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OPTIMISATION OF FRUIT VINEGAR PROCESS FROM *BACCAUREA LANCEOLATA* (MIQ.) MÜLL.ARG. USING CENTRAL COMPOSITE DESIGN WITH ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

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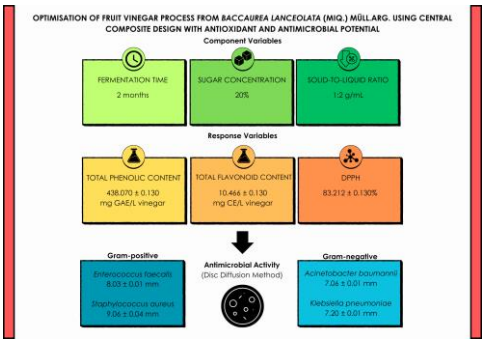
Graphical abstract



Abstract

Baccaurea lanceolata (Miq.) Müll.Arg., native to Southeast Asia, is valued in traditional practices and local diets for its potential medicinal properties. However, the biological activities of its fruit vinegar remain underexplored. This study aimed to optimise and evaluate the antioxidant and antimicrobial properties of *B. lanceolata* fruit vinegar (BLFV). A central composite design was applied to optimise fermentation time (FT), sugar concentration (SC), and solid-to-liquid ratio (SLR) as component variables, with total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as response variables. The antimicrobial activity of the optimised BLFV was assessed against Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii* and *Klebsiella pneumoniae*) bacteria using the disc diffusion method. The optimised BLFV process, using a 2-month FT, 20% SC, and 1:2 g/mL SLR, achieved the following response factors: 438.070 ± 0.130 mg GAE/L vinegar (TPC), 10.466 ± 0.131 mg CE/L vinegar (TFC), and 83.212 ± 0.134% (DPPH). Additionally, the optimised BLFV exhibited antimicrobial activity with inhibition zones measuring 8.03 ± 0.01 mm (*E. faecalis*), 9.06 ± 0.04 mm (*S. aureus*), 7.06 mm ± 0.01 (*A. baumannii*), and 7.20 ± 0.01 mm (*K. pneumoniae*). This study successfully optimised the BLFV process, highlighting its antioxidant and antimicrobial properties, which hold promise for applications in developing nutraceuticals and functional foods.

Keywords: *Baccaurea lanceolata*, fruit vinegar, antioxidant, antimicrobial, central composite design

Abstrak

Baccaurea lanceolata (Miq.) Müll.Arg., yang berasal dari Asia Tenggara, dihargai dalam amalan tradisional dan diet tempatan kerana potensinya sebagai sumber perubatan. Namun begitu, aktiviti biologi cuka buahnya masih belum diterokai sepenuhnya. Kajian ini bertujuan untuk mengoptimumkan dan menilai sifat antioksidan dan antimikrob cuka buah *B. lanceolata* (BLFV) Reka bentuk komposit berpusat telah digunakan untuk mengoptimumkan masa penapaian (FT), kepekatan gula (SC), dan nisbah pepejal-ke-cecair (SLR) sebagai pembolehubah komponen, dengan jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), dan aktiviti antioksidan melalui ujian 2,2-difenil-1-pikrilhidrazil (DPPH) sebagai pembolehubah respons. Aktiviti antimikrob BLFV yang dioptimumkan telah dinilai terhadap bakteria Gram-positif (*Enterococcus faecalis* dan *Staphylococcus aureus*) dan Gram-negatif (*Acinetobacter baumannii* dan *Klebsiella pneumoniae*) menggunakan kaedah penyebaran cakera. Proses BLFV yang dioptimumkan, menggunakan 2 bulan FT, 20% SC, dan 1:2 g/mL SLR, mencapai faktor respons berikut: 438.070 ± 0.130 mg GAE/L cuka (TPC), 10.466 ± 0.131 mg CE/L cuka (TFC), dan 83.212 ± 0.134% (DPPH). Selain itu, BLFV yang dioptimumkan menunjukkan aktiviti antimikrob dengan zon perencatan berukuran 8.03 ± 0.01 mm (*E. faecalis*), 9.06 ± 0.04 mm (*S. aureus*), 7.06 ± 0.01 mm (*A. baumannii*), dan 7.20 ± 0.01 mm (*K. pneumoniae*). Kajian ini berjaya mengoptimumkan proses BLFV, menonjolkan sifat antioksidan dan antimikrob, yang berpotensi untuk diaplikasikan dalam pembangunan nutraseutikal dan makanan fungsian.

Kata kunci: *Baccaurea lanceolata*, cuka buah, antioksidan, antimikrob, reka bentuk komposit pusat

1.0 INTRODUCTION

Vinegar, a traditional condiment with a rich history, has long been valued for its health-promoting properties. The historical use of fruit vinegar for medicinal and preservative purposes has spurred scientific investigation into its potential health impacts. A recent surge in popularity, driven by distinctive flavours and associated health benefits, has further heightened scientific interest [1]. Through fermentation, fresh fruits with limited shelf life are transformed into fruit vinegar, producing a liquid rich in melanoidins, organic acids, polyphenols, and tetramethylpyrazine [2]. These compounds contribute to its tangy taste and potential health benefits. Research indicates that fruit vinegar consumption may positively influence various health conditions, including diabetes, hypertension, hyperlipidaemia, cancers, and obesity [3].

