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OPTIMIZATION OF EXTRACTION EFFICIENCY OF ASCORBIC ACID FROM CABBAGE (BRASSICA OLERACEA) USING ORGANIC SOLVENTS

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Extraction efficiency of ascorbic acid from Brassica oleracea leaves and seeds was optimized by response surface methodology using organic solvents. The study design consisted of two independent variables (X_1: extraction time and X_2: solvent polarity) each at five levels affecting the total extractable components (TEC) and ascorbic acid content (AAC) of *B. oleracea* leaves and seeds. TEC (g/100 g dry wt.) and AAC (g/100 g extract) in leaves ranged from 2.42 to 40.84 and 3.20 to 8.30 and those in seeds ranged from 24.40 to 37.10 and 0.54 to 0.82 respectively. Statistical significant ($p<0.05$) variation in TEC of leaves and seeds and AAC of seeds was observed at various combinations of independent variables. A time and polarity dependent increase was observed in TEC and AAC of leaves and seeds. The optimum levels of extraction time and solvent polarity to achieve optimal value of TEC were found to be 46.24 h and 8.98D in leaves and in 16.20 h and 0.29D seeds respectively. The optimum value of AAC in leaves was predicted at extraction time 46.27 h and solvent polarity 6.02D and in seed 24.36 h and 7.97D respectively. The study would provide useful information to the researchers regarding the extraction of ascorbic acid from plants using relatively safe and cheap organic solvents of common use.

Keywords: Ascorbic acid, *Brassica oleracea*, Cabbage leaves and seeds, Central composite design, Response surface methodology, Solvent polarity

INTRODUCTION

Vitamin C chemically known as ascorbic acid, a six carbon lactone, has magical importance in nutritional and pharmaceutical fields due to its diverse biological functions. It possesses anticancer, antiproliferative and antioxidant activities (Belin *et al.*, 2009; Yeom *et al.*, 2009; Du *et al.*, 2010; and González *et al.*, 2013). High dose intravenous administration of vitamin C has been found to decrease the C-reactive protein level and reduce inflammation in cancer and rheumatoid arthritis patients (Mikirova *et al.*, 2012a and 2012b). It acts as cofactor for monooxygenase and dioxygenase enzymes involved in biosynthesis of collagen, carnitine and neurotransmitter and donor or accepter in electron transport at plasma membrane (Coles, 2013; and Riordan, 2014). Being a potent water soluble antioxidant compound, vitamin C protects biomolecules such as lipids, DNA, protein and amino acid from oxidative damage. It reduces a variety of reactive oxygen and nitrogen species such as superoxide, hydroxyl radical, peroxyl radical, singlet oxygen, ozone, nitrosamines and nitrous acids by its hydrogen donating ability. As an antioxidant, it has ability to chelate metal ions because of OH groups on adjacent carbon atoms and prevents from metal induced oxidative stress (Padayatty *et al.*, 2003; Flora, 2009; and Brewer, 2011).

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Ascorbic acid is synthesized mainly in plants but some animals except human also have ability to synthesize it. Human take it in their diets mainly from plant sources. Ascorbic acid is frequently used in nutritional and pharmaceutical formulations after extraction from plant sources. Ascorbic acids is usually found in very small amounts in plants and its extraction from plant material has been a common problem faced by the researchers and manufacturers. Generally inorganic solvents such as metaphosphoric acid and orthophosphoric acids are used for the extraction of ascorbic acid from cabbage and other plants tissues (Reiss, 1993; Rizzolo *et al.*, 2002; Yu *et al.*, 2009; and Fruits, 2016). Some workers have also reported the use of organic acids such as acetic acid, oxalic acid, citric acid, lactic acid trichloroacetic acid and ethylenediamin tetra acetic acid (EDTA) along with phosphoric acid for extraction of AA (Aydogmus *et al.*, 2002; Campos *et al.*, 2009; Kumar *et al.*, 2013; and Radulescu *et al.*, 2013). Others have used simply distilled water for extraction of AA from plant tissues (Machado *et al.*, 2013; and Fatariah *et al.*, 2015). A very few studies have been reported on the use of organic solvent such as alcohols, ethers or hydrocarbons for AA extraction from plant material (Dumbrava *et al.*, 2012; Machado *et al.*, 2013; and Ghafoor *et al.*, 2014). However, no data has been found on the optimization of extraction of ascorbic acids on the basis of polarity of organic solvents.

