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ORIGINAL ARTICLE

Optimization of vacuum impregnated nutmeg rind candy using RSM modeling: effect on functional and nutritional properties

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Abstract Vacuum impregnation (VI) stands as an innovative technique, used to create novel food formulations by impregnating vital nutritive compounds into natural food matrices. Process variables were syrup temperature ranging from (50 to 70 °C), syrup concentration (60° to 80° Brix), and VI duration (10 to 20 min). The optimal conditions emerged at 66.81 °C for syrup temperature, 71.58° Brix for syrup concentration, and a precise 11.59 min for the VI duration. At these paramount conditions, the candy exhibited striking attributes such as 50.0% water loss, 12.23% sugar gain, chewiness of 9 N, and lightness value of 22.17. The predicted values using the developed model were validated with experimental data and indicated the adequacy of the generated model. The functional characterization of the optimized nutmeg rind candy showcased a marked reduction in myristicin content, signifying an augmented level of safety for consumption. Furthermore in-vitro anti-bacterial assay, total phenolics, flavonoids and tannin content, anti-oxidant potential, proximate composition and microstructure of the optimized candy were analysed.

Keywords Vacuum impregnation · Optimization · Functional parameters · Nutmeg rind candy · Myristicin · Response surface methodology

Abbreviations

VI	Vacuum impregnation
RSM	Response surface methodology
FCCCD	Face centered centre composite design
WL	Water loss
SG	Sugar gain
NCCLS	National committee for clinical laboratory standards
SEM	Scanning electron microscopy

Introduction

Nutmeg (*Myristica fragrans Houtt.*) is widely grown in Indonesia, Malaysia, India, and South Africa (Putsakum et al. 2020). The nutmeg tree is the only spice with two economic products: nut (seed) and mace (aril). Typically, a single fruit weighs around 60g, with seeds accounting for 8 to 12g, mace 3 to 4g, and the remaining as rind or pericarp. Regrettably, about 30 to 40% of nutmeg rind is thrown as agricultural waste (Mini Raj et al. 2017). The fresh nutmeg rind which makes up 80 to 85% of the fruit's weight, has an astringent and aromatic flavour. These attributes limit its culinary use. However, due to its unique biological composition, nutmeg rind has been transformed into value-added products like candies, chewing gum, jam, syrups, and beverages. Nutmeg is also recognized for its therapeutic properties, offering anti-oxidant,

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anti-microbial, anti-inflammatory, and hepatoprotective benefits (Suwarda et al. 2021). Myristicin, a phenylpropanoid found in nutmeg rind, is associated with potential adverse effects. Doses ranging from 5 to 30g have been linked to hypertension, numbness, vomiting and paresthesia, extensively studied in recent years (Ehrenpreis et al. 2014). Therefore, it is imperative to evaluate and minimize myristicin content through various food processing techniques to ensure the safety of consuming nutmeg rind based products.

Vacuum impregnation (VI) has the potential to introduce substances which enhance fresh fruits and vegetables with nutrients or other bioactive compounds, resulting in the creation of novel food formulations. Gonzalez-Perez et al. (2022) recommended the application of VI technique on apples enriched with grape juice concentrate by employing various levels of vacuum time, pressure and solute concentrations and reported the effect of water loss, solid gain and colour impregnation in apple slices. Several techniques have been developed for the infusion of physiologically active compounds through VI into the plant matrices. Grape residue polyphenols enrichment in mango (Batista de Medeiros et al. 2019), and polyphenols enrichment in sweet potato (Abalos et al. 2020).

Since there is no scientific literature about the VI processing of nutmeg rind candy, the comparison of the myristicin concentration of fresh nutmeg rind and VI processed nutmeg rind candy is essential for the development of nutmeg processing in the future. Hence the present study was taken to optimize the VI conditions to develop nutmeg rind candy and to analyze the functional and nutritional attributes of the optimized product.

Materials and methods

Raw materials

Nutmeg rind (var: IISR-Vishwasree) was selected for VI experiments since it is a high-yielding variety. The fruits were collected from ICAR-IISR, Kozhikode. Food color-Natural pink (anthocyanin) was purchased from Symega Food Ingredients Pvt. Ltd. (Kochi, India). Table sugar, salt and citric acid were purchased from the local market at Kozhikode. Nutmeg rind was washed, peeled, and cut into cubes of the size of 1 × 1 × 1 cm. The cubes were soaked in brine solution (2% concentration) for 5 h. The cubes were then drained and blanched in hot water (75–85 °C) for 15 min to inactivate the enzymes. The initial moisture content of fresh nutmeg rind cubes was determined by toluene distillation as described by AOAC (2003).

Impregnation procedure

Preparation of impregnation solution

The impregnating solution was prepared by mixing natural food colour (Pink- anthocyanin) at a concentration of 1.5% sugar syrup (v/v) for all varying concentrations of 60,70 and 80° Brix experimented containing 2.5% citric acid as a preservative.

Vacuum impregnation of nutmeg rind cubes

Preliminary trials were conducted at the initial stages of study to screen the process conditions for preparing nutmeg rind candy. The selection of levels for VI was made from the preliminary experiments in which syrup concentrations (40, 50, 60, 70° Brix), syrup temperatures (30, 40, 50, 60, 70 °C) and vacuum durations (5, 10, 15, 20, 25 min) as done by other studies for various fruits. For example, mango and melon (Mujica-Paz et al. 2003), guava (Correa et al. 2010) and apple (Gonzalez-Perez et al. 2022). VI of nutmeg rind cubes was carried out in a VI system established at ICAR-Indian Institute of Horticultural Research, Bengaluru. The VI system consists of a pressure vessel with a jacketed kettle (capacity 25L), vacuum pump (Model M7NF, 0.5 hp power, 180 V DC, 2.6-amp, 1500 rpm), pressure gauge, heating unit (60–100 °C), control panel and platform.