Baccaurea lanceolata (Miq.) Müll.Arg., a plant native to Southeast Asia, holds significant value in traditional practices and local diets. Known as 'Limposu' among the Dusun people in Sabah, Malaysia, this medium-sized tree produces berry-like fruits that are consumed fresh, cooked, or occasionally dried. Various parts of *B. lanceolata*, including its leaves, bark, and fruit, are utilised in traditional herbal remedies, highlighting its cultural and pharmacological importance [4]. Indigenous communities such as the Iban, Bidayuh, Penan, and Kelabit in Sarawak, Malaysia, also use different parts of the plant for medicinal purposes, reflecting its diverse applications in local traditional medicine [5].

Response surface methodology (RSM), also referred to as design of experiments (DOE), is a widely used optimisation technique that integrates experimental design, modelling, and refinement to enhance processes using experimental data. This approach identifies optimal conditions to improve efficiency, reduce costs, enhance product quality, or minimise processing times [6]. In natural product research, advanced designs such as central composite design (CCD), Box-Behnken design (BBD), and Doehlert design are increasingly preferred over traditional three-level factorial designs. CCD is particularly recognised for its applications in optimising formulations, improving product quality, and refining separation techniques such as extraction and chromatography. This design allows researchers to analyse interactions between multiple factors, offering deeper insights into process dynamics [7].

Recently, fruit vinegar has garnered attention as a functional food due to its potential health benefits, including antioxidant and antimicrobial properties, as well as its effects on blood sugar management. However, the underutilisation of *B. lanceolata*, particularly in processed and functional foods, is exacerbated by a lack of innovative product development. This study aimed to optimise *B. lanceolata* fruit vinegar (BLFV) production using total phenolic content (TPC), total flavonoid content

(TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity for antioxidant activity through a CCD. The antimicrobial activity of the optimised BLFV was also evaluated. Addressing these gaps could enhance its potential as a functional food ingredient, promote sustainable usage and add value to the food industry, aligning with current trends towards health-oriented products in food science and consumer preferences.

2.0 METHODOLOGY

2.1 Raw Material

Fresh *B. lanceolata* fruits, locally sourced from vendors in Kota Belud, Sabah, Malaysia, were selected based on ripeness criteria, including a light brown colour and uniform *shape*.

2.2 Experimental Design

Following the method by Yıkmış [8] with modifications, 100 g of fresh *B. lanceolata* fruit purée was mixed with water and sugar in bottles, as recommended by Design-Expert (Version 13). The bottles were covered with muslin cloth and left to ferment at room temperature. During the initial alcoholic fermentation stage, the bottles were shaken daily for two weeks. On the 14th day, the supernatant was filtered and transferred to a second fermentation stage using acetic acid (5%), which lasted up to three months, with periodic shaking of the covered bottles. Key parameters for the BLFV process included fermentation time (FT) as A, sugar concentration (SC) as B, and solid-to-liquid ratio (SLR) as C. Table 1 outlines the parameter ranges, derived from previous studies [8–10]. Three levels (low, middle, high) of these variables were selected for CCD to analyse the experimental runs (Table 2), with the middle level serving as the constant value for the component variables.

Table 1 Range of components for BLFV process

Parameter	Low	Middle	High	References
FT (month)	1	2	3	[8,9]
SC (%)	15	20	25	[10]
SLR (g/mL)	1	2	3	[9]

Table 2 Component and response variables for BLFV process

Run	A	B	C	TPC (mg GAE/L vinegar)	TFC (mg CE/L vinegar)	DPPH (%)
1	1.00	25.00	1.00	379.00	9.94	61.61
2	2.00	28.41	2.00	397.91	5.98	80.54
3	1.00	25.00	3.00	490.00	9.14	83.17
4	1.00	15.00	3.00	318.00	7.70	77.89
5	2.00	20.00	2.00	465.00	11.78	85.80
6	2.00	20.00	2.00	472.00	10.45	88.85
7	2.00	11.59	2.00	296.00	6.01	81.98
8	3.00	25.00	1.00	448.00	4.06	84.86
9	3.00	15.00	1.00	460.00	2.75	93.17
10	2.00	20.00	2.00	462.63	10.47	90.85
11	1.00	15.00	1.00	321.00	10.87	57.45
12	0.32	20.00	2.00	380.21	13.88	44.70
13	3.00	15.00	3.00	292.12	2.17	88.21
14	2.00	20.00	3.68	382.00	3.31	94.28
15	3.00	25.00	3.00	380.00	3.66	85.35
16	2.00	20.00	0.32	485.10	7.75	76.69
17	3.68	20.00	2.00	373.00	1.44	85.01

2.3 Experimental Responses

TPC was determined using the Folin–Ciocalteu method as described by Benjamin *et al.* [11], with absorbance measured at 798 nm using a Lambda 25 spectrophotometer (PerkinElmer, Waltham, MA, USA). Results were expressed as milligrams of gallic acid equivalents per litre (mg GAE/L) of vinegar. TFC was determined using the aluminium chloride colorimetric assay based on the method by Jinin *et al.* [12], with absorbance measured at 510 nm. Results were expressed as milligrams of catechin equivalents per litre (mg CE/L) of vinegar. Antioxidant activity was evaluated through the DPPH assay following Awang *et al.* [13], with absorbance measured at 517 nm, and the radical scavenging activity of DPPH was subsequently calculated.

