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ORIGINAL ARTICLE

Aqueous extraction of bitter gourd (*Momordica charantia* L.) juice and optimization of operating conditions

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Abstract – Introduction. Bitter gourd (*Momordica charantia*), belonging to the Cucurbitaceae family, is a well-recognized therapeutic herbal plant with numerous nutritious benefits, improving the overall health of consumers. It possesses many phytonutrients with medicinal activities. Aqueous extraction of phytonutrients is the safest procedure, which can circumvent the challenges associated with other extraction techniques. **Materials and methods.** To optimize the process parameters for aqueous extraction of bitter gourd juice, three independent variables, *i.e.* the fruit:water ratio, extraction time and temperature, were selected within the ranges of 0.3–1.0 g mL^{-1}, 20–160 min and 30–90 °C, respectively. A Box-Behnken design of a numerical optimization technique was used to optimize these variables. The corresponding responses were estimated in terms of concentrations of protein, polyphenols and total solids, and juice clarity. **Results and discussion.** The effects of linear, interaction and quadratic terms of independent variables on the responses were investigated. The present study generated a regression model that could explain 99% of the total variability, with a coefficient of determination (R^2) of 0.99. **Conclusion.** The optimum conditions for aqueous extraction of bitter gourd fruit are: a fruit:water ratio of 0.48 g mL^{-1}, an extraction time of 95 min and an extraction temperature of 68 °C. The corresponding responses at optimum points were estimated to be 131.7 mg L^{-1}, 23.1 mg GAE 100 mL^{-1}, 0.89 g 100 mL^{-1} and 60.6% for concentrations of protein, polyphenols, total solids and juice clarity, respectively.

Keywords: India / bitter gourd / *Momordica charantia* / food technology / nutritional value / phenolics / response surface methodology

Résumé – Extraction aqueuse du jus de courge amère (*Momordica charantia* L.) et optimisation du procédé. Introduction. La margose ou courge amère (*Momordica charantia*), de la famille des cucurbitacées, est une plante herbacée thérapeutique bien connue pour ses nombreuses propriétés nutritionnelles globalement bénéfiques pour la santé des consommateurs. Elle doit également ses nombreuses activités médicinales à sa richesse en microéléments. L'extraction aqueuse de ces microéléments est la procédure la plus sûre permettant de contourner les problèmes liés aux autres techniques d'extraction. **Matériel et méthodes.** Afin d'optimiser les paramètres du procédé d'extraction aqueuse du jus de courge amère, trois variables indépendantes, à savoir le rapport fruit:eau, le temps d'extraction et la température, ont été sélectionnées dans les plages respectives de 0,3–1,0 g mL^{-1}, 20–160 min et 30–90 °C, respectivement. La modélisation Box-Benhken d'optimisation numérique de ces variables a été utilisée pour optimiser la technique. Les réponses correspondantes ont été estimées en termes de concentrations en protéines, en polyphénols, en solides totaux et de clarté de jus. **Résultats et discussion.** Les effets des termes de linéarité, d'interaction et quadratiques des variables indépendantes sur les réponses ont été étudiés. La présente étude a généré un modèle de régression qui pourrait expliquer 99 % de la variabilité totale, avec un coefficient de détermination (R^2) de 0,99. **Conclusion.** Les conditions optimales pour une extraction aqueuse des fruits de courge amère sont les suivantes : un rapport fruit:eau de 0,48 mg L^{-1}, un temps d'extraction de 95 min et une température d'extraction de 68 ° C. Les réponses correspondantes aux points optimums ont été estimées à 131,7 mg L^{-1}, 23,1 mg GAE 100 mL^{-1}, 0,89 g 100 mL^{-1} et 60,6 % pour les concentrations en protéines, polyphénols, solides totaux et pour la clarté du jus, respectivement.

Mots clés : Inde / courge amère – margose / *Momordica charantia* / technologie alimentaire / valeur nutritionnelle / composés phénoliques / méthode des surfaces de réponse

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1 Introduction

Fruit and vegetables are known to be the cheapest source of nutrients and are also termed "functional foods" [1]. They are low in salt, sugar and fat, and help to maintain a healthy weight. They reduce obesity and lower cholesterol, as well as blood pressure [2, 3]. Because of these merits, fruit and vegetable juices are now replacing ready-to-drink, health drinks and bottled cold drinks and are emerging as "New Age Beverages" in a health-conscious society.

Bitter gourd, botanically known as *Momordica charantia* L., is one of the vegetables belonging to the Cucurbitaceae family and is widely grown in Asia, Africa and South America. It is a well-known medicinal herbal plant with many nutritious benefits, improving the overall health of consumers. It is a good source of fiber, nutrients, moisture, amino acids, beta carotene, vitamin C, vitamin B, folate and minerals such as calcium, sodium, potassium, magnesium, phosphorus, zinc and iron, as well as folic acid, alkaloids, peptides and steroidal saponins [4, 5]. It is a well-known Indian traditional vegetable used by diabetic patients as it contains a component, charantin, that is a hypoglycemic and antidiabetic agent. Apart from this, bitter gourd possesses many other medicinal activities, such as antiviral, antioxidant, anti-inflammatory, antimutagenic, antiulcerogenic, antimicrobial, anticonstipation, analgesic and anticarcinogenic [4–7]. Many reports are available on the numerous medicinal effects of bitter gourd on animal models [4, 8, 9]. Chloroform- and alcohol-based extractions are efficient but unsuitable for human consumption [4, 10]. Extraction of phytonutrients using water is the safest and easiest procedure and can circumvent the challenges associated with other extraction techniques.

The present lifestyle and diet lead to major health issues, of which diabetes and obesity are the most common. Diabetes in general involves inadequate metabolism of fat, carbohydrate and protein. The highest number of diabetic patients is in India, followed by China and the USA, and it is predicted that India will have over 79 million diabetics by the year 2030. As the bitter gourd contains hypoglycemic and antidiabetic agents and the extraction of nutrients in juice form is advantageous due to ease of digestion and better effectiveness, a sustainable route of production of bitter gourd juice is therefore warranted.

