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Research Article

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Optimization of Antioxidant Extraction from Freeze-dried Pulp, Peel, and Seed of Burmese grape (*Baccaurea ramiflora* Lour.) by Response Surface Methodology

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ABSTRACT

This study aimed to attain the optimum condition necessary for extracting the maximum yield of antioxidants from the freeze-dried pulp, peel, and seed of Burmese grape using response surface methodology (RSM). Solvent (ethanol) concentration (%), temperature (°C), and time (min) were taken as independent variables by factorial screening for the extraction procedure. After extraction, the antioxidant activity of all samples was determined employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total phenolic compounds (TPC), and ferric reducing antioxidant power (FRAP) assay. The experiment's optimum conditions were 80% solvent concentration, 69.01°C temperature, and 30 min for pulp. The optimum extraction conditions were found at 80°C for 29.39 min incubation time using 52.12% concentrated solvent for seed. For peel, the solvent concentration of 41.62% was found optimum when the temperature of 50°C and 30 min incubation time were used. The actual values of TPC, FRAP, and DPPH for freeze-dried pulp, peel and seed extracts were close to the predicted values, which confirms the models' validity. The Analysis of Variance (ANOVA) showed that the models were significant for TPC, DPPH, and FRAP values of peel, pulp, and seed at different levels ($p < 0.001$ to $p < 0.05$). The composite desirability of pulp, seed, and peel were 0.94, 0.98, and 0.85, respectively, which suggest that the developed model could be effectively used for antioxidants' extraction from freeze-dried pulp, peel, and seed of Burmese grape.

Keywords: Burmese Grape, DPPH, Ferric Reducing Antioxidant Power, Optimization, Response Surface Methodology (RSM), Total Phenolic Compounds.

INTRODUCTION

Bangladesh has some exceptional natural features like 6-8 hours of constant daily sunlight over the year; therefore, this country is boasted with a great variety of fruits. About 70 different types of nutritious fruits are found in this tropical country. Among them, 3.01% of the land is occupied by minor fruits like *Baccaurea ramiflora*. But they produce more than 8.38% of the total fruit production of Bangladesh. In Bangladesh, the fruit plant is grown mainly in homestead conditions [1]. The tropical foods of Bangladesh are very rich in antioxidants. But some of them are appropriately utilized where some others are underexploited fruits; some natives only consume those. Burmese grape (*Baccaurea ramiflora* Lour.) is one of them. In Bangladesh, it is commonly known as lotkon. Geographically, Lotkon is grown in the sub-Himalayan track of South-east Asia region [2].

Taxonomical Classification (Cronquist, 1988)

Botanical Name	<i>Baccaurea ramiflora</i> Lour.
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Phyllanthaceae
Genus	<i>Baccaurea</i>
Species	<i>Baccaurea ramiflora</i>

The lotkon tree is semi-evergreen, reaching 8-10 meters with thick, broad, and ellipse-shaped leaves. In the summer season, this fruit grows on the tree stems and branches [3]. The yellow to

purple color fruit has leathery pericarp and a diameter of 2-3 cm. The pulp inside is pale rose in color and embeds 3-4 seeds inside it [4]. This mild acidic fruit is mainly consumed fresh (Pradhan *et al.*, 2015). There are numerous scientific shreds of evidence that plant and its product, like-fruits, peel, leaves, and seeds contain different bio-active compounds which offer countless health benefits and protection against degenerative diseases [5-8].

Previous studies revealed that consuming nutritious vegetables and fruits inversely affects the risk of degenerative disorders associated with cardio and cerebrovascular disorder morbidity and various kinds of cancers [910]. This is mainly due to the available antioxidant compounds, *e.g.*, flavonoids, carotenoids, and polyphenols in the fruits and vegetables. These compounds have some positive effects on the human body [11], and play an important role in balancing the ratio of free radicals and scavenging free radicals [12].

The ecological and traditional diversity refers that the plant in Bangladeshi locality might have a crucial source of pharmaceutical ingredients [1]. These fruits are taken mainly for a great source of vitamin A, C, and several other nutrients. It is rich in polyphenols and flavonoid contents [4]. The lotkon fruit has three different parts, *e.g.*, seed, juice/pulp, and peel. Besides pulp, it was reported that the seeds and peel of other fruits are rich in natural antioxidants [7, 13]. The enhanced various utilizations of the fruits meet the nutritional challenge. Lotkon fruits have been consumed since ancient times for health and medicinal purposes. However, it is significant that a tiny portion of lotkon fruit is edible, which means a large part of lotkon produce wastage [14, 15]. These waste materials are not easily degradable and thus produce environmental pollution. The use of lotkon seeds and skins may be a possible source of antioxidants and can help control ecological pollution if appropriately used. Several previous efforts were made to estimate the antioxidant activity of the freeze-dried seed, pulp, and skin of Burmese grape [16].

However, the process should be optimized before extraction because factors like extraction time, temperature, solvent concentration, solvent type, pressure, solid-to-liquid ratio, and pH can significantly affect the extraction process of fruits [17]. Solvent extraction methods have been considered as the easiest and simple method for antioxidant determination from plants' parts. In general, the solvent polarity, solvent concentration, extraction time, and extraction temperature are the most controlling factors [18-19]. Soong and Barlow (2004) used an identical extraction method for jackfruit, avocado, longan, mango, and tamarind to compare antioxidant activity between the edible portion and seeds [20]. However, according to Eberhardt *et al.* (2000), it would be difficult to establish a universally optimized extraction protocol due to the complex internal matrix and diversity of natural sources' antioxidant compounds [21]. Hence, the optimum extraction protocol is anticipated to differ with the type of fruits, and between the edible and non-edible portions. Very few previous research works were done to optimize the antioxidant activity of freeze-dried peel, pulp, and seed extract of Burmese grape to the best of our knowledge. Hence, this research work aimed to assess the antioxidant activity of lyophilized peel extract, pulp extract, and seed extract of lotkon samples in this context. The effects of extraction time, extraction temperature, and solvent concentration on the extraction of antioxidants from lotkon pulp, peels, and seeds were also determined.

MATERIALS AND METHODS

Sample collection and preparation of sample extracts

Lotkon fruits were purchased from the Agora super shop, Subidbazar, Sylhet, Bangladesh. Different parts of lotkon fruits were separated by maintaining the hygienic condition. All the parts of the Burmese grape were then freeze-dried. For this, the samples were firstly frozen in a blast freezer at -18°C followed by lyophilization in a freeze dryer ((Model- LYOQUEST-55) at

20 °C under vacuum condition. The dried parts were then powdered by laboratory type hammer mill and passed through a sieve of 1 mm size. The freeze-dried powders from peel, pulp, and seed of Burmese grape were extracted using 40-80% concentrated ethanol. Figure 01 illustrates the schematic overview of the research design.