Brassica oleracea, commonly known as cabbage, is frequently used vegetable all over the world. *B. oleracea* leaves are good source of AA present as biologically active form dehydroascorbic acid (Kurilich *et al.*, 1999; Podsedek, 2007; and Gacche *et al.*, 2010). Previously, researchers have used phosphoric acid and water for extraction of AA from *B. oleracea* leaves (Tee *et al.*, 1988; and Singh *et al.*, 2006). However, no data have been reported regarding the extraction of AA from *B. oleracea* leaves and seeds using organic solvents. The present study was, therefore, designed to optimize the process conditions for extraction of AA from *B. oleracea* leaves and seeds using response surface methodology (RSM). The data will contribute to the research on pharmaceutical extraction and medicinal importance of ascorbic acid present in brassica vegetable.

MATERIALS AND METHODS

Sampling

Leaves and seeds of *B. oleracea* were purchased from local market, transported to research laboratory at Institute of chemical sciences, Bahauddin Zakariya University, Multan, Pakistan and cleaned. The leaves were washed in distilled water and dried under shade. Leaves and seeds were ground using a low speed electric grinder to avoid temperature fluctuation beyond 30 ºC. The ground samples were sieved through fine cloth to obtain fine powder and stored in air tight containers till analysis.

Experimental Design for the Extraction of Ascorbic Acid

The effect of two independent variables X_1: Extraction time and X_2: Polarity of extracting solvents in terms of dipole moment (D) on the ascorbic acid content of leaves and seeds of *B. oleracea* was studied using RSM. A Central Composite Design (CCD) was employed which consisted of five levels of each of extraction time (12, 24, 36, 48, and 60 h) and solvent polarity (hexane 0.0, diethyl ether 2.80, ethyl acetate 4.40, methanol 5.10 and water 9.0 D). The levels of independent variables which showed the optimal response were searched by applying polynomial quadratic model containing nc = 3 centre points, nf = 4, factorial points and na-4 axial points.

The coded levels of both variables were calculated by following generalised equation:

$$Coded\ value\ of\ any\ level = \left[\frac{Selected\ level\ of\ variable - Center\ point}{Interval\ between\ the\ levels}\right]$$

The calculated values of coded levels of extraction time and solvent polarity and the randomized combinations of actual and coded levels of both variables as selected by CCD are given in Table 1.

The ascorbic acid in *B. oleracea* leaves and seeds samples was extracted in organic solvents (solid:solvent ratio 1:20) at various combinations of polarity and extraction time selected by experimental design. The extracts were evaporated to dryness and weighed to calculate extract yield in terms of Total Extractable Components (TEC).

TEC (g/100g dry wt.) = Weight of extract/Weight of sample x 100

Ascorbic Acid Analysis

The extracts were dissolved in respective solvents (100 mg/ 100 ml) and ascorbic acid content was determined by the method reported earlier using dichlorophenol indophenol as active reagent (JAOAC, 1984). Ascorbic Acid Content (AAC) was calculated as g/100 g extract.