2.4 Antimicrobial Activity

The antimicrobial activity of BLFV was evaluated following the methods described by Azelan *et al.* [14] and Jinoni *et al.* [15], targeting two Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Acinetobacter baumannii* and *Klebsiella pneumoniae*). Bacterial cultures were standardised to a 0.5 McFarland standard, corresponding to an inoculum of approximately 1.5 x 10^8 CFU/mL. Incubation was carried out at 37 °C for 24 h, and the

diameters of the zones of inhibition (ZOI) were measured in millimetres (mm). Ampicillin was used as the positive control, while a hexane-impregnated blank disc served as the negative control. The experiment was performed in triplicate, and results were reported as mean ± standard deviation (SD).

3.0 RESULTS AND DISCUSSION

3.1 Optimisation of BLFV Process

3.1.1 Model Fitting for Experimental Parameters

Statistical assessments and model evaluation metrics were employed, with data fitted to multiple models—linear, 2FI, quadratic, and cubic—as summarised in Table 3. The quadratic model was recommended based on sequential $p < 0.05$ and lack of fit $p > 0.05$ for TPC, TFC, and DPPH. Additionally, the adjusted R^2 (R^2adj) and predicted R^2 (R^2pred) values for all three responses were higher for the quadratic model compared to other models, although the cubic model displayed the highest overall values. Nonetheless, the cubic model exhibited aliasing, which preventing parameter estimation. Thus, the quadratic model was selected to describe the relationship between the component and response variables.

The significance of the model was evaluated using polynomial regression for analysis of variance (ANOVA), which analysed linear, interaction, and quadratic terms to assess model significance and lack of fit. Table 4 summarises the ANOVA results for both regression and residuals for TPC, TFC, and DPPH. The F-values were 36.87 for TPC, 34.05 for TFC, and 32.94 for DPPH, indicating robust model performance, with all analyses being highly significant ($p < 0.0001$). In ANOVA, a large F-value combined with $p < 0.05$ confirms statistical significance, consistent with the observed F-values [16]. For TPC, significant parameters included B and C, along with interactions AB, AC, BC, A^2, B^2, and C^2 ($p < 0.05$). For TFC, significant parameters were A and C, and interactions A^2, B^2, and C^2 ($p < 0.05$). For DPPH, significant parameters included A and C, with interactions AB, AC, and A^2 ($p < 0.05$). The lack of fit *p*-values for TPC (0.0775), TFC (0.4707), and DPPH (0.4180) were not significant ($p > 0.05$) compared to the pure error, confirming the adequacy of the model. In addition, R^2 values of 0.9793 for TPC, 0.9777 for TFC, and 0.9769 for DPPH indicate minimal fitting error, as R^2 approaches 1.0000. The close alignment of R^2 and R^2adj values further supports the inclusion of only significant terms.

Table 3 Model fitting summary of TPC, TFC, and DPPH

TPC						
Source	Sequential *p*-value	Lack of fit *p*-value	R^2adj	R^2pred	PRESS	
Linear	0.1498	0.0053	0.1709	–0.1397	82389.10	
2FI	0.0760	0.0075	0.4416	0.2917	51197.68	
Quadratic	0.0001	0.0775	0.9528	0.8460	11134.04	Suggested
Cubic	0.0637	0.1960	0.9901	0.7336	19259.64	Aliased
TFC						
Source	Sequential *p*-value	Lack of fit *p*-value	R^2adj	R^2pred	PRESS	
Linear	0.0007	0.0951	0.6572	0.6176	88.24	
2FI	0.9341	0.0730	0.5722	0.4227	133.20	
Quadratic	0.0004	0.4707	0.9490	0.8530	33.91	Suggested
Cubic	0.2537	0.7365	0.9712	0.9061	21.67	Aliased
DPPH						
Source	Sequential *p*-value	Lack of fit *p*-value	R^2adj	R^2pred	PRESS	
Linear	0.0055	0.0616	0.5191	0.3247	1941.01	
2FI	0.3053	0.0634	0.5577	0.4895	1467.32	
Quadratic	0.0004	0.4180	0.9473	0.8478	437.43	Suggested
Cubic	0.5960	0.2274	0.9406	–0.4790	4250.93	Aliased