The nutmeg rind cubes were immersed in different sugar syrup concentrations (60, 70 and 80° Brix) and at each concentration for different syrup temperatures of 50, 60 and 70 °C by maintaining a fruit-to-syrup ratio of 1:4. The VI was done for varying time durations of 10, 15 and 20 min inside the jacketed vessel at constant vacuum pressure (100 mbar). After completing the impregnation process, nutmeg rind cubes were removed from the VI unit and samples were given 10 min relaxation time at ambient conditions. The samples were immediately wiped with tissue paper to remove the excess syrup adhering to the surface then weighed and kept for moisture analysis. All the experiments were conducted in triplicates.

Determination of water loss

The percentage of water loss can be defined as the net loss of moisture from the nutmeg rind cubes at a time (ɵ) on the initial weight basis.

$$WL = \frac{W_i.X_i - W_\theta.X_\theta}{W_i} \times 100 \cdots \quad (1)$$

Determination of sugar gain

Sugar gain was the net gain in total solids by nutmeg rind cubes on an initial weight basis.

$$SG = \frac{W_\theta(1 - X_\theta) - W_i(1 - X_i)}{W_i} \times 100 \cdots \quad (2)$$

where, WL is the water loss (g water/100 g initial mass of nutmeg rind cubes), %, SG is the sugar gain (g/100 g initial mass of nutmeg rind cubes), %, W_i is the initial mass of nutmeg rind cubes, g, X_i is the initial moisture content of sample, W_θ is the mass of nutmeg rind cubes after each time interval ɵ, g, X_θ is the moisture content of nutmeg rind cubes after each time interval ɵ, g

Color measurement

CIE colour coordinates (L*, a*, b*) of nutmeg rind candy were measured using a colourimeter (Hunterlab, Colorflex: EZ, USA). The Hunter colour scale was expressed as L*, stands for lightness or darkness, a* represents redness or greenness and b* indicates yellowness or blueness. The equipment was calibrated using standard white and black ceramic tiles. Triplicate values of color parameters were noted and mean values were reported.

Texture profile analysis

Vacuum-impregnated nutmeg rind cubes were subjected to texture profile analysis (TPA) using a texture analyzer (Shimadzu, EZ-SX, Australia). The equipment was equipped with a load cell of 500 N during analysis. The physical characteristics of texture, such as hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness were calculated from the TPA curve measured using 6 mm diameter cylindrical probe.

Optimization of processing conditions using response surface methodology

Experimental design

Independent variables (factors) such as the temperature of the syrup (50–70 °C), the concentration of the syrup (60–80° Brix), and the duration of VI (10–20 min), and the dependent variables (responses) were water loss, sugar gain, chewiness, and lightness of the product. Every factor was selected based on the preliminary trials and available literature. Design Expert commercial statistical software package version 13.0 (Stat Ease Inc., MN, USA) was used to analyse the experimental data. Face Centered Central Composite Design (FCCCD) was used to optimize the process conditions. The experimental design consisted of 20 runs with six centre points (Table 1).

The quadratic model was used to describe the response variables as per the following equation:

$$\begin{aligned} Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \\ + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \cdots \end{aligned} \quad (3)$$

Analysis of variance (ANOVA) was performed to check the significance of the model and process variables.

Optimization technique

The numerical and graphical optimization techniques were used for simultaneous optimization of multiple responses. The desired goals set for each response were as follows:

Water loss: Maximize.
Sugar gain: Maximize.
Chewiness: In range.
Lightness: In range.

The independent factors were kept within the experimental range. The desirability function was used to search for a solution for multiple responses, where all the goals were combined into an overall composite function as given below (Sharma and Khanna 2013):

$$D(x) = \left(d_1 \times d_2 \times \ldots \ldots \times d_n\right) 1^{1/n} \cdots \quad (4)$$

where D(x) is the desirability function, $d_1, d_2..d_n$ are the responses and 'n' is the total number of responses.

The adequacies of the models were determined using model analysis, lack of fit test, coefficient of determination (R^2), coefficient of variation (CV) and mean relative percent deviation modulus (P).

Selection of critical limits

For optimization of processing conditions such as temperature of the syrup, concentration of the syrup and duration of VI, subjective evaluation was done to fix the desirable sensory attributes of nutmeg rind candy. The developed candy was then dried in a cabinet dryer (Pilotsmith. Ltd. India) at 45 °C for 2.5 h. The samples were subjected to sensory evaluation using a nine-point hedonic scale (ranging from 9 = like extremely to 1 = dislike extremely) by a trained panel of judges (Bergara-Almeida et al. 2002). The scores obtained were statistically analysed. The samples rated as good were selected for finding the critical limits of water loss, sugar gain, chewiness, and lightness.

Table 1 Experimental design and data for the response surface analysis (Face Centered Central Composite Design)

Run	Factor 1 syrup temperature (X_1), °C	Factor 2 syrup concentration (X_2), °Brix	Factor 3 duration of vacuum impregnation(X_3), min	Response 1 Water loss (Y_1), %	Response 2 Sugar gain (Y_2), %	Response 3 Chewiness (Y_3), N	Response 4 Lightness (Y_4)
1	70 (+1)	60 (−1)	10 (−1)	46.74	9.79	7.15	22.23
2	60 (0)	70 (0)	15 (0)	45.64	10.95	8.59	23.01
3	70 (+1)	80 (+1)	10 (−1)	51.41	14.14	10.02	20.98
4	60 (0)	70 (0)	15 (0)	47.83	10.99	8.17	23.71
5	70 (+1)	70 (0)	15 (0)	50.46	11.83	9.14	21.41
6	60 (0)	70 (0)	10 (−1)	41.89	10.53	7.99	24.89
7	60 (0)	80 (+1)	15 (0)	43.06	12.18	8.75	24.04
8	70 (+1)	60 (−1)	20 (+1)	48.2	10.05	9.58	21.92
9	50 (–1)	70 (0)	15 (0)	35.81	8.99	5.15	26.81
10	60 (0)	70 (0)	15 (0)	49.09	11.09	8.11	23.62
11	70 (+1)	80 (+1)	20 (+1)	53.91	14.68	10.14	19.73
12	50 (−1)	60 (−1)	10 (−1)	30.5	6.96	4.52	28.77
13	50 (−1)	80 (+1)	10 (−1)	38.03	9.81	5.52	27.4
14	60 (0)	70 (0)	15 (0)	46.02	11.5	7.92	23.89
15	50 (−1)	80 (+1)	20 (+1)	39.46	10.05	5.89	24.61
16	60 (0)	70 (0)	15 (0)	45.33	11.17	8.14	23.38
17	50 (−1)	60 (−1)	20 (+1)	34.08	7.14	4.99	27.84
18	60 (0)	70 (0)	15 (0)	48.17	11.12	8.05	23.43
19	60 (0)	70 (0)	20 (+1)	48.71	11.63	8.55	22.01
20	60 (0)	60 (−1)	15 (0)	40.03	9.23	6.44	24.94