Statistical Analysis

The process conditions for extraction of ascorbic acid from

Table 1: Coded and Actual Levels of Independent Variables as per Chosen by CCD

Experimental Runs	Coded Levels of Variables		Actual Levels of Variables		
	X_1	X_2	ξ_1 Extraction Time (h)	ξ_2 Solvent Polarity (D)	
1	0	-2	36	0	
2	-1	-1	24	2.8	
3	1	-1	48	2.8	
4	-2	0	12	4.4	
5*	0	0	36	4.4	
6*	0	0	36	4.4	
7*	0	0	36	4.4	
8	2	0	60	4.4	
9	-1	1	24	5.1	
10	1	1	48	5.1	
11	0	2	36	9	
Coded levels	**-2**	**-1**	**0**	**1**	**2**
ξ_1 : Extraction time (h)	**12**	**24**	**36**	**48**	**60**
ξ_2 : Solvent polarity (D)	**0**	**2.8**	**4.4**	**5.1**	**9**
*Central points					

B. oleracea leave and seed extracts were optimized by RSM using CCD. The generalized polynomial model for the prediction of variation in TEC and AAC was constructed as:

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Yi is the predicted response, β is a constant, β_1 and β_2 are the regression coefficients for linear effects, β_{11} and β_{22} are quadratic effects and β_{12} is the interaction effect of independent variables. The lack of fit (F-ratio) and probability ($p \leq 0.05$) tests were performed to check the significance of estimated regression coefficient for each response variable. The reduced model contained only those terms which were found statistically significant ($p < 0.05$). The coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) were also determined to find the adequacy of the response surface models and fairness of fit of the regression equation respectively. The Coefficient of Variation (CV) was determined to check the precision and reliability of the experiments carried out. The response surface analysis was performed using statistical software Design Expert 10 (Stat-Ease, Inc.).

RESULTS AND DISCUSSION

The experimental values of TEC and AAC of *B. oleracea* leaves and seeds at selected levels of extraction time and solvent polarity are given in Table 2. Experimental values of TEC (g/100 g dry wt.) and AAC (g/100 g extract) in leaves ranged from 2.42 to 40.84 and 3.20 to 8.30 and those in seeds ranged from 24.40 to 37.10 and 0.54 to 0.82 respectively. Comparatively lower values of TEC of and higher values of AAC were observed in leaves those in seeds. Statistically significant difference ($p<0.05$) was observed between TEC of leaves and seeds and AAC of seeds at various levels of design variables.

Response Surface Analysis and Optimization of Results

The effect of extraction variables including extraction time and solvent polarity was optimized by RSM using CCD. Response surface analysis of experimental data yielded the following polynomial regression equations which showed relationship between TEC and AAC of *B. oleracea* leaves and seeds.

Leaves

$$TEC\ \left({}^{g}/_{100\ g\ dry\ wt.}\right) = 7.0478 + 0.0711X_1 - 5.166X_2 - 2.110X_1^2 + 0.882X_2^2 + 0.029X_1X_2$$

Table 2: Experimental Values of TEC and AAC *B. oleracea* Leaves and Seeds at Various Levels of Extraction Time and Solvent Polarity as per Selected by CCD

S. No	X_1	X_2	Extraction Time (h)	Solvent Polarity (D)	TEC (g/100 g Dry wt)		AAC (g/100 g Extract)	
					Leaves	Seeds	Leaves	Seeds
1	0	-2	36	0	6.16	37.1	3.2	0.54
2	-1	-1	24	2.8	3.74	26.7	4.3	0.54
3	1	-1	48	2.8	5.41	25.7	3.7	0.78
4	-2	0	12	4.4	3.02	30.2	4.6	0.66
5*	0	0	36	4.4	2.42	25.5	8.3	0.82
6*	0	0	36	4.4	2.42	25.5	8.3	0.82
7*	0	0	36	4.4	2.42	25.5	8.3	0.82
8	2	0	60	4.4	2.4	27.4	6.7	0.73
9	-1	1	24	5.1	10.26	24.4	4.9	0.82
10	1	1	48	5.1	15.68	25.9	5.3	0.77
11	0	2	36	9	40.84	26.9	4.6	0.67

Table 3: Analysis of Variance (ANOVA), Coefficient of Variation (CV) and Correlation Coefficient (R^2) as Calculated by Response Surface Model for TEC and AAC of Leaves and Seeds of *B. oleracea*

