvent ratio significantly affected the overall yields of extraction of polyphenolic compounds. Optimised conditions of

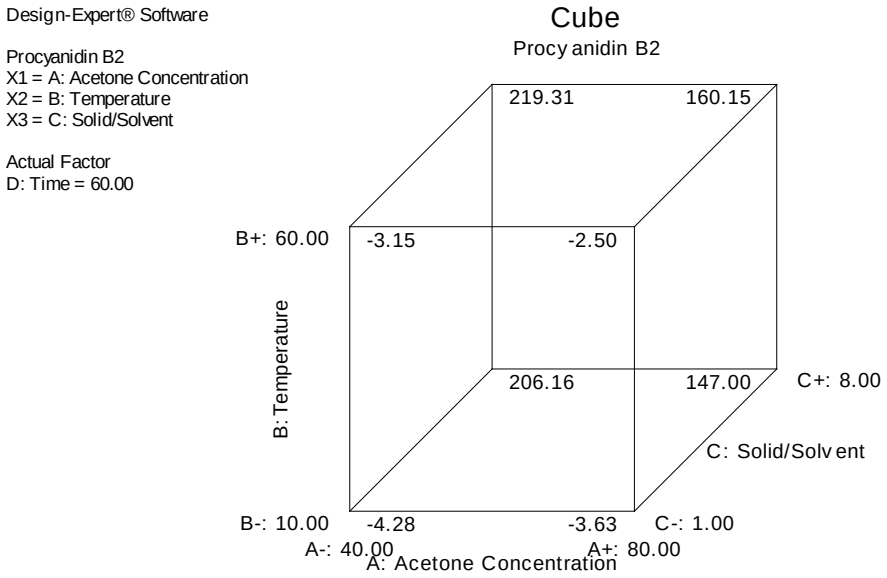

Figure 7. Effect of acetone concentration % (v/v), solid-to-solvent ratio % (w/v) and temperature (˚C) on the amount of Procyanidin B2 (mg/kg) dry weight for 60 minutes extraction time. of apple pomace.

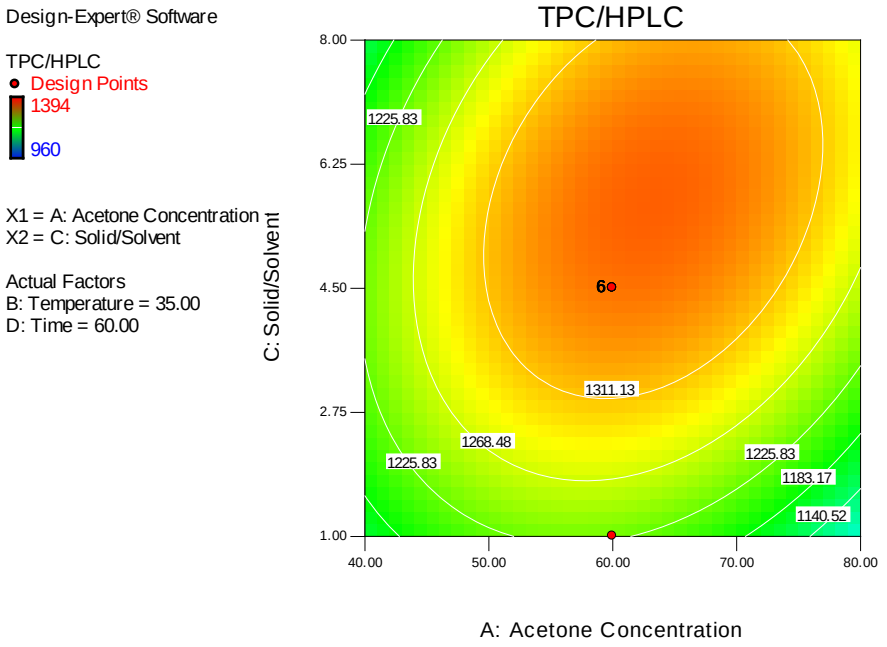

Figure 8. Effect of acetone concentration % (v/v) and solid-to-solvent ratio % (w/v) on total phenolic content (TPC, mg/kg) of acetone extracts of the apple pomace by the HPLC determination

Table 5. Diagnostic case statistics report of total phenolic content (mg/kg) dry weight.

Standard Order	Actual Value (mg/kg)	Predicted Value (mg/kg)	Residual	Leverage
1	1106.00	1101.12	4.88	0.445
2	1044.00	1036.84	7.16	0.470

Continued

3	1043.00	1136.00	−93.00	0.447
4	1103.00	1071.72	31.28	0.469
5	1158.00	1155.55	2.45	0.410
6	1222.00	1281.52	−59.52	0.457
7	1219.00	1190.43	28.57	0.417
8	1303.00	1316.40	−13.40	0.461
9	1120.00	1141.66	−21.66	0.445
10	1046.00	1077.38	−31.38	0.470
11	1314.00	1269.79	44.21	0.447
12	1174.00	1205.51	−31.51	0.469
13	1073.00	1071.34	1.66	0.410
14	1213.00	1197.31	15.69	0.456
15	1109.00	1199.47	−90.47	0.416
16	1366.00	1325.44	40.56	0.460
17	960.00	918.88	41.12	0.73
19	1301.00	1304.06	−3.06	0.145
21	1294.00	1228.78	65.22	0.148
22	1167.00	1142.19	24.81	0.74
23	1300.00	1322.10	−22.10	0.244
24	1340.00	1369.61	−29.61	0.266
25	1354.00	1344.82	9.18	0.097
26	1352.00	1344.82	7.18	0.097
27	1379.00	1344.82	34.18	0.097
28	1360.00	1344.82	15.18	0.097
29	1318.00	1344.82	−26.82	0.097
30	1394.00	1344.82	49.18	0.097

65% (v/v) of acetone, 6% solid-to solvent ratio for 60 minutes at 60˚C were suggested using the statistical model equation with optimal total phenolic content of 1394.01 mg/kg. Validation of the regression model was conducted using the conditions above. The experimental value was determined to be 1392.20 ± 2.9 mg/kg which was in agreement with that predicted by the model.

Higher amounts of phenolic compounds were mobilised around the optimised conditions. The chromatographic methods allowed quantification of individual phenolic compounds present in the extracts without any interference. The HPLC method may not well resolve all phenolic compounds in the extract. For instance, oligomeric flavanols which represent about 71% - 90% of polyphenolic content in apples [31], were not observed in extracts under HPLC used because they might not be retained by the stationary phase.

4. Conclusion

The research demonstrated the application of statistical tools to design experiments for optimisation of recovery of polyphenolic compounds from cider apple pomace using aqueous acetone as solvent. Model equations were generated for selected phenolic compounds by studying the influence of acetone concentration, solid-solvent ratio, temperature and extraction time on extraction of polyphenolic compounds. The independent variables have shown selectivity towards efficient recovery of selected polyphenolic compounds. Improving the polarity of acetone by adding water sufficiently improved recovery of Chlorogenic acid and Procyanidin B2. Quercetin-3-glucoside and Quercetin-3-galactoside exhibited different relationship with temperature and solid-to-solvent ratio although both are classified under Quercetin glycosides. The experimental design predicted 65% (v/v) acetone, 6% (w/v) solid-to-solvent ratio, 60 minutes extraction time at 60˚C as optimum conditions for extracting polyphenolic compounds from the by-product of apple juice and cider production.

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Conflicts of Interest

There are no conflicts of interest as regards this publication.

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