Figures in the parenthesis signify the coded values

Validation of models

For validation of the processing conditions, nutmeg rind candy was prepared at different concentrations, time and temperature combinations optimized by the software. The predicted response obtained from RSM software was compared with the experimental values. The validated model was confirmed by performing the experiments under the optimized conditions and determining the relative percent deviation modulus (P) using the following formula (Kaur and Zalpouri 2020).

$$\%P = \frac{Predictedvalue - Actualvalue}{Predictedvalue} \times 100 \cdots \quad (5)$$

Proximate composition

The nutmeg rind candy prepared at optimized conditions was subjected to proximate analysis. The samples were analyzed for moisture, fat, protein, ash and carbohydrate contents. Moisture content was estimated as mentioned in Sect. "Raw materials". Total ash content was analyzed by AOAC method (AOAC 2003). Total carbohydrate was determined by the Anthrone method (Shields and Burnett 1960). The protein content was estimated by using a semi-automated Kjeldhal apparatus. Fat content was determined as per AOAC (2003) method using soxhlet fat extraction apparatus. The crude fibre content was estimated by using a Fibra crude fibre analyzer (Fibraplus FES 06 E, Pelican equipment, Chennai, India).

Functional parameters of VI nutmeg rind candy

Biochemical analysis

The VI nutmeg candy was tested for various functional attributes. The optimized samples were analyzed for total phenolics, flavonoids, tannin content, antioxidant activity and *In-vitro* anti-bacterial assay. Total phenolic content and total flavonoid content were determined by protocol given by Fang et al. (2009) with some modifications. Tannin content was analysed by the Folin-Denis method, a procedure by Schandrel (1970). Anti-oxidant activity was estimated by the modified version of DPPH-free radical scavenging activity by Min et al. (2011).

Myristicin estimation

GC–MS of hexane extract from both fresh and VI nutmeg rind candy was carried out using Gas

Chromatography coupled with triple axis detector (Shimadzu GC-2010 gas chromatograph). Column use: DB 5MS 30 m × 0.250 mm × 0.25 um. Injection volume: 2 μL split ratio: 50:1. Helium gas (99.9995%) flow rate: 1 mL/min. The estimation was executed using an EI (electron impact) mode along with 70 eV of ionization energy. The injector temperature: 280 °C (constant). The column oven temperature program: 40 °C for 5 min, ramped to 100 °C @ 30 °C/min, ramped to 150 °C @ 25 °C/min, hold for 2 min, ramped to 280 °C @ 20 °C/min, hold for 5 min. The myristicin was identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST-08 SPECTRAL DATA) and was compared with the standard myristicin (Sigma cat no.102502288) injected at 100 ppm. The peak area reduction was used for the calculation of myristicin concentration.

In vitro anti-bacterial assay

Optimized nutmeg rind candy was tested for anti-bacterial assay against *E. coli* (ATCC 8739) and *Salmonella typhi* (MTCC 734) through a good diffusion method (NCCLS 1999). Methanol extract of nutmeg candy was prepared @1 g/mL, 0.9 g/mL and 0.8 g/mL and was sterilized by filtration through a 0.22 µm membrane filter (Millipore, Bedford, MA). One hundred microL portion of bacterial suspension containing 106 cfu/mL of bacteria was spread over the surface of the nutrient agar plate and allowed to dry. The 6 mm wells were cut from the agar with a sterile borer and 150 µL of each methanol extract solution was delivered into the plates. The inoculated plates were incubated for 24 h at 37 °C. After incubation, the diameter of the inhibition zone was measured in millimetres (mm). Silver nitrate was used as positive control and methanol was used as negative control.

Microstructure analysis

Microstructural analysis of the fresh nutmeg rind cubes and vacuum-impregnated nutmeg rind candy was analysed using scanning electron microscopy (SEM) (Model: HITACHI SV6600, Japan). The cubes were mounted on aluminium stubs followed by sputter coating with gold. The analysis was carried out using a 15 kV accelerating voltage. The magnification range of SEM was 600x.

Statistical analysis

The biochemical assay was performed using three replicates. The statistical analysis was done by one-way ANOVA using SPSS v.16.

Results and discussion

Model fitting

Face Centered Central Composite Design with six replications at the center point was used to optimize the process conditions during VI. The experimental design with various factors such as syrup temperature (X_1), syrup concentration (X_2) and duration of VI (X_3) along with responses such as water loss (Y_1), sugar gain (Y_2), chewiness (Y_3) & lightness (Y_4) is presented in Table 1. The water loss varied from 31.5% to 52.91%, sugar gain varied from 6.96% to 14.68%, chewiness varied from 4.52 to 10.14 N and lightness varied from 19.73 to 28.77 during VI with respect to variation in syrup temperature, syrup concentration and duration of vacuum treatment. To choose the best model for VI, the actual measured data was fit into various regression models.