	β_o	β_1	β_2	β_{12}	β_1^2	β_2^2	CV	R^2	Adjusted R^2	Adequate Precision
***B. oleracea* Leaves**										
TEC										
F-value	11.08	0.16	30.68	0.03	0.08	19.55	54.19	0.917	0.834	11.35
p-value	0.009	0.7	0.003	0.86	0.78	0.006				
AAC										
F-value	1.18	0.5	0.72	0.18	1.57	4.43	31.97	0.54	0.08	3.37
p-value	0.43	0.51	0.43	0.69	0.26	0.08				
***B. oleracea* Seeds**										
TEC										
F-value	7.92	0.46	15.34	0.19	5.49	23.42	6.19	0.88	0.77	9.09
p-value	0.02	0.53	0.01	0.67	0.06	0.005				
AAC										
F-value	12.67	0.85	9.94	13.19	10.31	34.24	5.76	0.92	0.85	10.49
p-value	0.007	0.39	0.03	0.02	0.02	0.002				

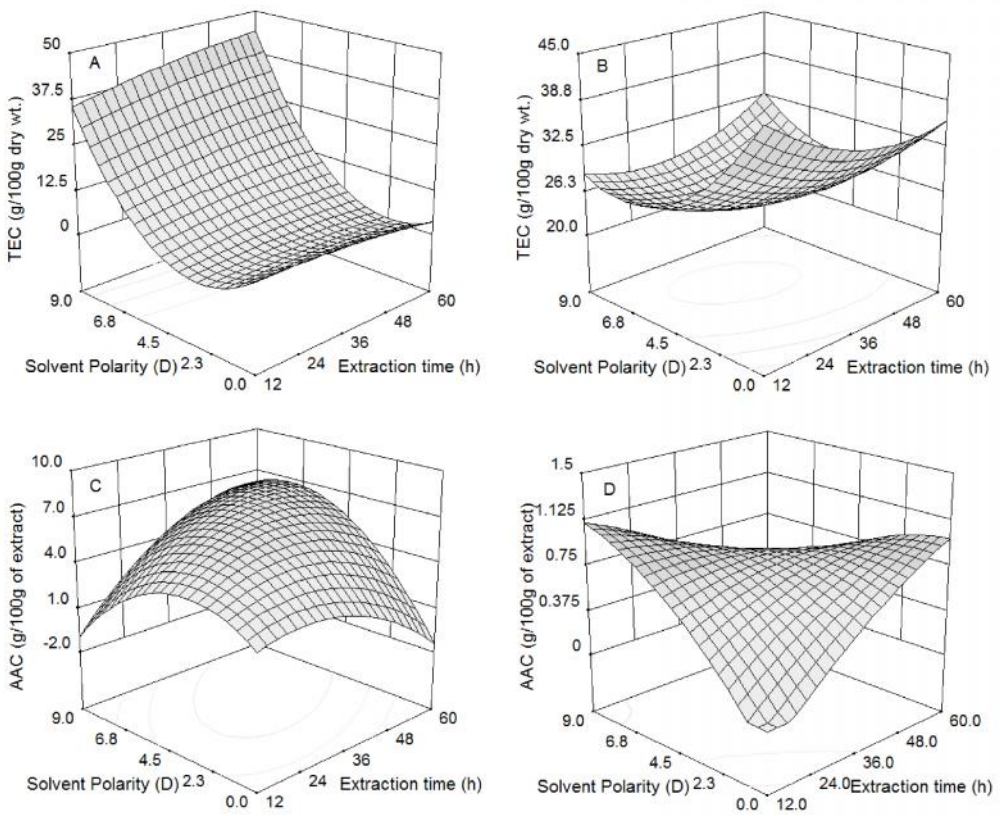
$$AAC\left({}^{g}/_{100\ g\ extract}\right) = 1.137 + 0.169X_1 + 0.744X_2 - 3.539X_1^2 - 0.163X_2^2 + 0.026X_1X_2$$

$$TEC\left({}^{g}/_{100\ g\ dry\ wt.}\right) = 49.361 - 0.592X_1 - 5.052X_2 + 6.198X_1^2 + 0.350X_2^2 + 0.0258X_1X_2$$