Extraction procedure

Firstly, 0.5 g of sample (dry powder) was taken for each extraction. For the extraction, 2³ full factorial screening design was used points where, X₁ = temperature (°C), X₂ = time (minute), and X₃ = solvent concentration (%) were independent variables. The samples were vortexed well before the extraction. A temperature-controlled water bath (JBA5 US, Tomas Scientific, USA) was used for maintaining temperature. Ethanol was used as solvent because of its low toxicity in contrast with other alcohols and ability to dissolve non-polar substances. The samples were kept as airtight as possible to prevent evaporation losses during extraction. At the end of each removal, the specimens were filtered with Whatman filter paper (Number 04). Then, the total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity of the filtrates were estimated.

Total phenolic contents (TPC)

The total polar phenolic compound was determined by the modified Folin-ciocalteu reagent (FCR) method described by Zzaman *et al.* (2021) with some moderations [22]. In a 10 mL flask, 0.5 mL of the ethanolic extract was placed. After that, 0.5 mL Folin-Ciocalteu reagent was added to the extracts. Following this, 1 mL of NaCO₃ solution was added, and the volume was made up to 10 mL with double-distilled water. The supernatant's absorbance was taken after keeping the mixture for 1 hr, using a UV Spectrophotometer (PG Instruments Ltd, Model - T60 U) at a wavelength of 725 nm against a reagent blank. The gallic acid (GA) calibration curve

($y=2692.4x$; $R^2 = 0.98$) was prepared by fitting the absorbance versus its corresponding pure gallic acid solutions, where the water blank was taken as zero (0). The results were calculated in mg GAE/100 grams of sample.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was measured using the updated method of Thaipong *et al.* (2006) [23]. At first, 0.3mL of extracted sample was mixed with 0.85mL of phosphate buffer (pH 6.6; 0.2 M) and 0.85mL of potassium ferricyanide (1%) and vortexed. After incubating the mixture at 50 °C for 20 minutes, 0.85mL of 10% trichloroacetic acid was added and vortexed well. Finally, 2.85mL of distilled water and 0.57mL of FeCl₃ (1%) were added, and the final mixture was kept at 25 °C for 30 minutes. After the second incubation, absorbance was measured at 700nm using a UV Spectrophotometer (PG Instruments Ltd., Model - T60 U). A blank was prepared in parallel, where distilled water was added instead of the extract. The standard ascorbic acid was prepared by serial aqueous dilution of stock solution. The standard curve ($y = 8.9855x$; $R^2 = 0.99$) was made by fitting the absorbance versus its corresponding standard ascorbic solutions, where the water blank was taken zero. The results were demonstrated as mg ascorbic acid equivalent antioxidant capacity/100g dry matter (mg AAE/100g DM).

DPPH radical scavenging activity

The method of Rahman *et al.* (2016) was slightly modified for the DPPH radical scavenging assay [24]. An aliquot of 1 mL from the ethanolic extract was added and vortexed with 4 mL DPPH solution in a tube. The tubes were allowed to stand untouched in the dark condition for 30 minutes. Hereafter, the mixtures' absorbance was taken at wavelength 517 nm by using a UV Spectrophotometer (PG Instruments Ltd., Model - T60 U). A DPPH radical solution without

adding the aliquot was used as a control. Finally, the scavenging activity was determined by using the following equation:

$$\text{DPPH radical scavenging effect (\%)} = \left(1 - \frac{\text{Absorbance}_{\text{SAMPLE}}}{\text{Absorbance}_{\text{CONTROL}}}\right) \times 100\% \text{ ----- (Equation 1)}$$

Statistical analysis and experimental design

The software package Minitab (version 19.2020.1) was used to design the experiments and statistical data analysis (ANOVA). The Box-Behnken design was used to optimize the experimental extraction process by Response surface methodology (RSM). The four most relevant variables, namely- solvent concentration, solvent types, time, and temperature, were initially selected. After a factorial screening test, only the temperature (X_1), time (X_2), and solvent concentration (X_3) were identified as the most influencing variables. Based on trial experiments and previous studies, every variable was encoded with three different values high (+1), medium (0), and low (-1), along with start points ($-\alpha$ to $+\alpha$) where the actual high, medium, and low values are given in Table 01.

Data were analyzed by multiple regressions using the least-square method, and Box-Behnken design was chosen to fit the experimental model with the standard second-order quadratic equation simply exhibited by Equation 2:

$$y_r = a_o + \sum_{i=1}^n a_i x_i + \sum_{i=1}^n a_{ii} x_i^2 + \sum_{i \neq j=1}^n a_{ij} x_i x_j \text{ ----- (Equation 2)}$$

Where, y_r represents the measured responses variables, while x_i and x_j are the levels of independent variables. a_o is a constant (predicted response at the center), a_i , a_{ii} , and a_{ij} are the linear, quadratic, and two-factor interactive coefficient of the model, respectively. All the statistical significance tests were based on the total error criteria with a confidence level of 95%.

RESULTS AND DISCUSSIONS

The linear models result from the response surface of TPC, FRAP and DPPH radical scavenging property for freeze-dried lotkon pulp, seed, and peel against different runs have been illustrated in Table 02.

Table 02 exhibits that freeze-dried lotkon pulp shows a higher concentration of TPC than seed and peel. According to the concentration of TPC they can be arranged like pulp>peel>seed. Where, pulp ranges from 10.56 to 15.15 mg GAE/100 g DM, seed ranges from 2.20 to 7.25 mg GAE/100 g DM, and peel ranges from 3.50 to 8.15 mg GAE/100 g DM. FRAP concentration was found to higher in the seed. According to FRAP assay, they can be arranged seed>peel>pulp. In pulp, the FRAP concentration ranges from 347.32 to 621.94 mg AAE/100 g DM; in seed, it is from 1662.00 to 3501.69 mg AAE/100 g DM, and in the peel, it is from 371.55 to 756.56 mg AAE/100 g DM. DPPH scavenging capacity ranges from 38.30 to 59.06 % for pulp, 39.39 to 62.63 % for seed, and 29.02 to 50.26 for the peel.