The quadratic response surface model was fitted to each response variable (Eq. 3). Regression analysis and ANOVA were used to fit the model data and to examine the statistical significance of terms. The values of R^2, Adj R^2 and CV were determined to evaluate the adequacy of the selected model. The p-values (level of significance) were used as the tool to check the significance of every coefficient, which was necessary to understand the pattern of mutual interactions of test variable (Koocheki et al. 2014). From ANOVA results (Table 2) it is evident that the quadratic model for each response was highly significant ($p \leq 0.05$). All the metrics of VI nutmeg rind candy were significantly dominated by higher F-values in positive quadratic terms, 16.90, 72.68, 41.55 and 46.62 for water loss, sugar gain, chewiness, and lightness respectively. From (Table 1) higher syrup temperature, higher syrup concentration, and a long duration of vacuum treatment resulted in more water loss, sugar gain, chewiness, and lightness. The lack of fit test did not result in a significant F-value (Table 2) for all responses indicating that the models were adequate for predicting the responses. The correlation between predicted and experimental values was found to be 0.9383, 0.9849, 0.9740 and 0.9767 for WL, SG, chewiness, and L*, respectively.

The coefficient of determination (R^2) value was obtained as 0.9383, 0.9849, 0.9740 and 0.9767 for water loss, sugar gain, chewiness, and lightness respectively, the corresponding Adj R^2 values were reported as 0.8828, 0.9714, 0.9505 and 0.9558 respectively. The R^2 value above 80% indicates a good fit for the model. The CV values of 4.81, 2.98, 4.92 and 2.08 for all the responses were found to be below 5% (less than 10% is a good fit). Higher R^2 value and low *p*-*p*-value (≤ 0.05) indicated the selected quadratic model for each response factor was highly significant and sufficient to represent the relationship between the process and response variables.

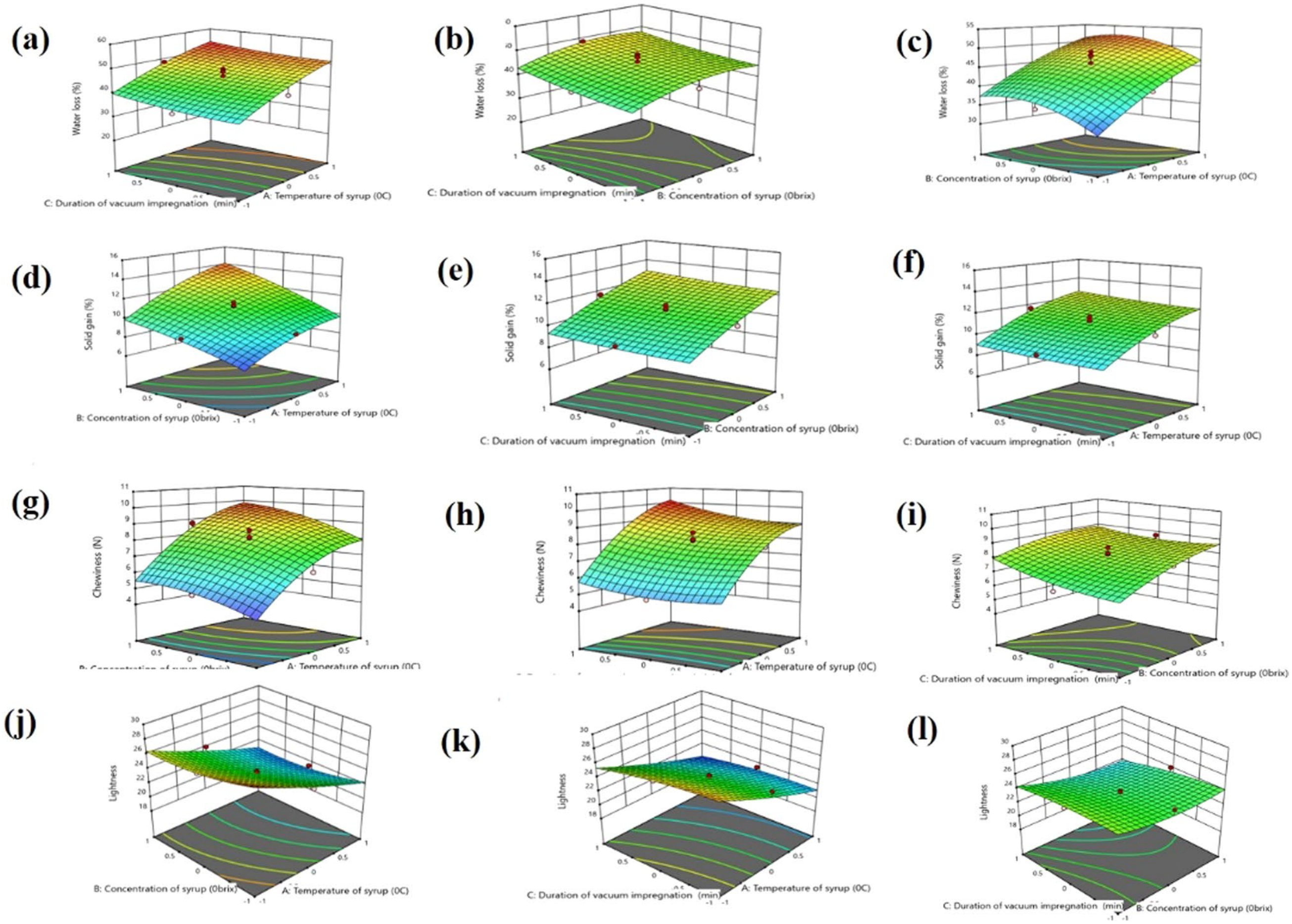


Fig. 1 Response surface plots showing the effects of water loss **a**–**c**, sugar gain **d**–**f**, chewiness **g**–**i** and lightness **j**–**l**

Effect of process conditions on water loss (WL) of nutmeg rind candy

From the results (Table 2), the highest water loss of 53.91% was recorded for a temperature of 70 ℃, at a concentration of 80° Brix, for 20 min while the lowest water loss was 30.5% for 50℃ at 60° Brix for 10 min duration. The quadratic equation (Eq. 6) was fitted with experimental data. The following quadratic equation in terms of actual units was generated to obtain the empirical relationship between the process variables such as syrup temperature (X_1), syrup concentration (X_2) and duration of VI (X_3).