Seeds

$$AAC\left({}^{g}/_{100\ g\ extract}\right) = -0.646 + 0.0396X_1 + 0.302X_2 - 2.095X_1^2 - 0.0104X_2^2 - 5.237X_1X_2$$

Figure 1: 3D Response Surface Plot of TEC and AAC of *B. oleracea* Leaves and Seeds at Various Levels of Extraction Variables as Selected by Experimental Design A: TEC of Leaves, B: TEC of Seeds, C: AAC of Leaves, D: AAC of Seeds



These equations included the main, linear, interaction and quadratic effects. The influence of each factor on the response was shown by the sign and magnitude of the main effect as determined by analysis of variance (ANOVA) as given in Table 3. The significance and adequacy of the model was measured in terms of *F*-value and *p*-value at 5% significance level ($p \leq 0.05$). The larger F-values (7.92-12.67) and smaller p-values (0.0202-0.0072) indicated the significant main effects of extraction time and solvent polarity on TEC of leaves and seeds and AAC of seeds**.** The linear and quadratic effects of solvent polarity were found to be significant on TEC of leaves and seeds and AAC of seeds while the interaction effect was found to be significant only on AAC of leaves. TEC of leaves and seeds and AAC of seeds were found to be increased in response to increase in solvent polarity. The polar solvents were found to be more suitable for the extraction of ascorbic acid from *B. oleracea* leaves and seeds. Three dimensional (3D) response surface plots were drawn to show the main and interaction effects of extraction time and solvent polarity on TEC, AAC and AAC/TEC of leaves and a seed extracts (Figure 1).

To test the applicability of the model, the predicted values of TEC and AAC were calculated from the polynomial regression equations and plotted against the experimental ones (Figure 2). A good agreement between the experimental and predicted values of TEC of leaves and seeds and AAC of seeds with high values of correlation coefficient (R^2 = 0.8879, 0.9269) and adjusted correlation coefficient (R^2_{adj} = 0.7758-0.8537). The model statistics showed that more than 88% of variability in TEC of leaves and seeds and AAC of seeds could be explained by suggested polynomial regression model with better prediction variability in response. Relatively low values of CV (6.19 and 5.76) and high values of adequate precision (9.089 and 10.496) showed good reliability and better precision of the model for TEC and AAC in seeds.

The extraction of ascorbic acid from *B. oleracea* leaves and seeds was optimized in terms of extraction time and

Figure 2: Agreement Between the Experimental and Predicted Values of TEC and AAC of *B. oleracea* Leaves and Seeds A: TEC of Leaves, B: TEC of Seeds, C: AAC of Leaves, D: AAC of Seeds