An appropriate multivariate technique, such as response surface methodology (RSM), helps construct a mathematical model to correlate the juice quality parameters with the operating conditions of extraction. The relevant process variables are the fruit:water ratio, extraction time and temperature, and they were optimized in terms of four responses, the concentration of proteins, polyphenols total solids, and juice clarity. Other characteristics of the juice, *e.g.*, the total dissolved solids (TDS), total soluble solids (TSS), and concentrations of sodium, potassium, calcium and magnesium, were also measured in optimum conditions and reported. The hypothesis is the optimum process parameters obtained will be used as the basis of primary water-based extraction of phytonutrients and subsequent advanced purification steps, in order to have an enhanced shelf life of bitter gourd extract without any additives/preservatives. As the concentration of phytonutrients, their purity and the clarity of the juice are critical in the commercialization of such juice, the above-mentioned characteristics were selected as important responses for bitter gourd in the current study.

The specific objective of this study is to determine the optimized extraction conditions of bitter gourd juice with the aim of larger-scale industrial applications. The importance of the study is associated with the assumption that water extraction of phytonutrients from bitter gourd would reduce the carbon footprint of the entire process since no external chemicals are required, no harmful streams will be generated and no auxiliary process is required to treat any resultant effluent streams.

2 Materials and methods

2.1 Materials

2.1.1 Description of plant material

Bitter gourd is a fast-growing, long-season, humid- and warm-climate creeping plant with flowering vine. It is a herbaceous climber with slightly pubescent stems and leaves and long, unbranched tendrils. This plant grows up to 6 feet tall and bears fruit with an irregular surface with warts and vertical ridges. It can be grown in any soil with a good drainage system with pH ranging from 5.5 to 6.7. It is an inexpensive vegetable accessible throughout India the whole year round on the local market. The main cultivated varieties of bitter gourd in Asia are 'Arka Harit', 'Konkan Tara', 'Priya', 'Hirkani', 'Phule Ujwal' and 'Preethi', but the most commonly available variety in India is 'Phule Ujwal'. The fruits can be stored for 2–3 weeks at 12–13 °C and 85–90% relative humidity. Since the harvesting conditions are not stringent for growing bitter gourd plants, the sustained and uniform quality of products is guaranteed.

2.1.2 Chemicals used

Sodium hydroxide pellets, Folin–Ciocalteu phenol reagent, anhydrous sodium carbonate, copper (II) sulfate pentahydrate and nitric acid were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Gallic acid standard was procured from Loba Chemie, Mumbai, India. Bovine serum albumin was used for calibration of the protein estimation and was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All chemicals were of analytical grade. HPLC-grade acetonitrile was supplied by Merck Specialities Pvt. Ltd., Mumbai, India. Pyridine-2,6-dicarboxylic acid, used for preparing the mobile phase in an ion chromatograph for analysis of cations, was purchased from Merck, Germany. The syringe filter, used for filtering the sample during analysis of cations, was procured from Whatman, GE Healthcare Ltd., UK. All glassware was obtained from Borosil Glass Works Ltd., Mumbai, India.

2.2 Methods

2.2.1 Preparation and sampling of aqueous extracted juice

Fresh, green and unripe bitter gourds were purchased from the local market in Kharagpur in West Bengal, India. These

Table I. Significance of ANOVA generated regression coefficients and fitting parameters on response functions used for optimization of operating conditions of aqueous extraction of bitter gourd juice.

Coefficients of regression equation	*F*- values			
	Protein (mg L^{-1})	Polyphenol (mg GAE 100 mL^{-1})	Clarity (%T)	Total solids (g 100 mL^{-1})
Linear effect coefficients				
A_0	58.01****	375.45****	106.54****	84.66****
A_1	75.04****	422.22****	378.39****	104.02****
A_2	22.34**	15.58**	25.55**	4.80
A_3	189.93****	1.811.02****	469.94****	430.16****
Interaction effect coefficients				
A_{12}	18.85**	35.58***	45.62***	9.01*
A_{13}	5.69*	108.16****	17.34**	29.39***
A_{23}	0.03	11.84*	21.07**	0.76
Quadratic effect coefficients				
A_{11}	24.95**	137.58****	0.35	33.18***
A_{22}	5.60*	72.47****	0.21	34.34***
A_{33}	166.54****	688.77****	0.43	98.64****
Fitting parameters	Protein (mg L^{-1})	Polyphenol (mg GAE 100 mL^{-1})	Clarity (%T)	Total solids (g 100 mL^{-1})
R^2	0.99	0.99	0.99	0.99
Adjusted R^2	0.97	0.99	0.98	0.98
Adequate precision	24.49	58.18	37.92	28.99

Subscripts : 1 – fruit:water ratio , 2 – extraction time, 3 – extraction temperature.
* Significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, *** significant at $p \leq 0.001$, **** significant at $p \leq 0.0001$.

were washed manually with water for removal of dirt or other particles on the outer part of the fruit. They were cut into small pieces, placed in a beaker along with water and heated in a water bath for a certain time. After completion of heating, the extracted juice was allowed to cool and then filtered using nylon mesh cloth to remove the fruit pulp and suspended solids, and the collected filtrate was used for various analyses. Three sets were prepared from each sample and experimental analyses were carried out in triplicate. All experimental results were reported as mean value ± standard deviation (*tables II–III*).