Table 4 ANOVA summary of TPC, TFC, and DPPH

TPC						
Source	Sum of square	dF	Mean square	F-value	*p*-value	Significance
Model	723588.29	9	7862.76	36.87	< 0.0001	Significant
A	263.52	1	263.52	1.24	0.3031	
B	16678.83	1	16678.83	78.18	< 0.0001	
C	6645.93	1	6645.93	31.15	0.0008	
AB	2969.24	1	2969.24	13.92	0.0074	
AC	14781.42	1	14781.42	69.28	< 0.0001	
BC	5717.92	1	5717.92	26.80	0.0013	
A^2	10583.38	1	10583.38	49.61	0.0002	
B^2	19065.22	1	19065.22	89.36	< 0.0001	
C^2	1244.67	1	1244.67	5.83	0.0464	
Residual	1493.43	7	213.35			
Lack of fit	1445.98	5	289.20	12.19	0.0775	Not significant
Pure error	47.45	2	23.72			
Cor total	72285.82	16				
R^2	0.9793					
R^2pred	0.8460					
R^2adj	0.9528					
TFC						
Source	Sum of square	dF	Mean square	F-value	*p*-value	Significance
Model	225.59	9	25.07	34.05	< 0.0001	Significant
A	154.40	1	154.40	209.76	< 0.0001	
B	0.7765	1	0.7765	1.05	0.3385	
C	11.29	1	11.29	15.34	0.0058	
AB	0.6543	1	0.6543	0.8890	0.3772	
AC	1.12	1	1.12	1.52	0.2576	
BC	0.8072	1	0.8072	1.10	0.3298	
A^2	13.86	1	13.86	18.84	0.0034	
B^2	32.51	1	32.51	44.17	0.0003	
C^2	39.11	1	39.11	53.13	0.0002	
Residual	5.15	7	0.7990			
Lack of fit	3.99	5	0.7990	1.38	0.4707	Not significant
Pure error	1.16	2	0.5789			
Cor total	230.74	16				
R^2	0.9777					
R^2pred	0.8530					
R^2adj	0.9490					
DPPH						
Source	Sum of square	dF	Mean square	F-value	*p*-value	Significance
Model	2807.83	9	311.98	32.94	< 0.0001	Significant
A	1420.18	1	1420.18	149.94	< 0.0001	
B	1.27	1	1.27	0.1337	0.7254	
C	329.75	1	329.75	34.81	0.0006	
AB	53.14	1	5.39	5.61	0.0497	
AC	269.97	1	688.50	28.50	0.0011	
BC	5.39	1	45.75	0.5692	0.4752	
A^2	688.50	1	3.06	72.69	< 0.0001	
B^2	45.75	1	45.75	4.83	0.0639	
C^2	3.06	1	3.06	0.3232	0.5874	
Residual	66.30	7	9.47			
Lack of fit	53.39	5	10.68	1.65	0.4180	Not significant
Pure error	12.91	2	6.45			
Cor total	2874.14	16				
R^2	0.9769					
R^2pred	0.8478					
R^2adj	0.9473					

Through multiple regression analysis, Equation 1 (TPC), Equation 2 (TFC), and Equation 3 (DPPH) describe the relationships between the component and response variables. The statistical findings demonstrate that the models in the equations effectively predict TPC, TFC, and DPPH within the specified design parameter ranges. The significant influence of the terms included in the models on the responses is validated by results with $p < 0.05$, indicating satisfactory model performance.

$$TPC = 466.21 + 34.95\,B - 22.06\,C - 19.27\,AB - 42.98\,AB + 26.73\,BC - 30.64\,A^2 - 41.12\,B^2 - 10.51\,C^2 \quad (1)$$

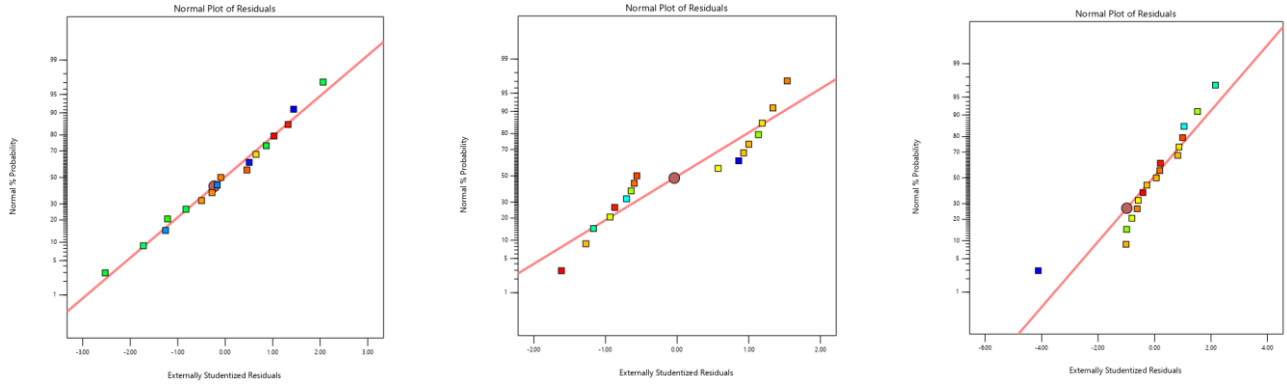
$$TFC = 10.89 - 3.36\,A - 0.9094\,C - 1.11\,A^2 - 1.70\,B^2 - 1.86\,C^2 \quad (2)$$

$$DPPH = 88.34 + 10.20\,A + 4.91\,C - 2.58\,AB - 5.81\,AC - 7.81\,A^2 \quad (3)$$

The analysis for TPC, TFC, and DPPH, as shown in Figures 1(a), 1(b), and 1(c), respectively, examined normal probability plots of residuals, which showed that the errors were approximately normally distributed and that no violations of assumptions were detected. Figures 2(a), 2(b), and 2(c) showed a strong correlation between predicted and actual values, demonstrating the reliability of the model in determining TPC, TFC, and DPPH, respectively. The accuracy of the model was further supported by R^2 values close to unity. Additionally, Figures 3(a) for TPC, 3(b) for TFC, and 3(c) for DPPH show all externally studentised residuals within the critical limits (±4.8196), indicating no outliers and supporting model adequacy.