Analysis of Total Phenolic Content

The Folin-ciocalteu reagent (FCR) method was used to estimate the total phenolic content (TPC) in Burmese grape. The regressions equation to determine the anticipated value of TPC of pulp, seed and peel are described as-

$$\begin{aligned} \text{TPC_pulp} &= 21.35 - 0.1784X_1 + 0.033X_2 - 0.135X_3 + 0.0005X_1^2 - 0.015X_2^2 - 0.0006X_3^2 \\ (\text{mgGAE}/100\text{g DM}) &+ 0.004X_1 * X_2 + 0.001X_1 * X_3 + 0.007X_2 * X_3; \\ &[R^2= 97.37\%; \text{p-value of Lack of fit}= 0.123] \text{----- (Equation 3)} \end{aligned}$$

$$\begin{aligned} \text{TPC_seed} &= 24.37 - 0.955X_1 + 0.107X_2 + 0.191X_3 + 0.008X_1^2 - .007X_2^2 - 0.002X_3^2 \\ (\text{mgGAE}/100\text{g DM}) &+ 0.003X_1 * X_2 - 0.00006X_1 * X_3 + 0.0005X_2 * X_3; \\ &[R^2= 97.31\%; \text{p-value of Lack of fit}= 0.056] \text{----- (Equation 4)} \end{aligned}$$

$$\begin{aligned} \text{TPC}_{\text{peel}} &= -19.21 + 0.513X_1 + 0.577X_2 + 0.053X_3 - 0.003X_1^2 - 0.0009X_2^2 + 0.001X_3^2 - \\ (\text{mgGAE}/100\text{g DM}) & 0.002X_1 * X_2 - 0.001X_1 * X_3 - 0.005X_2 * X_3; \\ & [R^2 = 95.25\%; \text{p-value of Lack of fit} = 0.053] \text{---(Equation 5)} \end{aligned}$$

The analysis of variance (ANOVA) was performed for second-order quadratic equation regression models with the response of total phenolic content for freeze-dried pulp, seed, and peel of Burmese grape. The results from Table 3 show that the models are highly significant ($p < 0.01$). The co-efficient of determination (R^2) value of TPC describes that these models could effectively describe 97.37%, 97.31%, and 95.25% data for pulp, seed, and peel, respectively.

The three-dimensional response surface, along with the contour plot for total phenolic content is presented in Figure 2.

The adjusted R-sq (R^2_{Adj}) values for pulp, seed, and peel were 92.64%, 92.48%, and 86.70%, respectively. This means there are only 4.73%, 4.83%, and 8.55% significant differences between actual values and predicted values, and hence, models reliability. The lack of fit values from Equation 3, 4, and 5 were also insignificant ($p > 0.05$), indicating the models' appropriateness. The estimated coefficients of the fitting model and their statistical significance by ANOVA test for TPC has also been presented in the Table 3. It is clear from the table that the linear term of temperature (X_1) and time (X_2), the quadratic term for time (X_2^2), and the interaction of time to temperature ($X_1 * X_2$) and time to solvent concentration ($X_2 * X_3$) had significant effect ($p < 0.05$) on the extraction of TPC of freeze-dried pulp. For seed, the linear term of temperature (X_1), time (X_2) and the quadratic term for temperature (X_1^2) showed significant effect ($p < 0.05$) on TPC extraction. However, the linear term of temperature (X_1), and time (X_2) exhibited significant effect ($p < 0.05$) on total phenolics extraction.

The TPC was found between 10.56 – 15.15 mg GAE/100g DM for pulp, 2.20 – 7.25 mg GAE/100g DM for seed, and 3.50 – 8.15 mg GAE/100g DM for peel (Table 2). The response surface graph of Figure 2a represented that the extraction of TPC in pulp was increased diagonally by maintaining high temperature and time. It was also found that increased extraction time favors polyphenolic compounds extraction. The combination of more than 75 °C temperature and 20 min was found TPC to be greater than 14 mg GAE/100g DM (Figure 2b). Beyond considering time and solvent concentration for seed, the extraction of TPC tends to decrease with an increase in temperature of up to 65 °C; above it, TPC tends to increase with temperature.

The 3D response surface graph indicates that the TPC decreases with time but increases with the solvent concentration. In contrast, the maximum extraction of TPC from peel was found from 50-60% solvent concentration and 20-25 minutes. However, the temperature had a proportional relation with TPC, and solvent concentration had a quadratic relationship (Figure 2c).

Most other authors also stated that increasing extraction temperature increases extraction, diffusion coefficient, and solute solubility. However, TPC can be degraded above a particular temperature for different samples [17, 25]. A previous study reveals that some phenolics bonded by protein might cause TPC loss in the pulp at higher temperature [26]. Dorta *et al.* (2012) also demonstrated a consistent result in mango peel [27]. Moreover, the heat can enhance the recovery of phenolic compounds. Shi *et al.* (2003) described that enhanced extraction temperature loosens the plant tissue and interrupts the association between phenolic compounds and proteins, or polysaccharides, therefore, increase the solubility, which also improves the diffusion rate [28]. It was also observed that time has the most considerable effect on TPC yield. Inevitably, solute solubility and phenolic extraction increase with time. It might be because of

the time required to increase the medium's solubility and subsequently dissolve out from the dried powder.

Analysis of Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) was found higher in seed in contrast with pulp and peel. The frequency of FRAP ranged between 347.32 to 621.94 mg AAE/100g DM for the flesh, 1662.00 to 3501.69 mg AAE/100g DM for seed, and 371.55 to 756.56 mg AAE/100g DM for peel (Table 02). The regression equations to compute the predicted value of FRAP of pulp, seed and peel are described as-

$$\begin{aligned} \text{FRAP}_{\text{pulp}} &= 694 - 12.92 X_1 - 5.24 X_2 + 0.44 X_3 + 0.1236 X_1^2 - 0.054 X_2^2 - 0.0404 X_3^2 - \\ &(\text{mgAAE/100g DM}) \quad 0.0215 X_1 * X_2 + 0.0183 X_1 * X_3 + 0.226 X_2 * X_3; \\ &[R^2 = 96.94\%; \text{p-value of Lack of fit} = 0.008] \text{----- (Equation 6)} \end{aligned}$$

$$\begin{aligned} \text{FRAP}_{\text{seed}} &= 4665 - 164.6 X_1 - 61.2 X_2 + 66.0 X_3 + 1.450 X_1^2 - 2.543 X_2^2 - 0.727 X_3^2 - \\ &(\text{mg AAE/100g DM}) \quad + 1.466 X_1 * X_2 - 0.219 X_1 * X_3 + 1.699 X_2 * X_3; \\ &[R^2 = 98.70\%; \text{p-value of Lack of fit} = 0.703] \text{----- (Equation 7)} \end{aligned}$$

$$\begin{aligned} \text{FRAP}_{\text{peel}} &= 477 + 9.4 X_1 - 17.3 X_2 + 5.1 X_3 - 0.132 X_1^2 + 0.318 X_2^2 - 0.136 X_3^2 - \\ &(\text{mg AAE/100g DM}) \quad 0.201 X_1 * X_2 + 0.0924 X_1 * X_3 + 0.253 X_2 * X_3; \\ &[R^2 = 93.18\%; \text{p-value of Lack of fit} = 0.133] \text{----- (Equation 8)} \end{aligned}$$