2.2.2 Experimental design and statistical analysis

As discussed earlier, the three independent variables were the fruit:water ratio (X_1), extraction time (X_2) and extraction temperature (X_3). During the experiments, the ranges of these independent variables were X_1: 0.3–1.0 g mL^{-1}, X_2 : 20–160 min and X_3: 30–90 °C. The corresponding responses were the concentrations of protein, polyphenols and total solids, and juice clarity. The Box-Behnken design was used as a response surface methodology (RSM) to generate optimum parameter conditions, and the number of experiments required (N) in this design was calculated by the following relation:

$$N = 2K(K-1) + CP \quad (1)$$

where K is the number of independent variables and CP is the number of central points. Thus, seventeen experimental sets were generated including five at the central points.

The following second-degree polynomial expression was used to correlate the relation between response functions and independent variables:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 \quad (2)$$

where Y is the experimental response; A_0 is the intercept; X_1, X_2 and X_3 are independent variables; A_1, A_2 and A_3 are coefficients of linear effects; A_{11}, A_{22} and A_{33} are coefficients of quadratic effects, and A_{12}, A_{13} and A_{23} are coefficients of interaction effects.

Statistical analyses were performed using Stat-Ease Design-Expert 9.0.5.1 software. Analysis of variance (ANOVA) was applied to identify the effects of linear terms, interaction terms and quadratic terms of independent variables on the responses, and their impact was reviewed by coefficients of determination (R^2) and F-values at different probability levels of 0.0001, 0.001, 0.01 or 0.05 (*table I*).

2.2.3 Biochemical analyses

The temperature, pH and TDS of the samples were measured by a Multiparameter PCSTestr 35 (Eutech Instruments Ltd., Oakton, Singapore). The TSS were determined by Sper scientific digital refractometer (Model: SKU#02941-34; 0–95° Brix, Cole Parmer Instrument Company). A modified Folin-Ciocalteu method [11] was used to measure the polyphenol concentration at 750 nm and Lowry's method [12] was adopted to measure the protein concentration at 660 nm. A Lambda 35 UV-Vis spectrophotometer (M/s Perkin Elmer, Connecticut, USA) was used to measure polyphenol and protein

concentrations by using distilled water as a blank. The polyphenol concentration was expressed in mg gallic acid equivalents (GAE) 100 mL^{-1}. The color of the sample was measured on absorbance (A) mode at a wavelength of 420 nm and the clarity of the sample was determined in terms of percentage transmittance (%T) at 660 nm using the UV-Vis spectrophotometer [13]. Total solids of samples were measured gravimetrically using a hot air oven at 104 ± 2 °C for 24 h. The D445 method of the American Society for Testing and Materials (ASTM) was used to measure the viscosity of the sample at room temperature with the help of a U-tube reverse-flow viscometer (BS/IP/RF, Size: 3, No.-359).

The cation concentration was analyzed by ion chromatography. The sample was injected into the Metrosep C-4 150/4.0 column of a Metrohm 883 Basic IC Plus Ion Chromatograph, supplied by M/s Metrohm Herisau, Switzerland. A solution of 1.7 mL 1M nitric acid and 0.117 g pyridine dicarboxylic acid was used as the mobile phase at a flow rate of 20 μL min^{-1}. Before injecting the sample into the column, it was filtered through a 13-mm disposable syringe filter with polytetrafluorethylene filter media with polypropylene housing and a pore size of 0.45 μm.

2.2.4 Numerical optimization of the juice extraction

The Stat-Ease Design-Expert 9.0.5.1 software (trial version) was used to optimize processing of the independent variables, *i.e.* the fruit:water ratio, extraction time and temperature. Four responses, as already discussed, the concentrations of proteins, polyphenols and total solids, and juice clarity, were examined in the set of experimental conditions generated. The target conditions for numerical optimization were: (a) the maximum concentration of polyphenols, (b) the maximum clarity, (c) the minimum protein concentration and (d) the minimum concentration of total solids.

3 Results and discussion

The implementation of response surface methodology (RSM) to optimize the experimental and analytical processes is very advantageous over the one-factor-at-a-time method. RSM with a Box-Behnken design was used to generate various experimental and optimum conditions and mathematical models using ANOVA. RSM generates a three-dimensional plan representing the variation of one response with respect to two independent variables, while the other variables are kept constant at center points. The F-value provides information on how well the factors describe the statistical variation in the data from its mean. This value for all four responses is reported in *table I*.

The significance of the model can be judged statistically by computing the value of the coefficient of determination (R^2) and adequate precision. R^2 is the ratio of explained variation to total variation and its value varies from 0 to 1, signifying the closeness of data to the best fit curve. ANOVA indicates that the response variables are quite adequate as the value of R^2 is close to unity, *i.e.* 0.99, for all four response functions (*table I*).

Adequate precision indicates the signal to noise ratio. The value of this factor should be more than 4 as a desired limit, so that the model can be used to navigate the design space. The recorded values of adequate precision were 24.49, 58.18, 37.92 and 28.99 (units in *table I*) for the concentrations of protein and polyphenols, juice clarity and total solids, respectively, indicating the best model representation.

The effects of linear, interaction and quadratic terms of independent variables on the responses were also investigated. The linear effect of the fruit:water ratio on all four responses was highly significant. The linear effect of the extraction temperature was also quite significant, but the linear effect of the extraction time did not show any effect on the total solids. No quadratic terms showed any significant effect on the clarity of the sample. Among the quadratic terms, the extraction temperature showed a high significance. The interaction term of the extraction time – temperature combination did not show any effect on the concentrations of protein and total solids.