$$WL = -228.6 + 2.66X_1 + 4.67X_2 - 0.26X_3 - 0.01X_1^2 - 0.03X_2^2 + 0.03X_3^2 - 3.16E - 003X_1X_2 - 2.62E - 003X_2X_3 - 2.77E - 003X_1X_3 \cdots \quad (6)$$

The RSM plots showing the interactions between the temperature of the syrup, concentration of the syrup, and duration of impregnation on water loss are shown in Fig. 1 (a, b, c), it is clear from the figure that water loss increased from 30.5% to 53.91% with an increase in syrup temperature from 50 to 70 °C and increase in syrup concentration from 60 to 80° Brix followed by increase in duration of vacuum treatment from 10 to 20 min.

The ANOVA results (Table 2) revealed that the response surface model of WL was highly significant ($p \leq 0.05$) for linear effects of the temperature of the syrup, concentration of the syrup duration of vacuum treatment and quadratic effects of the syrup concentration. It was found that the temperature and concentration of the syrup have a positive correlation, and the duration of vacuum treatment has a negative correlation with water loss. Similar trends were seen in the interactions observed for papaya candy (Islam et al. 2019), and honey ginger candy (Gupta et al. 2012). The osmotic dehydration process with vacuum application increased water loss due to enhanced mass transfer rate through hydrodynamic mechanisms (Mujica-Paz et al. 2003). Elevated temperature further amplified water loss, as it led to increased food porosity, promoting rapid water release. Additionally, higher temperatures facilitated water loss by swelling and plasticizing cell membranes. Correa et al. (2010) explored osmotic dehydration of guava slices in different syrup concentrations and vacuum pressures, finding vacuum enhanced gas and liquid removal from

Table 2 ANOVA results for various responses using quadratic response surface model

F-values for responses					
Variables/factors	df	Water loss (Y_1), %	Sugar gain (Y_2), %	Chewiness (Y_3), N	Lightness (Y_4)
Quadratic model	9	16.90*	72.68*	41.55*	46.62*
X_1- Syrup temperature	1	117.37*	302.32*	282.17*	343.56*
X_2- Syrup concentration	1	15.32*	307.51*	41.34*	32.29*
X_3- Duration of vacuum impregnation	1	5.52*	5.29*	11.05*	26.90*
X_1^2	1	1.18	9.06*	13.13*	0.96
X_2^2	1	5.41*	2.18	2.68	5.04*
X_3^2	1	0.36	0.22	1.80	1.49
$X_1 X_2$	1	0.18	12.74*	2.07	0.68
$X_1 X_3$	1	0.030	0.18	2.59	2.36
$X_2 X_3$	1	0.034	0.14	5.14*	3.96
Residual	10	–	–	–	–
Lack of fit	5	2.76	4.30	4.48	4.27
Pure error	5	–	–	–	–
Total	19	–	–	–	–

*Significance at $p \leq 0.05$; Lack of fit is non-significant at $p \geq 0.05$

capillary pores. Kuo et al. (2018) observed that processing time (10–50 min) during vacuum-assisted osmotic dehydration of watermelon rind at 100 mmHg significantly impacted water loss.

Effect of process conditions on sugar gain of nutmeg rind candy

From the results (Table 1), the highest sugar gain of 14.68% was observed for a temperature of 70 °C, concentration of 80° Brix, duration of 20 min and lowest sugar gain was 6.96% for 50 °C, at 60° Brix and 10 min. As the solid gain of the product is directly related to its concentration of the syrup, an increase in syrup concentration has enhanced the solid gain of the products (Gupta et al. 2012). The ANOVA results (Table 2) revealed that the linear effects of syrup temperature, syrup concentration and duration of VI, quadratic effects of syrup temperature and interaction effect of syrup temperature and concentration were highly significant ($p \leq 0.05$). The following quadratic equation in terms of actual units was generated to obtain the empirical relationship between the sugar gain and process parameters.

$$SG = -27.82 + 0.57X_1 + 0.32X_2 - 0.17X_3 - 5.8X_1^2 - 2.84X_2^2 + 3.63X_3^2 + 4.02\text{E} - 003X_1X_2 + 9.50\text{E} - 004X_2X_3 + 8.50\text{E} - 004X_1X_3 \cdots \quad (7)$$

The 3D surface graph in Fig. 1 (d, e, f) illustrates the impact of process variables on sugar gain. Syrup concentration and temperature exhibit a positive correlation, while treatment duration shows a negative correlation. Higher syrup concentration (60° Brix) led to a notable solute gain (11.28%) in osmotically dehydrated pomegranate arils, compared to 7.16% and 7.63% at 40° Brix and 50° Brix respectively (Mundada et al. 2011). Increased syrup concentration resulted in enhanced water removal from nutmeg rind tissues, filling pores with solute content. These findings were in consistent with the literature (Silva et al. 2015; Islam et al. 2019). Mujica-Paz et al. (2003) noted increased solid gain in mangoes, melons, and apples with longer vacuum durations. Chafer et al. (2003) and Correa et al. (2010) observed similar trends in pear fruits and osmotically dehydrated guava slices respectively through vacuum treatment.