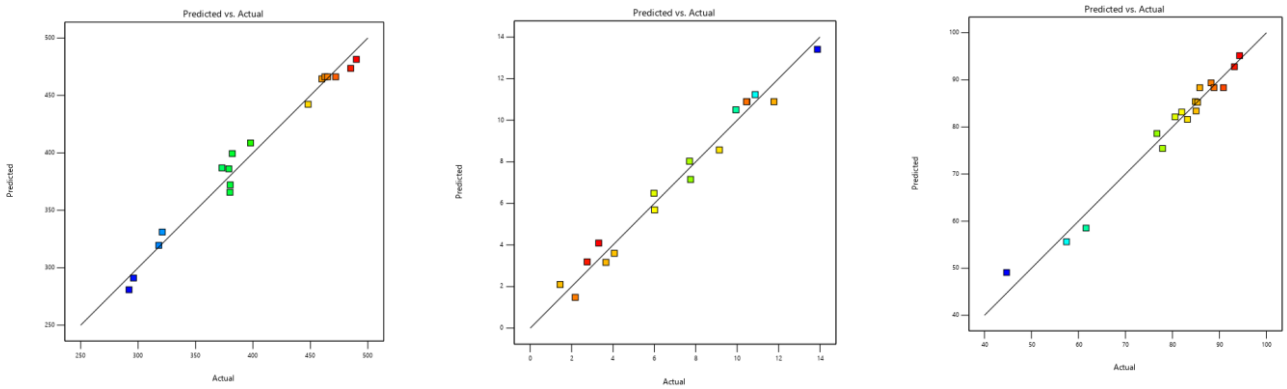
3.1.2 Effect of TPC on the BLFV Process

A 3D RSM plot of TPC proportion against FT (A), SC (B), and SLR (C) was generated by varying two variables while keeping the third constant. Figure 4 illustrates the interaction between FT and SC at a constant 1:2 g/mL SLR. SC emerged as the most significant factor in the regression model for TPC proportion, supported by both linear (B) and quadratic (B^2) terms ($p < 0.05$). Payet *et al.* [17] highlighted that SC was the most significant factor in TPC, emphasising its role as a commonly used foodstuff for sweetening, which may contribute antioxidant properties due to phenolic molecules. In contrast, the linear term (A) for FT showed no significant effect on TPC ($p > 0.05$) while the quadratic term (A^2) had a significant effect ($p < 0.05$). Maximum TPC values were observed within 1.5 to 2.5 months for FT and 19% to 25% for SC, aligning with studies linking TPC enhancement in fruit vinegar [18].



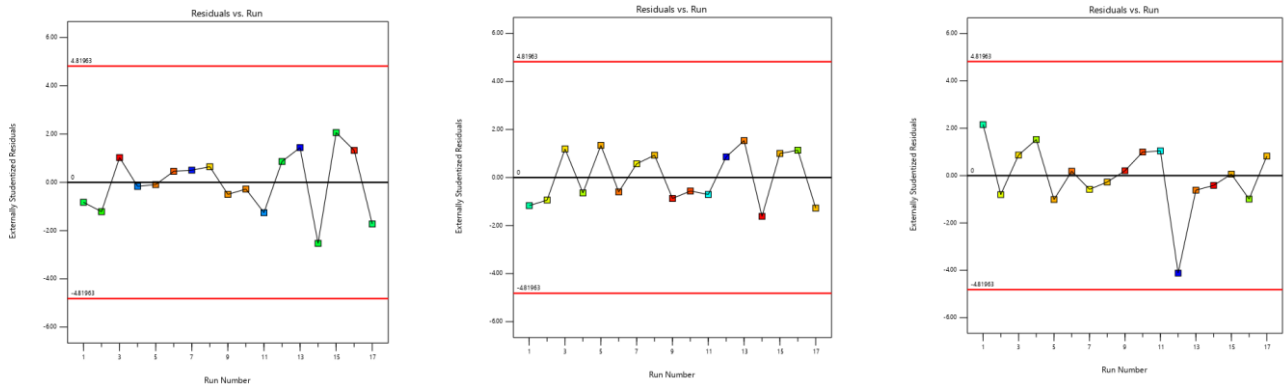
(a) (b) ©

Figure 1 Normal plot of residual for (a) TPC, (b) TFC, and (c) DPPH



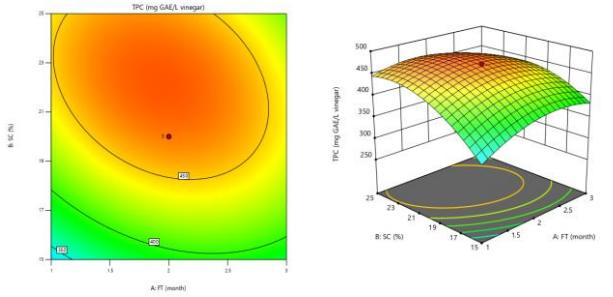
(a) (b) ©

Figure 2 Relationship between predicted and actual values for (a) TPC, (b) TFC, and (c) DPPH