3.1 Effect of independent variables on concentrations of proteins and polyphenols

The regression models representing the effect of X_1, X_2 and X_3 on the concentration of proteins and polyphenols, in terms of their actual level, are given as:

$$\begin{aligned} \textit{Protein}\ (\mathrm{mg\,L^{-1}}) = {} & -157.07 + 158.34X_1 + 0.02X_2 + 6.45X_3 \\ & + 0.65X_1X_2 + 0.83X_1X_3 + 2.74\times10^{-4}X_2X_3 - 152.37X_1^2 \\ & - 1.65\times10^{-3}X_2^2 - 0.05X_3^2 \quad (3) \end{aligned}$$

$$\begin{aligned} \textit{Polyphenols}\ (\mathrm{mg\,GAE\,100\,mL^{-1}}) = {} & -42.56 + 30.69X_1 \\ & + 0.09X_2 + 1.27X_3 + 0.08X_1X_2 + 0.34X_1X_3 - 5.36\times10^{-4}X_2X_3 \\ & - 33.30X_1^2 - 5.54\times10^{-4}X_2^2 - 9.29\times10^{-3}X_3^2 \quad (4) \end{aligned}$$

The coefficients of all linear terms have positive values, indicating the concentrations of proteins and polyphenols increased with these independent parameters. The fruit:water ratio had the most significant effect ($P \leq 0.0001$) among all linear parameters. All quadratic factors were significant, but their negative coefficients reflected a negative influence on the response. It is clear from *table I* that all quadratic factors for polyphenols were reasonably significant, with $P \leq 0.0001$, while the quadratic factor of time for the protein concentration showed the least significant effect ($P \leq 0.05$). Among the interaction terms, the combination of fruit:water ratio and temperature of extraction is the most significant for the polyphenol concentration. In the case of the protein concentration, the combination of extraction time and temperature is not significant, as the corresponding probability is >0.05. The coefficients of determination (R^2), adjusted R^2 values, are also presented in *table I*. R^2 values were 0.99 in both cases, indicating the adequacy of the regression models. It is clear from *figures 1a* and *2a* that the concentrations of protein and polyphenols linearly increased as a function of the time of extraction, indicating more extraction with time. For example, the protein

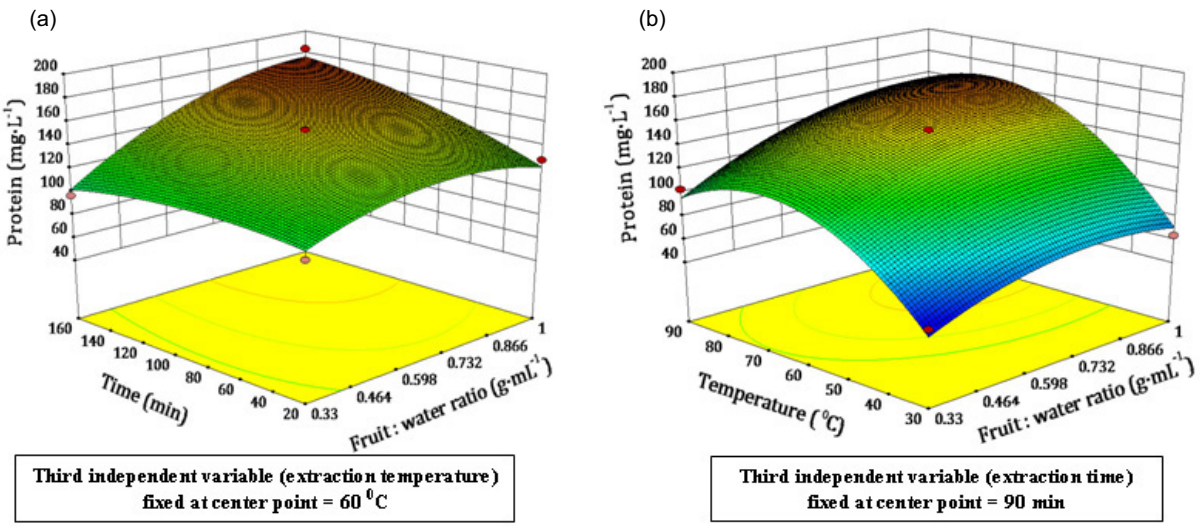

Figure 1. Variation of the protein concentration of aqueous-extracted bitter gourd juice as a function of (a) the fruit:water ratio and time; (b) the fruit:water ratio and temperature.

concentration increased from 120 to 180 mg L^{-1} as the extraction time increased from 20 to 160 min for a fruit:water ratio of 1. In the case of polyphenols, the concentration increased from 20 to 27 mg L^{-1} corresponding to a similar range of extraction times.

Figures 1a and *1b* presents a three-dimensional plot representing the variation of protein concentrations with respect to two independent variables, keeping the third variable constant at the center point. *Figures 2a* and *2b* shows that of the concentration of polyphenols. At a fixed extraction temperature and time, the concentration of both proteins and polyphenols increased with the fruit:water ratio. With a fruit:water ratio between 0.33 and 0.73, more proteins and polyphenols were extracted due to a higher availability of fruit. Beyond 0.73, the increase in the concentration became slower due to the limited solubility of these solutes in a fixed amount of solvent. The protein concentration increased from 100 to 150 mg L^{-1} as the ratio changed from 0.3 to 1.0 g mL^{-1}, at 90 °C (*figure 1b*). Sagu *et al.* [14] reported that protein concentrations increased from 500 to 1,600 mg L^{-1}, as the enzyme dose varied from a lower to higher limit, *i.e.* 0.01–0.05% (v/w) at 60 °C in the case of enzymatic extraction of banana juice.

Similarly, as the fruit:water ratio changed from 0.3 to 1.0 g mL^{-1}, the polyphenol concentration increased from 12 to 26 mg GAE 100 mL^{-1} for 160 min of extraction at 60 °C (*figure 2a*). This result is in concurrence with the extraction of another phytochemical, *i.e.* stevioside, during its water-based extraction [15]. The concentration of extracted stevioside increased from 8.5% to 11.2% as the stevia leaf:water ratio increased from 5 to 20 g mL^{-1} for an extraction time of 120 min at a temperature of 60 °C. Cho *et al.* [16] reported similar results during the ethanol-based extraction of isoflavone from soybean sprout. It was reported that after 20 min of extraction at 85 °C, the concentration of isoflavone increased from 8.5 to 10.8 mg g^{-1} as the ethanol concentration increased from 60% to 95%. A similar relationship was reported by Ghafoor *et al.* [17] between the concentration of extracted polyphenols and carbon dioxide pressure during the supercritical extraction of polyphenols from grape. In all these references, a lower rate of solute extraction was reported in higher operating conditions.