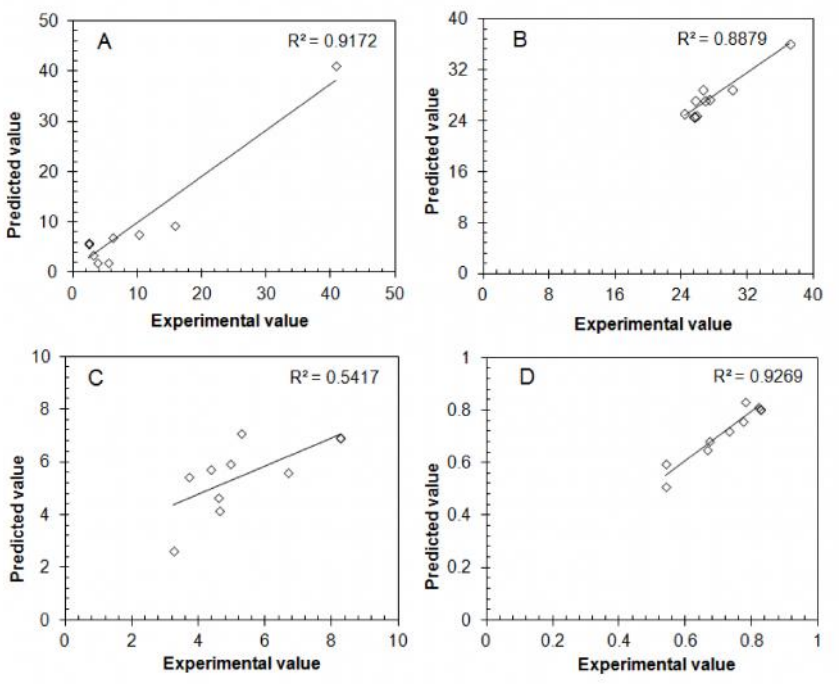
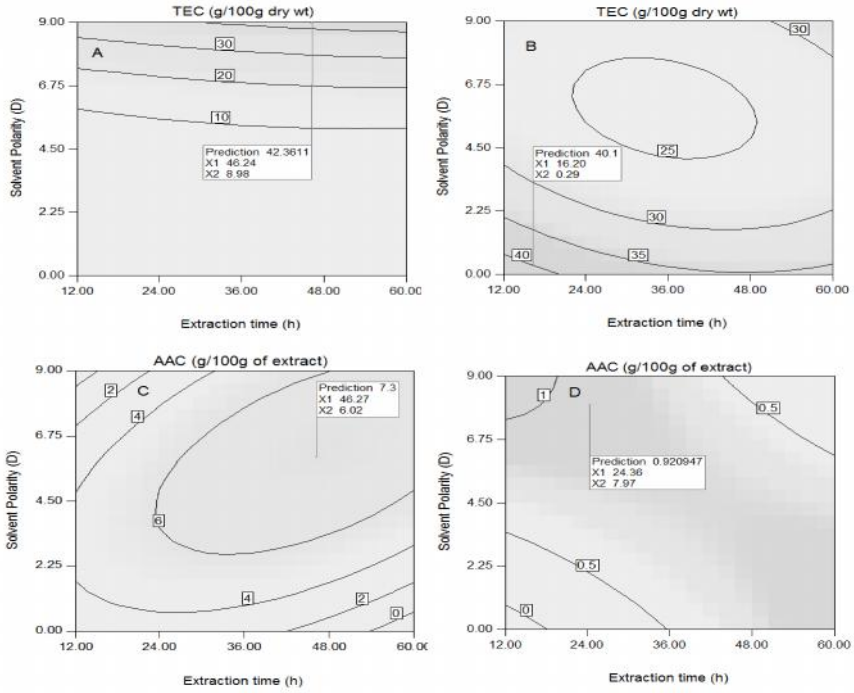


Figure 3: Optimum Values of Extraction Variables in the Area of Optimal Prediction of TEC and AAC of *B. oleracea* Leaves and Seeds A: TEC of Leaves, B: TEC of Seeds, C: AAC of Leaves, D: AAC of Seeds



solvent polarity using RSM. The optimization process predicted an optimum level for each of independent variables in the area of optimal response. The optimum levels of independent variables at prediction of maximal response are presented in Figure 3. The optimum conditions of extraction time and solvent polarity are: TEC of leaves 46.24 h and 8.98D, TEC of seeds 16.20 h and 0.29D, AAC of leaves 46.27 h and 6.02D and AAC of seeds 24.36 h and 7.97D respectively. The extraction from leaves required longer duration to reach the optimal response than that from seeds.

CONCLUSION

The response surface analysis indicated that the relationship between extraction conditions and TEC of *B. oleracea* leaves and seeds and AAC of seeds could be explained by suggested second order polynomial regression models. The extraction yield from leaves and seeds is significantly increased with an increase in extraction time and solvent polarity. AAC of seed extracts is also increased as a function of extraction time and solvent polarity. The polar solvents were found to be more suitable for the quick extraction of ascorbic acid from *B. oleracea* leaves and seeds. However, the effect of studied variables on AAC of leaves extracts could not be explained significantly by the suggested model.

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