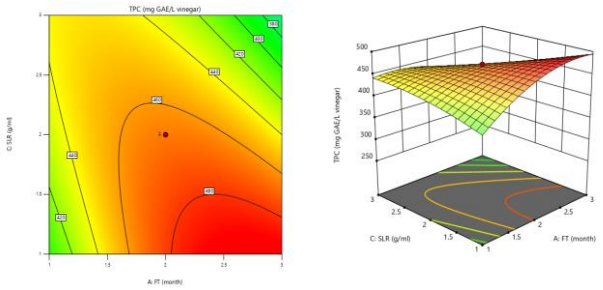
(a) (b) ©

Figure 3 Comparison of residuals versus experimental runs for (a) TPC, (b) TFC, and (c) DPPH



(a) (b)

Figure 4 (a) Contour plot and (b) 3D RSM of FT (A) and SC (B) on TPC at constant SLR (C)

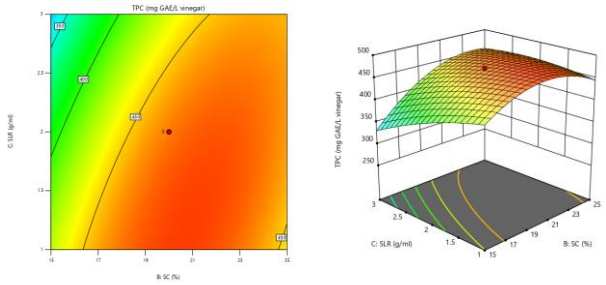


(a) (b)

Figure 5 (a) Contour plot and (b) 3D RSM of FT (A) and SLR (C) on TPC at constant SC (B)

Figure 5 illustrates the effects pf FT (A) and SLR (C) on TPC proportion at a constant 20% SC (B). The interaction between these parameters (AC) significantly influenced TPC ($p < 0.05$). Significant linear (C) and quadratic (A^2 and C^2) terms on TPC were observed ($p < 0.05$), except for the linear term of A ($p > 0.05$). The highest TPC values were achieved at approximately 2 to 3 months of FT and an SLR of 1:0 to 1:1.5 g/mL. Hammouda *et al.* [19] highlighted the significant impact of prickly pear vinegar fruit presence on TPC, which aligns with the observed changes in TPC with FT, enhancing bioactive compound content. TPC increased up to 3 months of FT but declined thereafter due to yeast activity, which contributes to the conversion of simple phenolics and the depolymerisation of high molecular weight compounds. This observation is consistent with studies on TPC in solid-state yeast fermentation of wheat and oat bran [20]. Similarly, Wang *et al.* [21] reported a TPC increase in noni juice during specific FT, peaking at optimal points.

Figure 6 illustrates the relationship between SC (B), SC (B) and SLR (C) at a constant 2-month FT (A) on TPC proportion. The interaction between BC was significant ($p < 0.05$). Both linear (B and C) and quadratic (B^2 and C^2) terms on TPC were also significant ($p < 0.05$). The highest TPC was observed at approximately 17 to 24% SC and 1:0 to 1:2 g/mL SLR. TPC increased notably with SLR but declined beyond a certain point. Hammouda *et al.* [19] reported a significant increase in TPC with increasing SLR during prickly pear vinegar production. Similarly, Jayasree Radhakrishnan and Venkatachalam [22] observed higher TPC in *Ficus benghalensis* fruits with increasing SLR. Conversely, Yaghoobi *et al.* [23] found increased TPC in myrtle leaves with decreasing SLR, while Okur *et al.* [24] reported reduced TPC and oleuropein content in olive leaves with increasing SLR. These trends are attributed to SLR saturation, where the liquid medium becomes unable to extract additional phenolics, or to plant material degradation, which reduces phenolic content [25]. Collectively, these findings suggest that TPC increases with SLR up to a threshold before declining.

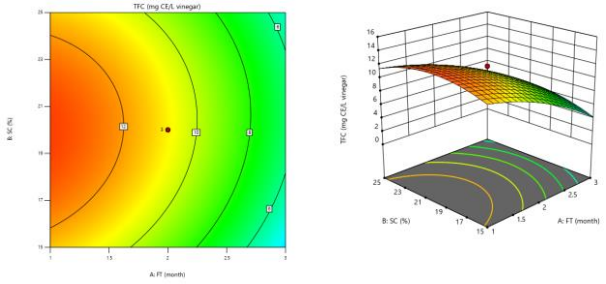


(a) (b)

Figure 6 (a) Contour plot and (b) 3D RSM of SC (B) and SLR (C) on TPC at constant FT (A)

3.1.3 Effect of TFC on the BLFV Process

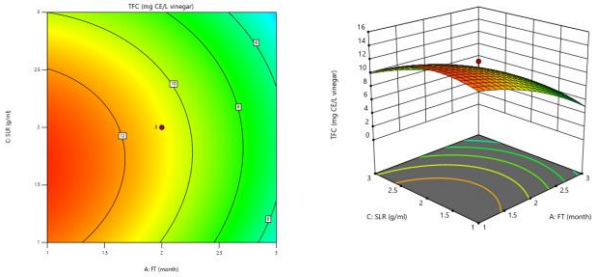
A 3D RSM plot was created to analyse TFC proportion against FT (A), SC (B), and SLR (C), with Figure 7 depicting the interaction between FT and SC at a constant 1:2 g/mL SLR. FT significantly influenced TFC and was identified as the most impactful factor in the regression model, supported by significant linear (A) and quadratic (A^2) terms ($p < 0.05$). However, the linear term (B) of SC was not significant ($p > 0.05$) although its quadratic terms (B^2) were significant ($p < 0.05$). The RSM plot revealed that within 1.0 to 1.5 months of FT and 17 to 23% SC, decreasing FT enhances TFC. Zhao *et al.* [26] highlighted that extending FT can degrade TFC in some food materials, emphasising its role in enhancing bioactive compounds. These findings suggest that optimising FT within certain thresholds can maximise TFC during the fruit vinegar process.