For a particular fruit:water ratio and extraction time, the concentrations of both proteins and polyphenols increased with the extraction temperature, and beyond 70 °C their concentrations remained invariant. As the temperature increased, the concentrations of both solutes also increased due to enhanced solubility. However, since the quantity of fruit per unit volume of solvent was the same, almost all solutes in the fruit were extracted, resulting in a further increase in the solute concentration. The protein concentration increased from nearly 70 to 150 mg L^{-1}, as the temperature changed from 30 to 90 °C, for a fruit:water ratio of 1 g mL^{-1} (*figure 1b*). A similar result was reported by Sagu *et al.* [14] during the enzymatic extraction of banana juice. The protein concentration increased from 1,000 to 1,600 mg L^{-1} as the temperature changed from 30 to 60 °C, with an enzyme dose of 0.05 (%v/w). Similarly, for polyphenols, our results are in accordance with those of Cho *et al.* [16], who reported that isoflavones increased from 8.2 to 9.2 mg g^{-1} as the temperature varied from 75 to 95 °C at an ethanol concentration of 60%.

The increase in the polyphenol concentration with respect to the extraction time was small at a low fruit:water ratio (*figure 2a*) and this result is similar to that of Rai *et al.* [15] during water extraction of stevioside from stevia leaves. Likewise, Cho *et al.* [16] reported that at a particular ethanol concentration, the total isoflavones increased from 8.0 to 10 mg g^{-1} as the extraction time changed from 20 to 100 min.

3.2 Effect of independent variables on juice clarity

The regression model representing the effect of X_1, X_2 and X_3 on the clarity of extracted juice, in terms of their actual

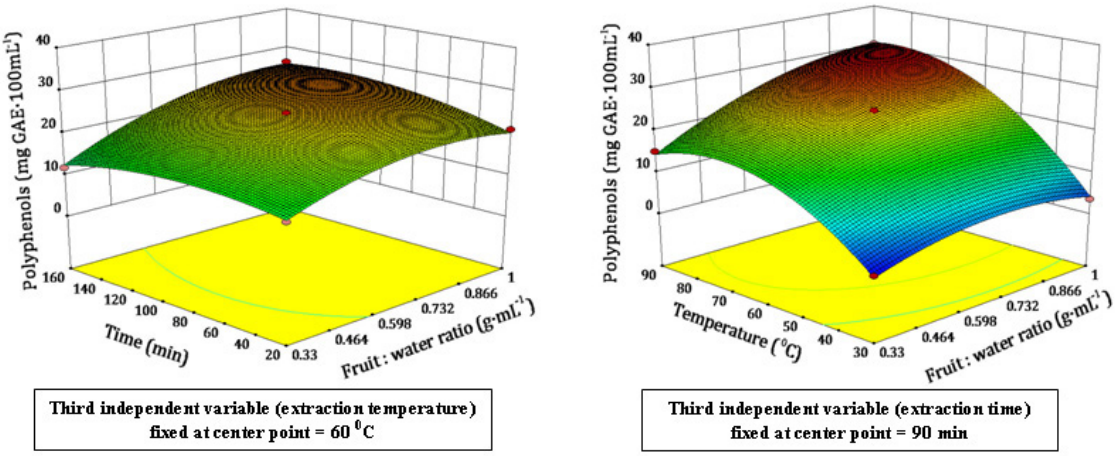

Figure 2. Variation of the concentration of polyphenols of aqueous-extracted bitter gourd juice as a function of (a) the fruit:water ratio and time; (b) the fruit:water ratio and temperature.

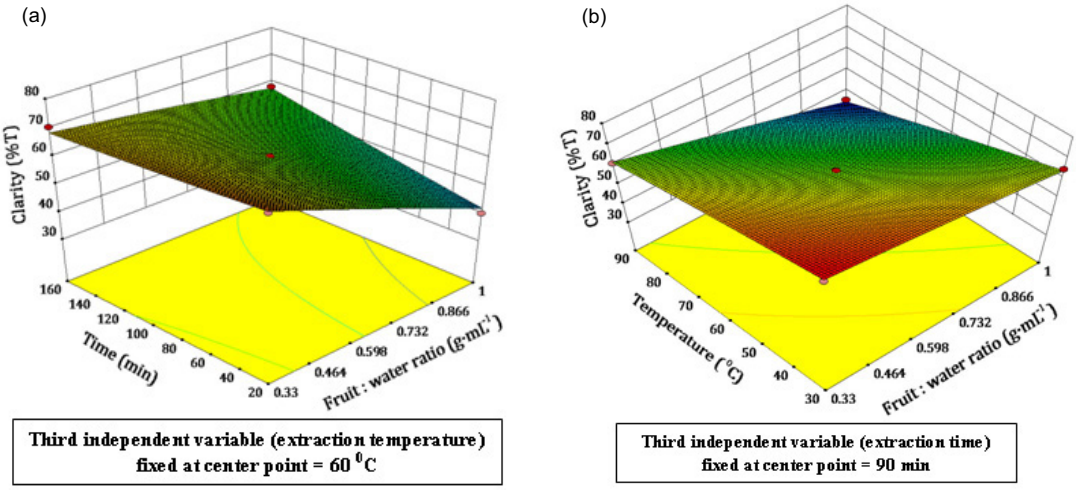

Figure 3. Variation of the clarity of aqueous-extracted bitter gourd juice as a function of (a) the fruit:water ratio and time; (b) the fruit:water ratio and temperature.

level, is given as:

$$Clarity\,(\%\mathrm{T}) = 107.71 - 26.93X_1 - 0.22X_2 - 0.26X_3 + 0.22X_1X_2 - 0.32X_1X_3 + 1.67\times10^{-3}X_2X_3 - 3.90X_1^2 + 6.89\times10^{-5}X_2^2 - 5.42\times10^{-4}X_3^2 \quad (5)$$

All linear and interaction factors significantly affected the clarity of the sample, as their probability level was less than 0.01, whereas the quadratic factors were not significant ($P > 0.05$). All three linear terms had negative coefficients, indicating the negative influence of the responses. Among all the interaction terms, the combination of fruit:water ratio and extraction temperature had a negative effect and the remaining two interaction terms, *i.e.* the combination of fruit:water ratio – extraction time and extraction time – temperature, had positive effects on the juice clarity. The regression model could explain 99% of the total variability, with a value of $R^2 = 0.99$.