The analysis of variance (ANOVA) was executed for second-order quadratic regression models with the response of ferric reducing antioxidant power (FRAP) for freeze-dried pulp, seed, and peel of Burmese grape. Table 4 shows that the models are significant for seed ($p < 0.001$), pulp ($p < 0.005$), and peel ($p < 0.01$). However, it was insignificant for the peel. The co-efficient of

determination (R^2) values of FRAP were 0.96, 0.98, and 0.93 for pulp, seed, and peel, respectively, which describes the models' efficiency. The adjusted R-sq (R^2_{Adj}) values for pulp, seed and peel were 91.43%, 96.36%, and 80.91%, respectively. The small significant differences between actual values and predicted values reflect the models' reliability. The lack of fit values from Equation 6, 7, and 8 exhibited that they were also insignificant ($p>0.05$) for seed and peel, however, significant for pulp.

Table 4 represents the estimated coefficients of the fitting model and their statistical significance by ANOVA test for FRAP. It has been found that the linear term of temperature (X_1) and time (X_2), and solvent concentration (X_3) significantly affect ($p<0.05$) the ferric reducing antioxidant power of freeze-dried pulp of Burmese grape. Besides, the linear term of temperature (X_1), time (X_2), the quadratic term for temperature (X_1^2), time (X_2^2), and solvent concentration (X_3^2), and the interaction of time-to-temperature (X_1*X_2) and time-to-solvent concentration (X_2*X_3) had significant effect ($p<0.05$) on FRAP of seed. However, the linear term of temperature (X_1) showed significant effect ($p<0.05$) on peels' FRAP.

On the other hand, the model obtained from the response surface assay of FRAP for pulp and seed showed that the extraction temperature and time were significant. Therefore, an increase in these variables can promote the growth of FRAP. The response surface graph illustrated that with the rise in temperature and time, the FRAP value was increased for pulp as well as seed (Figure 3a). The contour plot indicates that the maximum FRAP in pulp was achieved when the temperature and time were maintained at 80 °C and 30 min, respectively.

The combination of 80 °C and 30 min extraction time was required to get a FRAP concentration of more than 3500 mg AAE/100g DM (Figure 3b). In peel, the highest FRAP value was achieved at about 60% solvent concentrations, and FRAP value were reduced at temperature more than 60

°C (Figure 3c). Maybe, it is due to the denaturation of the phenolic compounds that shows FRAP activities. Figure 3c also represents that at a fixed 20 min extraction time, the FRAP values for peel extract were increased with temperature. This can be due to the possession of iron ion binding capacity by polymeric phenols, thus increasing FRAP strength. Our results are in agreement with the findings reported by Soong and Barlow (2004) [20], Thaipong *et al.* (2006) [23], Shanmugapriya *et al.* (2011) [29], Loizzo *et al.* (2010) [30], and Jagtap *et al.* (2010) [31].

DPPH radical scavenging activity

The scavenging activity of DPPH radical (%) was found highest in the 14th run (59.06 %) for pulp, 3rd run for seed (62.63%), and 1st run for skin (50.78%) as shown in Table 02. The regression equations to compute the predicted value of DPPH radical scavenging activity of pulp, seed and peel are described as-

$$\begin{aligned} \text{DPPH_pulp} &= -65.8 + 3.62 X_1 + 1.012 X_2 - 0.636 X_3 - 0.0241X_1^2 + 0.0095 X_2^2 + 0.0092 X_3^2 - \\ (\%) & \quad 0.0094X_1*X_2 - 0.0057X_1*X_3 - 0.0057X_2* X_3; \end{aligned}$$

$$[R^2= 87.02\%; \text{p-value of Lack of fit}= 0.030] \text{-----} \text{---(Equation 9)}$$

$$\begin{aligned} \text{DPPH_seed} &= -45.5 + 6.79X_1 + 2.52X_2 - 5.156X_3 - 0.0490X_1^2 + 0.1489 X_2^2 + 0.06866 X_3^2 - \\ (\%) & \quad 0.0022 X_1*X_2 - 0.00825 X_1*X_3 - 0.1317 X_2*X_3; \end{aligned}$$

$$[R^2= 95.33\%; \text{p-value of Lack of fit}= 0.709] \text{-----} \text{---(Equation 10)}$$

$$\begin{aligned} \text{DPPH_peel} &= 1.5 + 1.776X_1 + 0.481X_2 - 0.064X_3 - 0.0153X_1^2 + 0.0060 X_2^2 \\ (\%) & \quad + 0.00448 X_3^2 - 0.00505 X_1*X_2 - 0.00357 X_1*X_2 - 0.0109 X_2*X_3; \end{aligned}$$

$$[R^2= 97.40\%; \text{p-value of Lack of fit}= 0.054] \text{-----} \text{---(Equation 11)}$$

The results of analysis of variance (ANOVA) (Table 5) show that the models are significant at different for DPPH radical scavenging activity of freeze-dried pulp ($p<0.05$) seed ($p<0.01$), and peel ($p<0.01$) and insignificant for pulp. The higher co-efficient of determination (R^2) values of DPPH radical scavenging activity describes that these models could describe 87.02%, 95.33%, and 97.40% data for pulp, seed, and peel, respectively. The adjusted R-sq (R^2_{Adj}) values for pulp, seed, and peel were 79.67%, 86.92%, and 92.72%, respectively, which reflect the models' reliability. The lack of fit values from Equation 9, 10, and 11 shows that the models for seed and peel were insignificant ($p>0.05$); that is, the seed and peel models were correct. However, for pulp, it was not appropriate.