(a) (b)

Figure 7 (a) Contour plot and (b) 3D RSM of FT (A) and SC (B) on TFC at constant SLR (C)

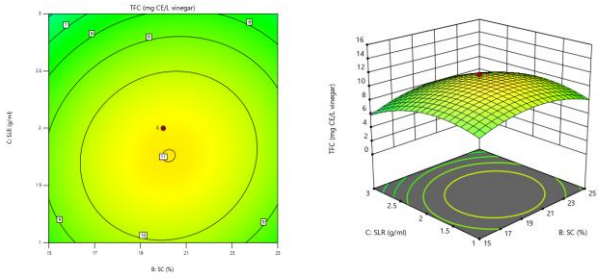
Figure 8 illustrates the impact of FT (A) and SLR (C) on TFC proportion at a constant 20% SC (B). The interaction between these parameters (AC) was not significant for TFC proportion ($p > 0.05$). However, the linear terms of A and C, as well as the quadratic terms of A^2 and C^2, were significant ($p < 0.05$), demonstrating a strong influence on TFC. The highest TFC was observed within the range of 1 to 1.5 months of FT and an SLR of 1:0 to 1:2.5 g/mL. TFC decreased with increasing FT but increased with higher SLR, peaking at 1:2.5 g/mL. Cao *et al.* [27] reported a gradual TFC increase in *Apocynum venetum* leaves during solid-state fermentation up to day 6, followed by degradation by day 12. Furthermore, TFC concentrations in *F. benghalensis* fruits were influenced by SLR, supporting the concept that higher SLR can enhance TFC [24].



(a) (b)

Figure 8 (a) Contour plot and (b) 3D RSM of FT (A) and SLR (C) on TFC at constant SC (B)

Figure 9 illustrates the relationship between SC (B) and SLR (C) at a constant 2-month FT (A) on TFC proportion. The interaction between these variables (BC) was not significant for TFC outcomes ($p > 0.05$). The linear term of B was also not significant ($p > 0.05$), whereas the linear term of C was significant ($p < 0.05$). Additionally, the quadratic terms of B^2 and C^2 had a significant impact on TFC outcomes ($p < 0.05$). The highest TFC was achieved at 20% SC and a 1:1.7 g/mL SLR. This trend aligns with Choommongkol *et al.* [28], who reported an increase in the flavonoid compound (2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethyl chalcone) with SLR at an optimal point. Similarly, Yu *et al.* [29] observed an increase in TFC with SLR in *Crinum asiaticum* at 1:10 to 1:20 g/mL SLR, followed by a decrease.



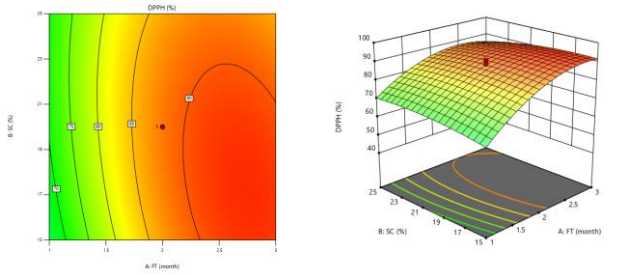
(a) (b)

Figure 9 (a) Contour plot and (b) 3D RSM of SC (B) and SLR (C) on TFC at constant FT (A)

3.1.4 Effect of DPPH on the BLFV Process

A 3D RSM plot of DPPH proportion against FT (A), SC (B), and SLR (C) was generated, with Figure 10 illustrating the significant interaction between FT and SC at a constant 1:2 g/mL SLR. FT emerged as the most influential factor for DPPH proportion, supported by significant linear (A) and quadratic (A^2) terms ($p < 0.05$), while B also showed no significant linear (B) and quadratic (B^2) terms ($p > 0.05$). The maximum DPPH activity was observed within 2.25 to 3 months of

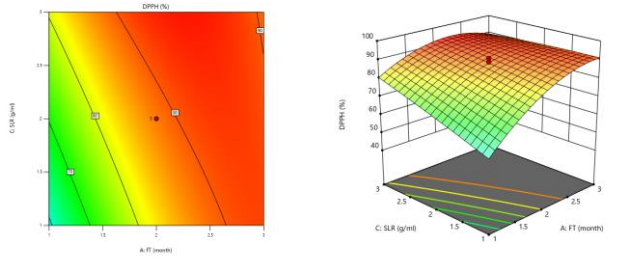
FT and 15 to 23% SC, as increasing FT enhanced the DPPH proportion. Previous studies have highlighted that extending FT plays a major role in enhancing antioxidant activity during fruit vinegar production [1,17].