The fruit:water ratio was less, and the clarity level was higher due to the dilution of juice with water (*figures 3a* and *3b*). As this ratio varied from 1.0 to 0.3 g mL^{-1}, the juice clarity increased from 35% to 60% at 90 °C (*figure 3b*). Similar results were reported by Chen *et al.* [18] during the enzymatic clarification of green asparagus juice. As the enzyme concentration varied from 0.6% to 1.8%, the asparagus juice clarity decreased by 16% at 45 °C.

Since the mixture of fruit and water was heated, more coloring pigments were extracted, thereby decreasing the clarity of the juice. The juice clarity decreased from 64% to 35% as the temperature increased from 30 to 90 °C at a fruit:water ratio of 1 g mL^{-1} (*figure 3b*). Rai *et al.* [15] reported a similar result that at a leaf:water ratio of 20 g mL^{-1}, the color increased from 9.8 to 13.0, deteriorating the clarity of the extract as the temperature varied from 30 to 90 °C.

The juice clarity varied almost linearly from 40% to 58% as the extraction time increased from 20 to 160 min

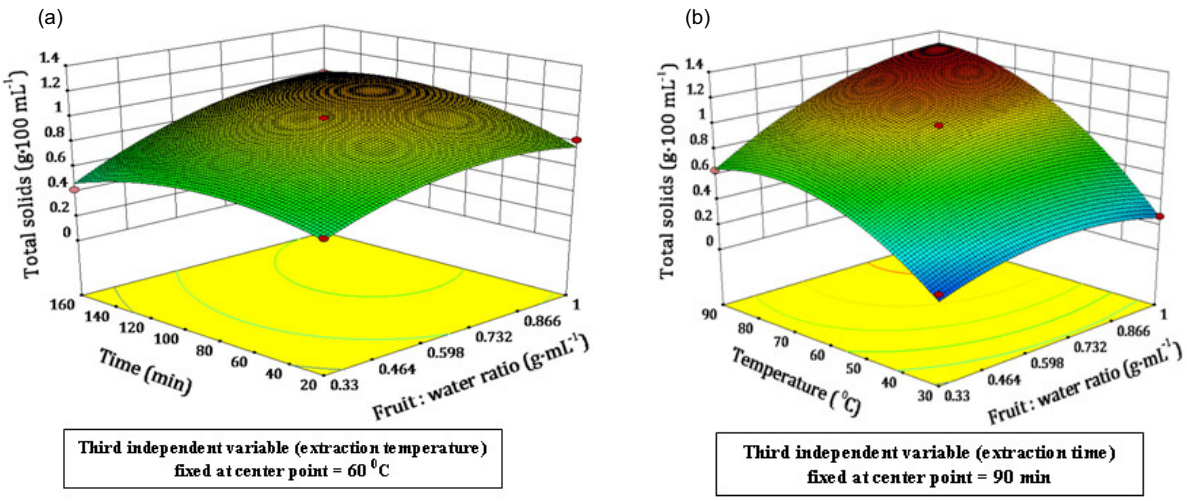

Figure 4. Variation of the total solids concentration of aqueous-extracted bitter gourd juice as a function of (a) the fruit:water ratio and time; (b) the fruit:water ratio and temperature.

(*figure 3a*). The possible reason is that the extraction of coloring components was fast at a particular temperature and the fruit:water ratio, and thereby reduced the clarity later on. Sagu *et al.* [14] similarly reported that banana juice clarity increased from 50% to more than 80%, as the time varied from 20 to 120 min for a particular enzyme dose of 0.05 (%v/w). Rai *et al.* [19] also reported a significant increase in the clarity of sweet lime juice (*Citrus limetta* Risso), when the extraction time changed from a lower to higher limit, at a high enzyme dose.

3.3 Effect of independent variables on total solids

The regression model representing the effect of X_1, X_2 and X_3 on the total solids concentration of extracted juice, in terms of their actual level, is given as:

$$\begin{aligned} \textit{Total solids}\ (100\ \mathrm{mL}^{-1}) = {} & -1.31 + 1.14X_1 + 4.43\times10^{-3}X_2 \\ & + 0.04X_3 + 3.30\times10^{-3}X_1X_2 + 0.01X_1X_3 - 1.07\times10^{-5}X_2X_3 \\ & - 1.29X_1^2 - 3.01\times10^{-5}X_2^2 - 2.78\times10^{-4}X_3^2 \quad (6) \end{aligned}$$

All three linear terms have a positive influence of response since their coefficients are positive. It is clear from *table I* that the linear term of the fruit:water ratio had the most significant effect, followed by the temperature, and that of the extraction time was not significant ($P > 0.05$). All three quadratic factors had significant effects, as their probability levels were <0.001, and they showed a negative influence due to the negative coefficients. Among the interaction terms, only the extraction time and temperature combination did not show a significant effect ($P > 0.05$).

The coefficients of both significant linear terms have positive values, indicating that the total solids concentration increases with the fruit:water ratio and the extraction temperature. The coefficients of all significant quadratic terms have negative values, indicating a negative influence on the extraction response. Both significant interaction terms had positive effects. As observed from *table I*, the R^2 value of correlation proposed in Equation (6) was 0.99, indicating that the regression model could explain more than 99% of the total data.