Effect of process conditions on chewiness of nutmeg rind candy

From the results (Table 1), the highest chewiness value of 10.14 N reported for a temperature of 70 °C, concentration of 80° Brix and duration of 20 min and the lowest chewiness value 4.52 N for 50 °C, at 60° Brix and 10 min. The ANOVA of the response surface model revealed that the linear effects of syrup temperature, syrup concentration and duration of VI, the quadratic effect of syrup temperature and the interaction effect of syrup concentration and duration of VI were highly significant ($p \leq 0.05$). However, among the three main factors, syrup temperature had a profound positive impact on chewiness followed by positively correlated syrup concentration and negatively correlated duration of vacuum treatment. The relationship between independent and dependent variables is illustrated in 3D representations of the response surface generated by the model presented in

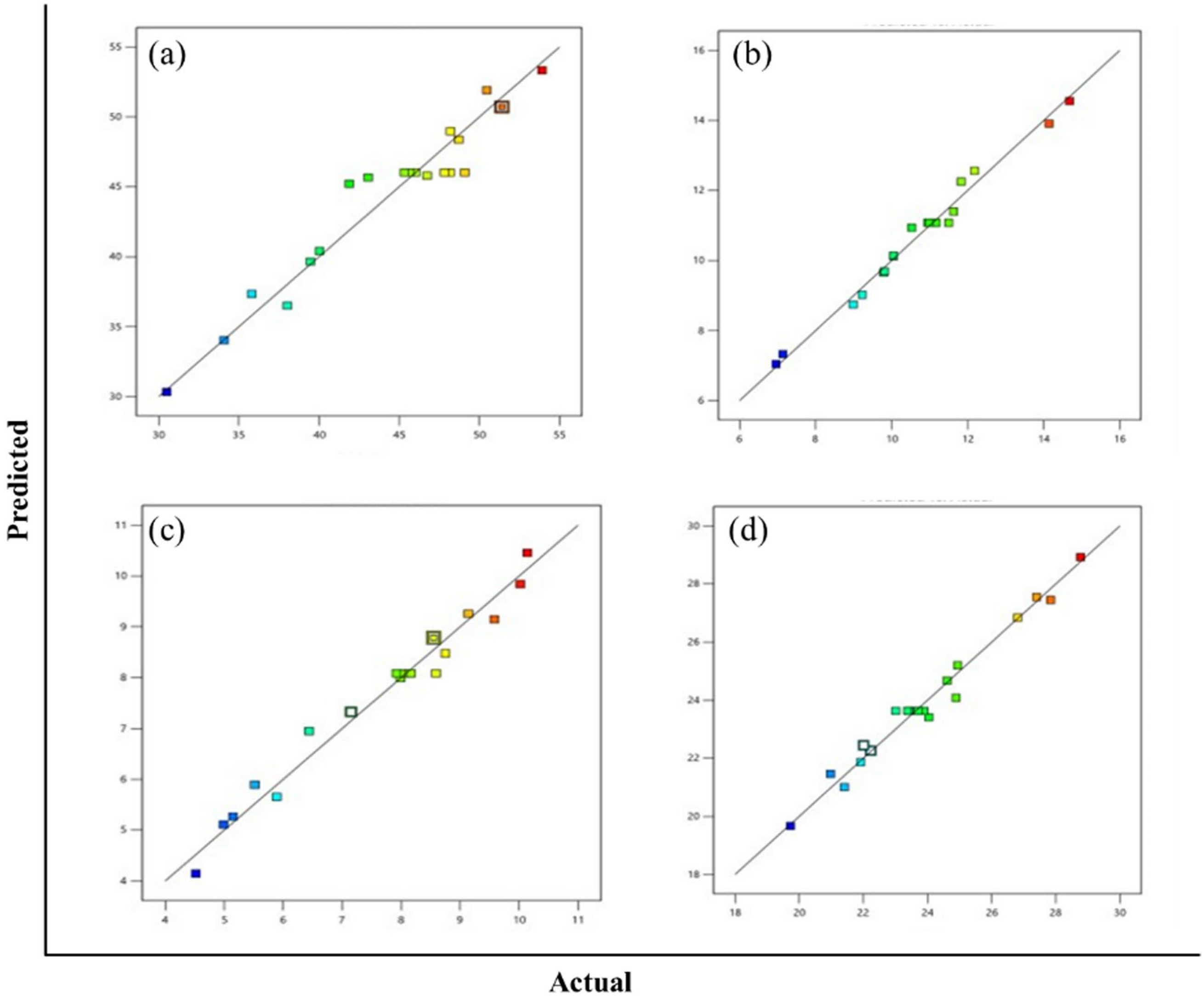


Fig. 2 Comparison between predicted and actual values of water loss **a**, sugar gain **b**, chewiness **c** and lightness **d**

Fig. 1 g, h, i. The following quadratic equation in terms of actual units explained the empirical relationship between the chewiness and process conditions.

$$Chewiness = -49.86 + 0.98X_1 + 0.57X_2 - 0.12X_3 - 8.20E - 003X_1^2 - 3.70E - 003X_2^2$$
$$+0.01X_3^2 + 1.91E - 003X_1X_2 + 4.27E - 003X_2X_3 - 6.02E - 003X_1X_3 \cdots \quad (8)$$

As the chewiness of the product is directly related to its loss of water, an increase in syrup temperature facilitated an increase in water loss which in turn enhanced the chewiness of the nutmeg rind candy. The increase in chewiness might be due to an increase in hardness and decrease in cohesiveness of nutmeg rind candy after water loss and sugar gain. These results follow the findings obtained by Barragan-Iglesias et al. (2019), a study on the textural characteristics of minimally treated papaya cubes and concluded that a high chewiness value was 3.64N obtained for the sample having higher water loss during the osmotic dehydration process. The corresponding hardness value for the sample was 26.3N. The results show that more energy will be needed to prepare the cubes for swallowing.