Table 5 shows the estimated coefficients of the fitting model and their statistical significance by ANOVA test for DPPH radical scavenging activity (%) of Burmese grape. From Table 5, the linear term of time (X_2), and the quadratic term of temperature (X_1^2) and solvent concentration (X_3^2) had significant effect ($p<0.05$) on the DPPH radical scavenging activity (%) freeze-dried pulp of Burmese grape. On the other hand, the linear term of time (X_2), the quadratic term for temperature (X_1^2), time (X_2^2), and solvent concentration (X_3^2), and the interaction of time-to-solvent concentration (X_2*X_3) had significant effects ($p<0.05$) on the DPPH radical scavenging activity (%) of seed. However, the linear term of temperature (X_1) and time (X_2), and the quadratic term for temperature (X_1^2) exhibited significant effects ($p<0.05$) on peels' DPPH radical scavenging activity.

Between 60-70 °C temperature and more than 26 minutes, the DPPH scavenging effect was found to be more than 66% for pulp (Figure 4a). At higher temperatures and extended extraction time, maybe the cells' breakdown is more as reflected by the higher antioxidant activity. It might be the reason behind the increase in DPPH activity with an increase in time and temperature

(Figure 4). Figure 4b demonstrates that in the seed, the DPPH activity reached the highest peak at 65 °C using 60% solvent concentration. Scavenging activity decreased when the temperature was increased further. The combination was found to be 60-70 °C temperature and 28-30 min time from the contour plot (Figure 4b). As like as the FRAP response, a negative slope was found for the peel. This demonstrated that, at 60% solvent concentration and 50-52 °C temperature, the DPPH scavenging effect was found to be more than 45% (Figure 4c). The DPPH value increased with rising solvent concentration, but with very high temperature, it reduced gradually. The polarity of the antioxidant plays a significant role in increasing the DPPH scavenging activity. However, with the increase in temperature, the peel's cell wall may be disrupted early, and then further increase in temperature may be denatured the structure of antioxidant. According to Pinelo *et al.* (2005), extraction temperature more than 50 °C may alter the phenolic compounds, stability and affects integrity of plants' membrane [32]. This might be a fundamental reason behind decreasing the activity [33-35]. Hossain *et al.* (2020a, b) also reported similar results for antioxidant extraction from moringa leaves and jackfruit pulp and seed [7, 12].

Optimization of the extraction process

An optimization study was done based on the findings mentioned above to determine the best operating condition to extract from the pulp, seed, and peel. The target was set to maximize FCR, FRAP, and DPPH; where the feasibility of the study was also taken into account. Table 6 provides information about the target and fit values of TPC (mg GAE/100g DM), FRAP (mg AAE/100g DM), and DPPH radical scavenging activity (%) of freeze-dried pulp, seed, and peel of Burmese grape.

Figure 5 shows the result of the study of the optimization of individual parts of the Burmese grape. Optimum conditions for pulp to get maximum TPC, FRAP, and DPPH were solvent concentration of 80%, the temperature of 69.01 °C and 30 min incubation time, where the predicted values of TPC, FRAP, and DPPH were 13.19 mg GAE/100 g DM, 561.2 mg AAE/100 g DM and 51.73% with the desirability of 1, .95, and 0.86, respectively (Figure 5a). The actual values of TPC, FRAP, and DPPH of pulp were 13.01 ± 0.14 mg GAE/100 g DM, 549.32 ± 5.63 mg AAE/100 g DM, and 49.32 ± 0.51 %, respectively, which reflects the accuracy and reliability of the fitted model. Whereas, the optimum conditions for the seed to get maximum TPC, FRAP, and DPPH were solvent concentration of 52.12%, the temperature of 80 °C and 29.39 min incubation time (Figure 5b). In this condition the actual value of TPC, FRAP, and DPPH were 7.35 ± 0.08 mg GAE/100 g DM, 3301.34 ± 85.50 mg AAE/100 g DM and 63.95 ± 0.72 %, respectively. The actual values were similar to those of predicted values of TPC, FRAP, and DPPH were 7.47 mg GAE/100 g DM, 3381.36 mg AAE/100 g DM and 62.86% with the desirability of 1, 0.93, and 1, accordingly, which denotes the reliability of the model.

In the case of peel, optimum conditions for maximum extraction of TPC, FRAP, and DPPH were solvent concentration of 41.62%, the temperature of 50 °C and 30 min incubation time. At this condition, the predicted values of TPC, FRAP, and DPPH were 8.10 mg GAE/100 g DM, 569.16 mg AAE/100 g DM and 48.32% with the desirability of 0.99, 0.69 and 0.91, respectively (Figure 5c). The actual values of TPC, FRAP, and DPPH of pulp were 8.26 ± 0.09 mg GAE/100 g DM, 550.25 ± 7.32 mg AAE/100 g DM, and 47.45 ± 0.65 %, respectively, which are very close to the predicted values and thereby confirms the appropriateness and reliability of the fitted model. In comparison, the composite appropriateness of pulp, seed, and peel were 0.94, 0.98, and 0.85, respectively.

CONCLUSIONS

Response surface methodology was applied to estimate the optimum operating conditions to extract bioactive compounds, namely by determining FCR, FRAP, and DPPH scavenging effect in Burmese grapes (*Baccaurea ramiflora* Lour.). Quadratic models were used to predict the response of the parameters. The response surface model, contour plot, and the optimization study were performed to find the optimum conditions for the pulp, seed, and peel portion of the berry. For, pulp the optimum condition was 80 °C, 30 min, and 58.59% solvent concentration. For pulp, it was 80 °C, 23.13 min, and 43.23% solvent concentration. Lastly, the optimum condition for seed was found at 50 °C, 30 min, and 64.24%. The desirability values of all the predicted models were satisfactory. This model may be efficiently used for the optimization of antioxidants' extraction process from Burmese grapes.