A higher fruit:water ratio indicates a more concentrated juice. It is clear from *figure 4a* that as the fruit: water ratio varied from 0.3 to 1.0 g mL^{-1}, the total solid concentration ranged from 0.5 to 0.8 g 100 mL^{-1} for an extraction time of 20 min. Similarly, the total solids increased from 0.6 to 1.3 g 100 mL^{-1} and from 0.1 to 0.3 g 100 mL^{-1} for an extraction temperature of 90 and 30 °C, respectively, as the fruit:water ratio varied from 0.3 to 1.0 g mL^{-1} (*figure 4b*).

As the temperature increased, more solids were extracted into the solution, increasing the solid concentration. For a fixed fruit:water ratio of 1 g mL^{-1}, the concentration of total solids increased from 0.3 to 1.3 g 100 mL^{-1} as the extraction temperature varied from 30 to 90 °C (*figure 4b*).

At a lower fruit:water ratio (0.33), the total solids concentration attained a limit value of nearly 0.4 g 100 mL^{-1} (*figure 4a*). At this ratio, the amount of fruit was more than that of water, thereby approaching the solubility limit of total solids.

3.4 Numerical optimization of the extraction of the bitter gourd juice

The numerical optimization generated the optimum conditions of the independent parameters as: fruit:water ratio: 0.48 g mL^{-1}, extraction time: 95 min and extraction temperature: 68 °C. The corresponding responses at optimum points were: 131.7 mg L^{-1}, 23.1 mg GAE 100 mL^{-1}, 0.89 g 100 mL^{-1} and 60.6% for concentrations of proteins, polyphenols and total solids, and juice clarity, respectively. An independent experiment was conducted in the optimum conditions and the responses were found to be within 5% of the numerically calculated values, indicating the predictive capability of the model.

Table II. Experimental conditions of three independent variables generated by Box-Benhken design (in actual levels) and their effect on corresponding responses used for optimization of operating conditions of aqueous extraction of bitter gourd juice. Values are means ± standard deviations (n = 3). X_1 – fruit:water ratio , X_2 – extraction time, X_3 – extraction temperature.

Exp. No.	Independent variables			Responses			
	Experimental conditions			Experimental results			
	X_1 (g mL^{-1})	X_2 (min)	X_3 (°C)	Protein (mg L^{-1})	Polyphenol (mg GAE 100 mL^{-1})	Clarity (%T)	Total solids (g 100 mL^{-1})
1	1.00	90	90	150.0 ± 6.1	30.3 ± 1.4	35.2 ± 1.7	1.25 ± 0.05
2	0.66	90	60	153.0 ± 7.5	24.8 ± 1.1	60.6 ± 3.2	0.99 ± 0.03
3	0.33	90	30	50.2 ± 6.2	1.9 ± 0.4	77.8 ± 3.9	0.22 ± 0.05
4	0.66	160	30	77.5 ± 3.8	6.3 ± 0.5	70.3 ± 3.5	0.28 ± 0.02
5	0.66	90	60	153.0 ± 7.5	24.8 ± 1.1	60.6 ± 3.2	0.99 ± 0.03
6	0.66	90	60	153.0 ± 7.5	24.8 ± 1.1	60.6 ± 3.2	0.99 ± 0.03
7	0.66	20	90	122.8 ± 6.2	23.4 ± 1.2	43.6 ± 2.3	0.95 ± 0.03
8	1.00	90	30	63.5 ± 7.5	3.5 ± 0.7	64.6 ± 3.5	0.27 ± 0.02
9	1.00	160	60	183.6 ± 8.2	26.3 ± 1.2	58.4 ± 2.9	0.99 ± 0.03
10	0.33	160	60	97.1 ± 4.8	11.8 ± 0.8	70.5 ± 4.2	0.42 ± 0.05
11	1.00	20	60	128.0 ± 7.3	21.0 ± 1.1	40.2 ± 2.6	0.82 ± 0.06
12	0.33	90	90	103.2 ± 7.7	15.1 ± 0.9	61.1 ± 3.2	0.64 ± 0.03
13	0.66	90	60	153.0 ± 7.5	24.8 ± 1.1	60.6 ± 3.2	0.99 ± 0.03
14	0.33	20	60	102.5 ± 8.8	14.3 ± 0.7	72.9 ± 4.8	0.56 ± 0.02
15	0.66	90	60	153.0 ± 7.5	24.8 ± 1.1	60.6 ± 3.2	0.99 ± 0.03
16	0.66	160	90	145.8 ± 6.4	23.4 ± 1.3	53.6 ± 2.9	1.05 ± 0.06
17	0.66	20	30	56.8 ± 3.2	1.8 ± 0.2	74.3 ± 4.5	0.09 ± 0.06

Table III. Effect of fruit:water ratio, extraction time and temperature on various characteristics of aqueous extracted bitter gourd juice. Values are means ± standard deviations (n = 3).