Effect of process conditions on the lightness of nutmeg rind candy

The L* value increased from 19.73 (70 °C, 80° Brix and 20 min) to 28.77 (50 °C, 60° Brix and 10 min). Table 1 showed that the L* value of VI nutmeg rind candy was darker for increased syrup temperature, and concentration of the syrup followed by duration of vacuum treatment. The ANOVA results (Table 2) showed that the quadratic model developed for the lightness of nutmeg rind candy, temperature of the syrup, concentration of the syrup and duration of vacuum treatment were highly significant ($p \leq 0.05$). Among the three, syrup temperature and concentration have a negative correlation, and the duration of vacuum treats a positive correlation with lightness Fig. 1 j, k, l. The following quadric equation in terms of actual units explained the empirical relationship between the L* and process conditions.

$$\begin{aligned} Lightness = &+93.71 - 0.82X_1 - 1.01X_2 + 0.44X_3 + 2.93\mathrm{E}-003X_1{}^2 \\ &+ 6.73\mathrm{E}-003X_2{}^2 - 0.01X_3{}^2 + 1.45\mathrm{E}-003X_1X_2 + 5.40\mathrm{E} \\ &- 003X_2X_3 - 7.00\mathrm{E}-003X_1X_3 \cdots \end{aligned} \quad (9)$$

Low syrup temperature along with low syrup concentration resulted in degradation of the colour of nutmeg rind candy, which became less pinkish than the candy obtained for higher syrup temperature and syrup concentration. Moreover, the intense colour of nutmeg cubes can be attributed to the increased water loss and higher sugar gain with increased syrup concentration, resulting in better luminosity. Pereira et al. (2006) obtained comparable results with papaya and melon pieces. For them, the better colour of the L* value was directly related to the fact that the sample showed higher water loss and sugar gain during the osmotic dehydration process.

Optimization of syrup temperature, syrup concentration and duration of VI

The optimization of processing conditions was done by setting the criteria. The desired goal for each response was selected based on the sensory evaluation. The optimized solution having the highest desirability value was selected. A desirability value of 0.891 was obtained for VI at 66.81 °C, 71.58° Brix and 11.59 min. The corresponding responses were recorded as 50.00% water loss, 12.23% sugar gain, 9 N chewiness and 22.17 lightness.

Validation of optimized processing conditions

The model was validated by conducting experiments using the derived optimum processing conditions and their responses were also determined. The percentage error (%P) of various responses such as water loss, sugar gain, chewiness and lightness were 0.035, 0.134, 0.141 and 0.098 respectively, suggesting that the experimental data were in good agreement with the predicted values, and the response surface optimization model was adequate (Fig. 2).

Proximate composition of fresh nutmeg rind and VI nutmeg rind candy

Proximate composition of fresh rind was observed as moisture 86.02%, total carbohydrate 7.31%, crude protein 0.84%, crude fat 0.47%, total ash 1.36% and crude fibre 2.98%. These compositions were in agreement with the results obtained by (Mini Raj et al., 2017). For VI optimized nutmeg rind candy was found to be moisture 31.57%, total carbohydrate 62.91%, crude protein 0.63%, crude fat 0.39%, total ash 1.14%, crude fibre 2.49%. From the results, it was noted that total carbohydrate was increased with the addition of impregnation solution into the nutmeg matrix.

Functional attributes of VI nutmeg rind candy

The biochemical analysis of VI nutmeg rind candy revealed total phenolics content of 54.63 ± 0.439 mg gallic acid equivalent (GAE)/100 g sample, total flavonoid content of 86.23 ± 0.057 mg Catechol equivalent (CE)/100 g, tannin content of 231.35 ± 0.493 µg of tannic acid equivalent (TAE)/100 g and anti-oxidant activity expressed as IC_{50} value of 48.73 ± 0.226 µg/mL. All the functional attributes tested in the study exhibited lower values when compared to those of fresh rinds, such as 57.6, 91.76, 313.3 and 81.23 for total phenolics, flavonoids, tannin content and antioxidant activity respectively (Mini Raj et al. 2017; Rahman et al. 2017). This might be due to the loss during processing or due to altered proximate composition.

Myristicin estimation of fresh nutmeg rind and VI nutmeg rind candy

GC–MS profiling of the volatiles from optimized nutmeg rind candy revealed a 57.28% reduction in myristicin content after vacuum impregnation (VI), decreasing from 80.80 in fresh rind to 34.52 ppm in the candy (Fig. 3). Myristicin is reported to produce hallucinogenic effects, and it can be converted to 3,4-methylenedioxymethamphetamine (MMDMA) under controlled chemical synthesis. Moreover, dose-dependent cytotoxicity in living cells is also reported. Myristicin is listed as a hazardous substance in the Data Bank of National Center for Biotechnology Information, (2023). The study indicates that the ill effects of myristicin can be greatly reduced due to the VI process, which can bring down the content of myristicin to make it well within the permitted safe limit (684 µg/day) (Alajlouni 2017).