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DECLARATION OF CONFLICTING INTERESTS

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Figures

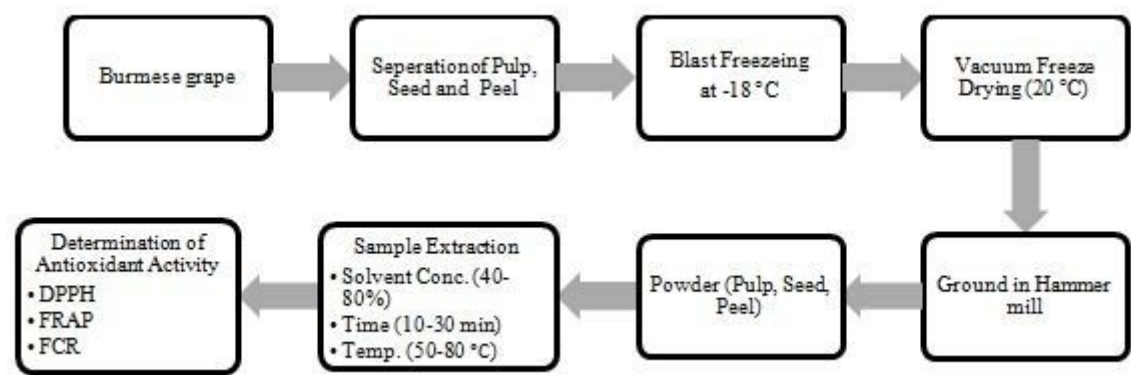
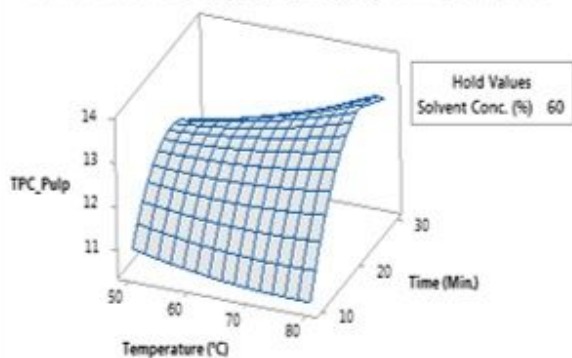


Figure 1

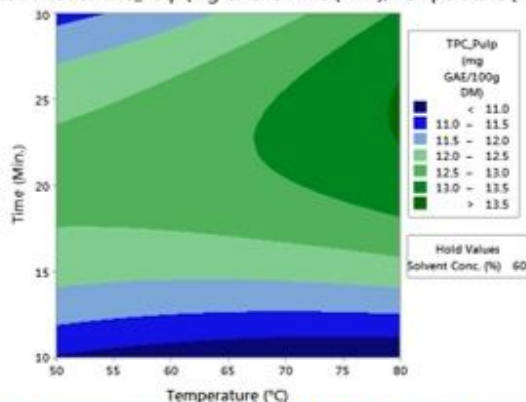
Schematic diagram of research design

(2a) Surface plot and contour plot of total phenolic content (TPC) of pulp

Surface Plot of TPC_Pulp (mg GAE/100g DM) vs Time, Temperature

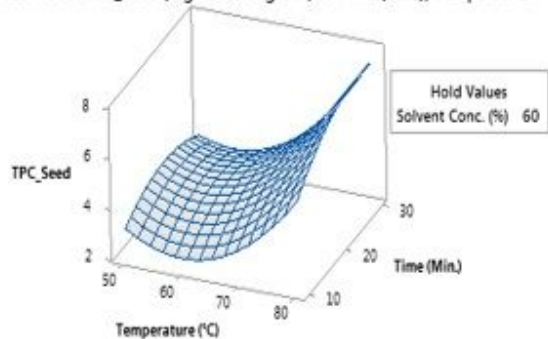


Contour Plot of TPC_Pulp (mg GAE vs Time (Min.), Temperature (°C))

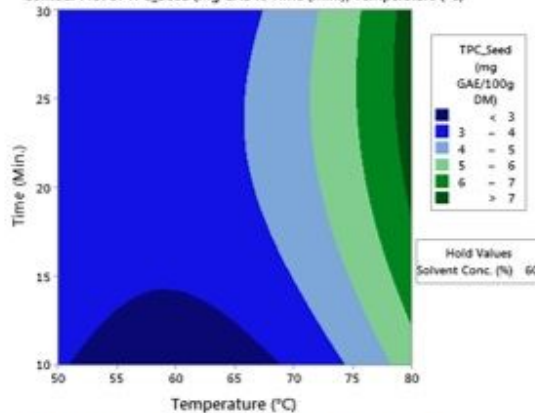


(2b) Surface plot and contour plot of total phenolic content (TPC) of seed

Surface Plot of TPC_Seed (mg GAE/100g DM) vs Time (Min.), Temperature

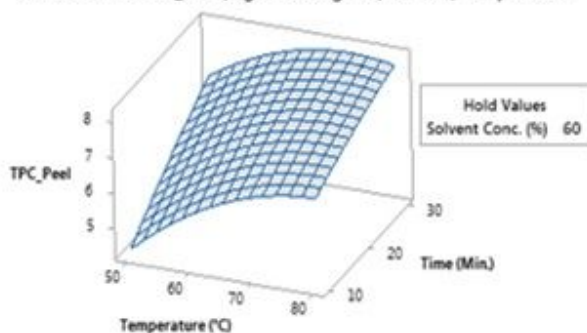


Contour Plot of TPC_Seed (mg GAE vs Time (Min.), Temperature (°C))



(2c) Surface plot and contour plot of total phenolic content (TPC) of peel

Surface Plot of TPC_Peel (mg GAE/100g DM) vs Time, Temperature



Contour Plot of TPC_Peel vs Time (Min.), Temperature (°C)

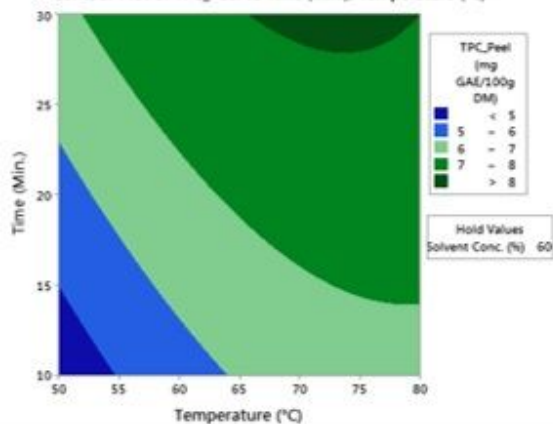
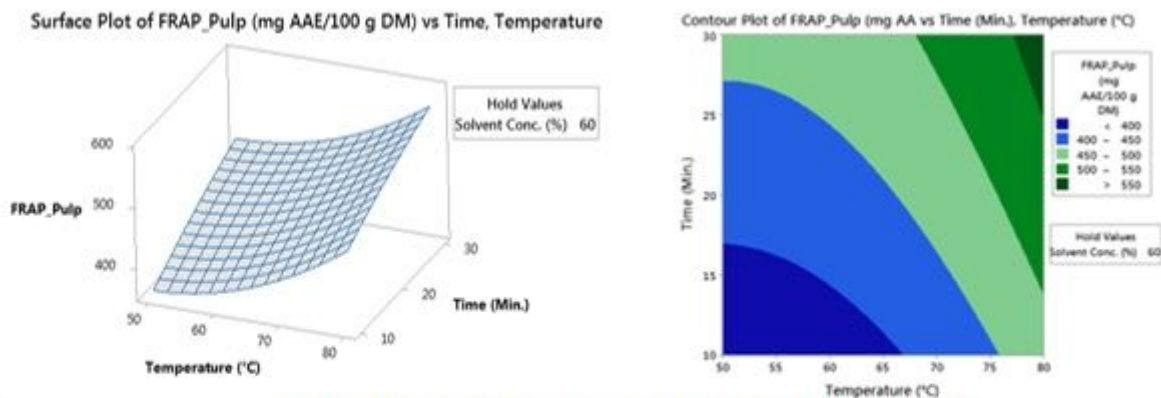