Exp. No.	pH	TDS (mg L^{-1})	TSS (°Brix)	Color (A)	Na (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Viscosity (mPa.s)
1	5.28 ± 0.3	1,860 ± 55	1.4 ± 0.1	1.03 ± 0.05	31.1 ± 1.2	1086 ± 39	135.8 ± 6.2	10.4 ± 0.6	1.12 ± 0.05
2	5.58 ± 0.4	1,680 ± 40	1.0 ± 0.1	0.35 ± 0.03	30.7 ± 1.1	994 ± 38	91.6 ± 3.4	4.9 ± 0.4	0.87 ± 0.04
3	5.35 ± 0.2	232 ± 11	0.1 ± 0.0	0.18 ± 0.01	4.7 ± 0.2	89 ± 10	9.2 ± 1.2	0.6 ± 0.1	0.85 ± 0.03
4	5.35 ± 0.3	690 ± 15	0.3 ± 0.1	0.32 ± 0.02	15.2 ± 0.6	306 ± 11	35.4 ± 2.1	1.8 ± 0.1	0.85 ± 0.03
5	5.58 ± 0.4	1,680 ± 40	1.0 ± 0.1	0.35 ± 0.03	30.7 ± 1.1	994 ± 38	91.6 ± 3.4	4.9 ± 0.4	0.87 ± 0.04
6	5.58 ± 0.4	1,680 ± 40	1.0 ± 0.1	0.35 ± 0.03	30.7 ± 1.1	994 ± 38	91.6 ± 3.4	4.9 ± 0.4	0.87 ± 0.04
7	5.55 ± 0.3	1,790 ± 44	1.1 ± 0.1	0.56 ± 0.04	30.7 ± 0.7	505 ± 17	99.1 ± 5.9	7.5 ± 0.5	0.96 ± 0.04
8	5.82 ± 0.2	418 ± 12	0.1 ± 0.0	0.22 ± 0.02	6.9 ± 0.4	106 ± 10	14.2 ± 1.0	0.6 ± 0.1	0.83 ± 0.03
9	5.37 ± 0.3	1,750 ± 45	1.1 ± 0.1	0.43 ± 0.03	32.4 ± 1.3	990 ± 35	101.8 ± 5.2	12.7 ± 0.6	0.89 ± 0.04
10	5.32 ± 0.3	1,180 ± 35	0.6 ± 0.1	0.15 ± 0.01	4.6 ± 0.2	436 ± 14	33.7 ± 1.8	4.2 ± 0.3	0.86 ± 0.04
11	5.74 ± 0.4	1,540 ± 35	1.0 ± 0.1	0.81 ± 0.03	31.7 ± 1.2	551 ± 20	82.4 ± 4.6	5.1 ± 0.4	0.85 ± 0.03
12	5.26 ± 0.3	1,260 ± 33	0.8 ± 0.1	0.42 ± 0.03	18.1 ± 0.7	656 ± 24	67.1 ± 2.9	5.4 ± 0.4	0.92 ± 0.04
13	5.58 ± 0.4	1,680 ± 40	1.0 ± 0.1	0.35 ± 0.03	30.7 ± 1.1	994 ± 38	91.6 ± 3.4	4.9 ± 0.4	0.87 ± 0.04
14	5.77 ± 0.3	1,130 ± 25	0.6 ± 0.1	0.20 ± 0.01	13.9 ± 0.5	623 ± 19	46.7 ± 2.3	5.2 ± 0.4	0.84 ± 0.04
15	5.58 ± 0.4	1,680 ± 40	1.0 ± 0.1	0.35 ± 0.03	30.7 ± 1.1	994 ± 38	91.6 ± 3.4	4.9 ± 0.4	0.87 ± 0.04
16	5.30 ± 0.4	1,730 ± 45	1.3 ± 0.1	0.55 ± 0.04	12.6 ± 0.5	511 ± 18	120.8 ± 4.7	10.2 ± 0.6	1.10 ± 0.05
17	5.84 ± 0.2	235 ± 10	0.1 ± 0.0	0.21 ± 0.02	6.3 ± 0.3	72 ± 10	9.2 ± 1.1	0.8 ± 0.1	0.84 ± 0.03

3.5 Effect of variables on pH, TDS, TSS, color, viscosity, sodium, potassium, calcium and magnesium

Experimental analyses of four responses are reported in *table II* and additional parameters, such as pH, TDS, TSS, etc., are reported in *table III*. The pH did not change considerably, as its values varied between 5.26 and 5.84. The TSS varied from 0.1 to 1.4 °Brix and the TDS varied from 232 to 1,860 mg L^{-1}. The value of absorbance for color varied from 0.15 to 1.03. It was also observed from our results that the concentration of potassium was the highest among all cations, *i.e.* sodium, potassium, calcium and magnesium. The highest concentration of potassium was estimated as 1,086 mg L^{-1}, whereas the maximum concentrations of sodium, calcium and magnesium were 32.4, 135.8 and 12.7 mg L^{-1}, respectively. The viscosity of the extracted solution varied from 0.83 to 1.12 mPa s, indicating easy processing in subsequent clarification steps.

3.6 Comparison between water and alcohol based extraction

Alcohol-based extraction was also carried out to compare the results with water-based extraction. It was observed that the concentrations of protein, polyphenols and total solids were nearly 18–20% higher in the alcoholic extract as compared with the aqueous extract. Also, the clarity of the alcoholic extract was only 8% compared with 61% for the aqueous extract. Although the concentrations of proteins, polyphenols and total solids from an alcohol extract are higher than from an aqueous extract, the use of alcohol as an extraction medium makes it unsuitable for human consumption, or requires an additional post-processing step for removing the alcohol.

4 Conclusion

Response surface methodology was used to optimize the process variables (fruit:water ratio, extraction time and temperature) of aqueous extraction of bitter gourd juice, and ANOVA was applied to identify the effects of various terms. ANOVA indicated that the response variables are quite adequate and the regression model can explain nearly 98% of the total variability, as the value of R^2 is close to unity, *i.e*. 0.99, for all four response functions. The optimum conditions generated by the RSM are a fruit:water ratio of 0.48 g mL^{-1}, an extraction time of 95 min and an extraction temperature of 68 °C, resulting in concentrations of 131.7 mg L^{-1} proteins, 23.1 mg GAE 100 mL^{-1} polyphenols, 0.89 g 100 mL^{-1} total solids and clarity of the extracted juice of 60.6%. These optimum values were examined for the model validation and it was confirmed that they fit in the significant range from the predicted values. Further optimization of the process parameters will be beneficial for the subsequent advanced purification steps and for upscaling its industrial application for commercializing bitter gourd juice.

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