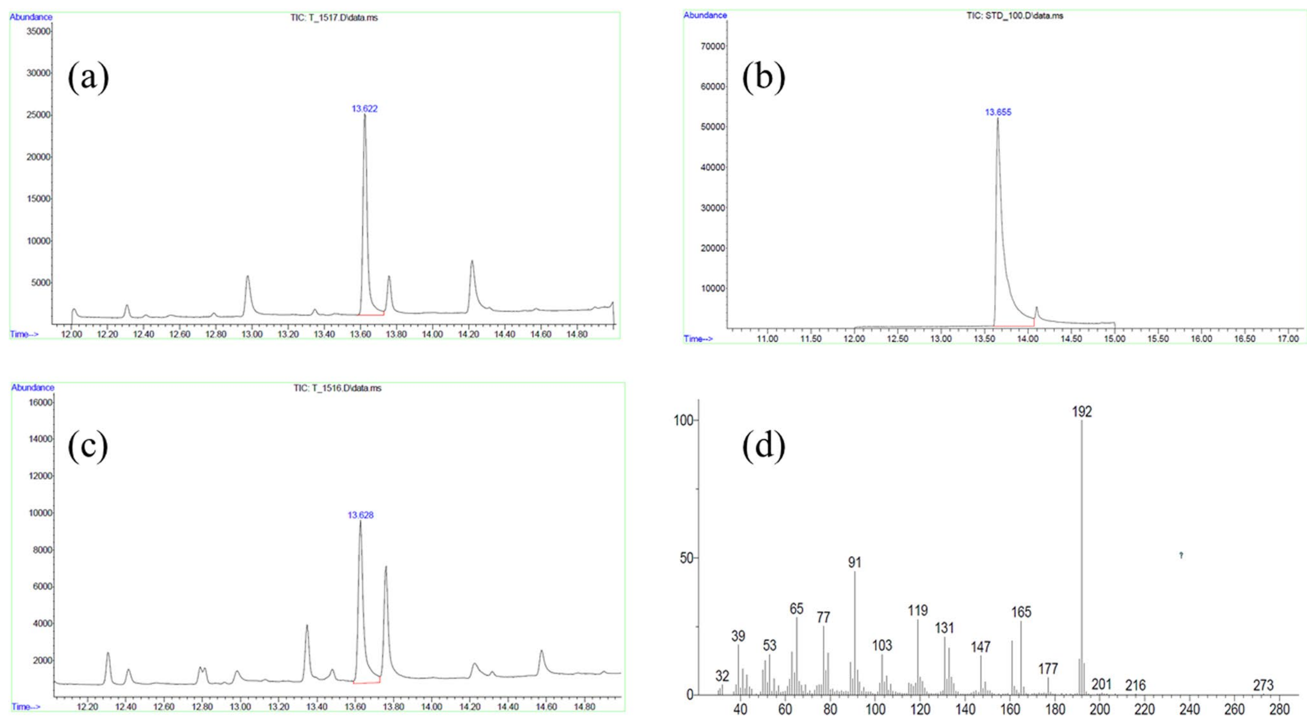


Fig. 3 Myristicin content before and after the VI process in nutmeg rind. **a** GC Chromatogram of fresh nutmeg rind; **b** GC Chromatogram of VI nutmeg rind candy; **c** GC Chromatogram of myristicin standard (100 ppm); **d** Mass spectra of myristicin

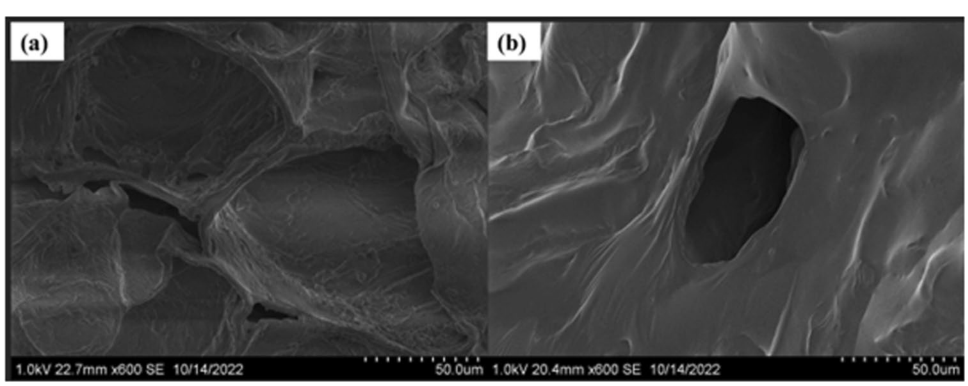
In-vitro anti-bacterial assay

The anti-bacterial assay was performed to investigate the anti-bacterial potential of the nutmeg rind candy. It was found that the 1 g/mL extract exhibited 100% inhibition when compared to positive control. 0.9 g/mL and 0.8 g/mL were able to show 93.3% and 83.3% inhibition respectively. This indicates that the nutmeg rind candy possesses significant antibacterial activity against *E.coli (*ATCC 8739) as reported by (Aliakbarlu et al. 2014). However, no inhibition was exhibited against *Salmonella typhi (*MTCC 734) even at a higher dosage of the extract i.e., 1 g/mL.

Microstructure analysis

The microstructure of fresh nutmeg rind was compared with the VI nutmeg rind candy samples. Due to the application of the vacuum and subsequent restoration of atmospheric pressure, the impregnating solution was able to fill the intercellular spaces in VI samples, thickening the cell wall in comparison to fresh rind samples (Bellary et al. 2016). Hence it is clear from Fig. 4 b that the pores are filled with impregnation solution by hydrodynamic mechanism irrespective of the fresh sample.

Fig. 4 Microstructural observations of **a** fresh nutmeg rind **b** VI nutmeg rind candy



Conclusion

The present study was effective in optimizing the VI conditions for nutmeg rind candy production through the FCCCD module of Response Surface Methodology. Three important variables (syrup temperature, syrup concentration, and duration of vacuum treatment) were analyzed against four responses (water loss, sugar gain, chewiness, and lightness). Quadratic equations were derived to predict these responses. At the optimized conditions (syrup temperature 66.81 °C, syrup concentration 71.58° Brix, vacuum duration 11.59 min), desirable outcomes were achieved as 50% water loss, 12.23% sugar gain, 9 N chewiness, and a lightness value (L*) of 22.17, yielding a desirability value of 0.891.VI nutmeg rind candy offers a notable advantage of 33% reduction in myristicin content, enhancing consumer safety by eliminating the risk of excessive myristicin intake. This reduction ensures compliance with permissible limits. Additionally, this technology aligns with the 'wealth from waste' initiative, benefiting nutmeg growers. The optimization study employing RSM establishes foundational data and process guidelines. This paves the way for future development of diverse value-added products utilizing nutmeg rind.

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Author contributions Saleena P: Carried out the VI experiments, data analysis, writing (original draft); Jayashree E: conceptualization, methodology, supervision, writing (original draft); Neethu KC: conceptualization, data analysis, writing (original draft); Bhuvaneswari: conceptualization, methodology, supervision; Alfiya PV: critical review, editing (final draft); Anees K: critical review, editing (final draft).

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest There are no conflicts of interest associated with this publication.

Ethical approval Not Applicable.

Consent for publication I hereby declare that the present work described has not been published before and it is not under consideration for publication elsewhere, its submission to JFST publication has been approved by all authors as well as the responsible authorities—tacitly or explicitly—at the institute where the work has been carried out, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder, and JFST will not be held legally responsible should there be any claims for compensation or dispute on authorship.

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