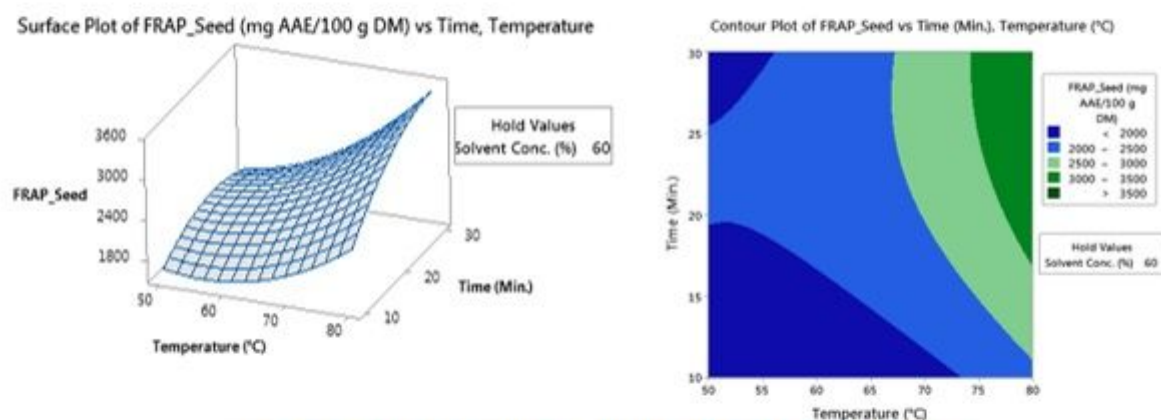
Figure 2

Response surface and contour plot for total phenolic content (TPC) in (a) pulp, (b) seed, and (c) peel of Burmese grape.

(3a) Surface plot and contour plot of FRAP of pulp



(3b) Surface plot and contour plot of FRAP of seed



(3c) Surface plot and contour plot of FRAP of peel

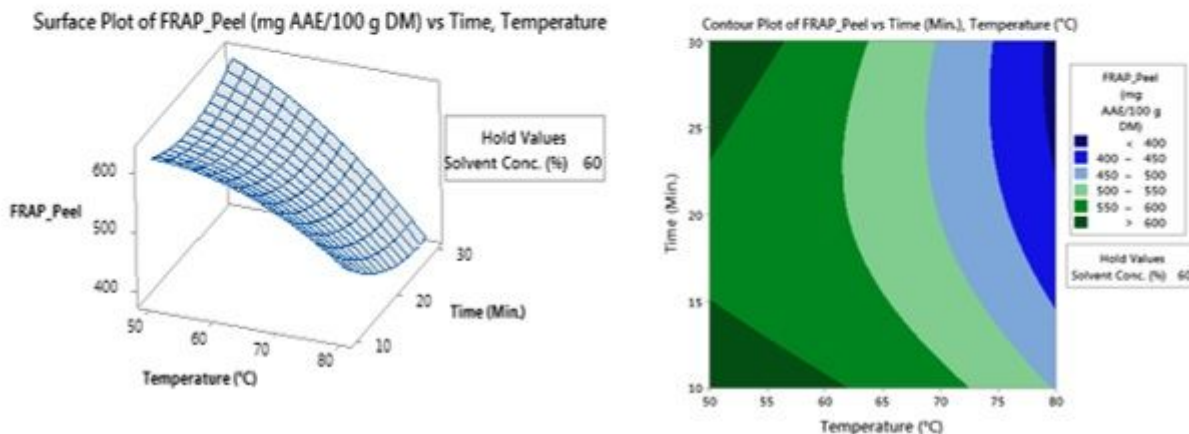
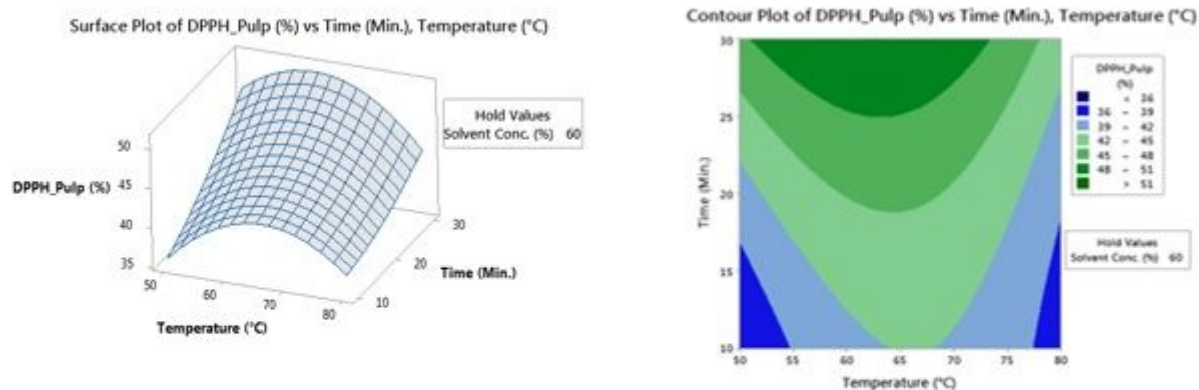


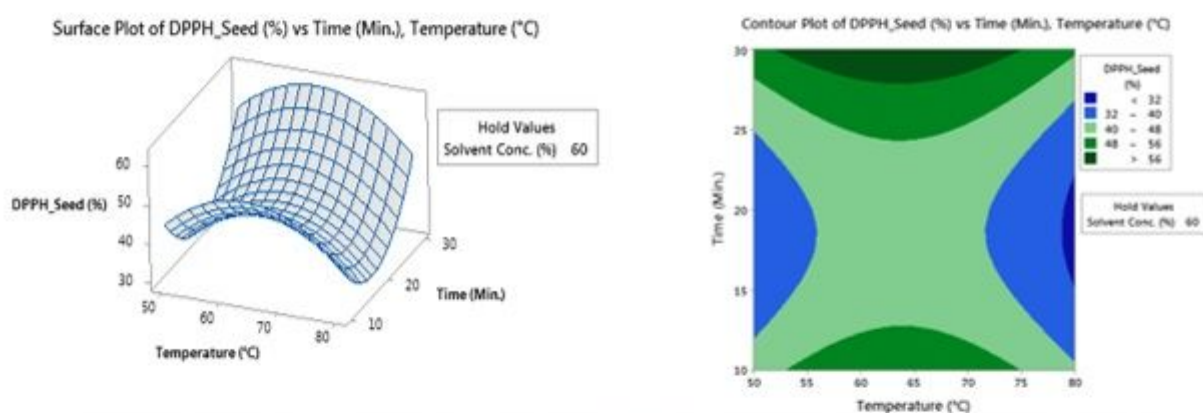
Figure 3

Response surface and contour plot for Ferric reducing antioxidant power (FRAP) in (a) pulp, (b) seed, and (c) peel of Burmese grape.

(4a) Surface plot and contour plot of DPPH radical scavenging activity of pulp



(4b) Surface plot and contour plot of DPPH radical scavenging activity of seed



(4c) Surface plot and contour plot of DPPH radical scavenging activity of peel

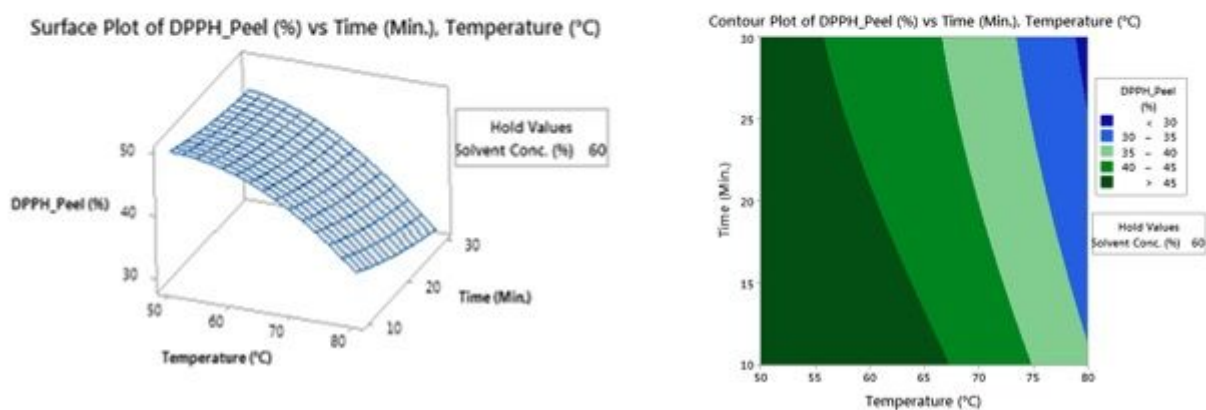


Figure 4

Response surface and contour plot for DPPH radical scavenging activity in (a) pulp, (b) seed, and (c) peel of Burmese grape.

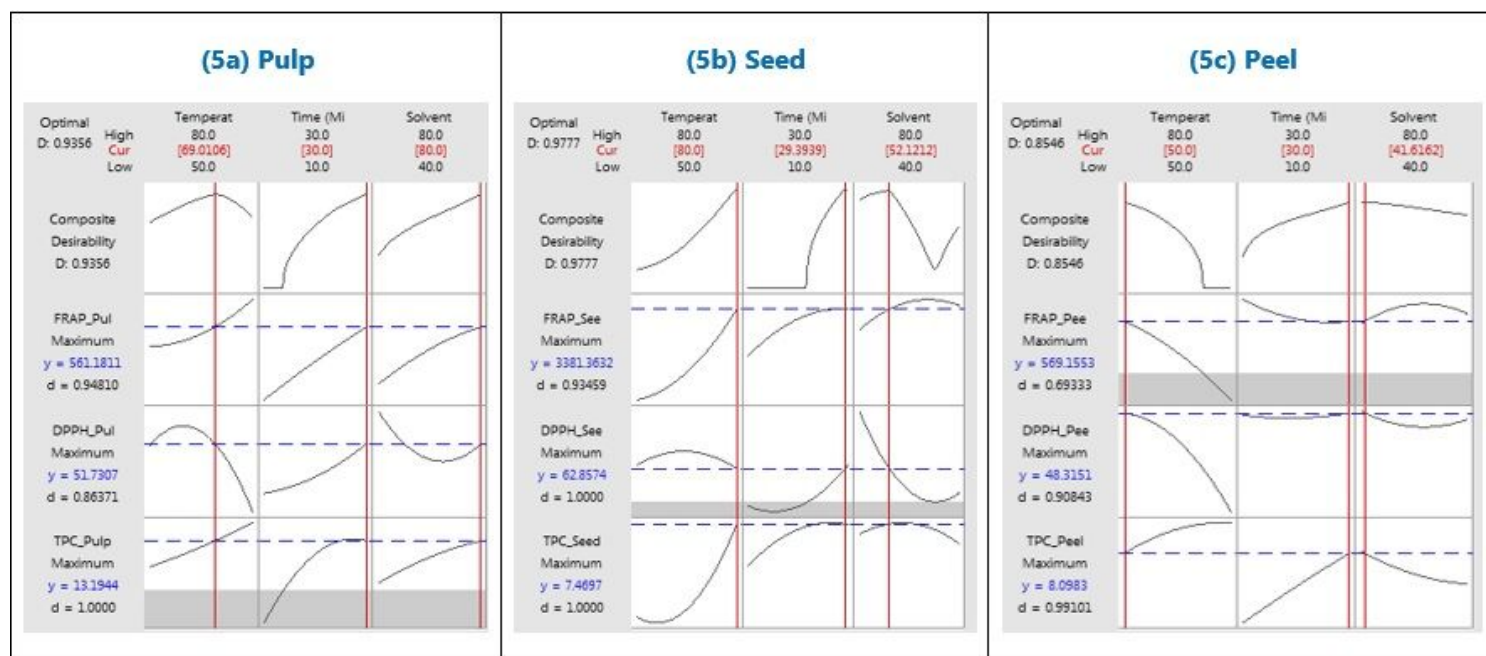


Figure 5

Optimum condition for antioxidant extraction of freeze-dried pulp, seed, and peel of Burmese grape