(a) (b)

Figure 10 (a) Contour plot and (b) 3D RSM of FT (A) and SC (B) on DPPH at constant SLR (C)

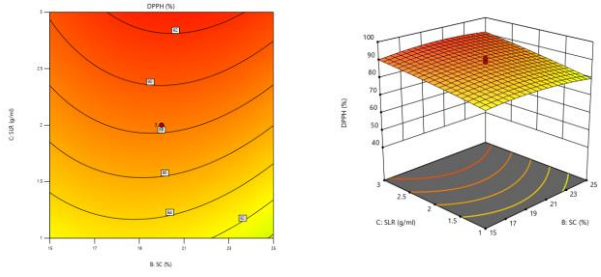
Figure 11 illustrates the impact of FT (A) and SLR (C) on DPPH proportion at 20% SC (B). Both linear terms of A and C, as well as the quadratic term of A^2, significantly influence DPPH proportion ($p < 0.05$), whereas the quadratic term of C^2 was not significant ($p > 0.05$). The highest DPPH value was observed within 2 to 2.5-month FT and 1:2 to 1:3 g/mL SLR. While FT increases with SLR, DPPH values rose to approximately 90%. This direct relationship between SLR and DPPH was also noted by Khan *et al.* [30], who reported stable DPPH values in corn silk with higher SLR. Additionally, FT significantly influences the antioxidant properties of fruit vinegars. Zou *et al.* [31] similarly observed an increase in the DPPH of persimmon with FT ranging from 2 to 3 months, indicating that longer FT enhances antioxidant capacities in fruit vinegar.



(a) (b)

Figure 11 (a) Contour plot and (b) 3D RSM of FT (A) and SLR (C) on DPPH at constant SC (B)

Figure 12 illustrates the relationship between SC (B) and SLR (C) at a fixed 2-month FT (A) on DPPH proportion. The interaction of BC was non-significant ($p > 0.05$), indicating no significant effect on DPPH outcomes. The linear term of B was also insignificant ($p > 0.05$), whereas C had a significant linear term ($p < 0.05$). The quadratic terms (B^2 and C^2) did not significantly influence DPPH outcomes ($p > 0.05$). The highest DPPH value was observed at 21% SC and 1:2.25 g/mL SLR. Recent studies highlight the complexity of relationships among SC, and DPPH. Gong *et al.* [32] reported an increasing trend in DPPH scavenging rates with higher SC in *Siraitia grosvenorii* polysaccharides.



(a) (b)

Figure 12 (a) Contour plot and (b) 3D RSM of SC (B) and SLR (C) on DPPH at constant FT (A)

3.1.5 Verification of Predictive Model on BLFV

Experimental validation under optimal conditions confirmed the accuracy of the regression model (Table 5). Results closely matched predictions: TPC 466.208 mg GAE/L vinegar (predicted) vs. 438.070 ± 0.130 mg GAE/L vinegar (actual), TFC 10.891 mg CE/L vinegar (predicted) vs. 10.466 ± 0.131 mg CE/L vinegar (actual), and DPPH 88.338% (predicted) vs. 83.212 ± 0.134% (actual). Percentage errors were minimal: 6.423% for TPC, 4.061% for TFC, and 6.160% for DPPH. Optimal conditions included 2-month FT, 20% SC, and 1:2 g/mL SLR.

Table 5 Component and predicted vs actual response values for TPC, TFC, and DPPH

	Component variables	
Optimised process	FT (months)	2
	SC (%)	20
	SLR (g/mL)	1:2
	Response variables	
Predicted value	TPC (mg GAE/L vinegar)	466.208
	TFC (mg CE/L vinegar)	10.891
	DPPH (%)	88.338
*Actual value	TPC (mg GAE/L vinegar)	438.070 ± 0.130
	TFC (mg CE/L vinegar)	10.466 ± 0.131
	DPPH (%)	83.212 ± 0.134
Error (%)	TPC	6.423
	TFC	4.061
	DPPH	6.160

*The values indicate the mean ± SD from three replicates.

3.2 Effect of Optimised BLFV on Antimicrobial Activity

The Kirby-Bauer Test, also referred to as the disc diffusion assay, evaluates bacterial susceptibility to antibiotics by measuring the clear ZOI around antibiotic-impregnated discs, with larger zones indicating higher susceptibility [33]. This method is widely favoured for its efficiency in detecting antimicrobial activity by assessing bacterial growth or

inhibition. Table 6 summarises the antimicrobial effectiveness of BLFV using the disc diffusion method. At 1000 ppm, hexane showed no ZOI for any bacterial strain. For Gram-positive bacteria, ampicillin produced ZOI measurements of 17.23 mm and 16.93 mm, whereas BLFV produced ZOI measurements of 8.03 mm and 9.06 mm for *E. faecalis* and *S. aureus*, respectively. For Gram-negative bacteria, ampicillin produced ZOI measurements of 17.03 mm and 16.10 mm, whereas BLFV produced ZOI measurements of 7.06 mm and 7.20 mm for *A. baumannii* and *K. pneumoniae*, respectively.

S. aureus and *E. faecalis*, components of the human microbiota, along with *A. baumannii* and *K. pneumoniae*, which are associated with hospital-acquired infections and antibiotic resistance, were chosen for their clinical relevance and the growing concern of antimicrobial resistance in healthcare settings [34,35]. Previous studies have highlighted the potent antimicrobial effects of apple cider vinegar against resistant strains such as *Escherichia coli* and methicillin-resistant *S. aureus* [36], suggesting that fruit vinegars, particularly those containing acetic acid, can effectively inhibit the growth of both Gram-positive and Gram-negative bacteria. Moreover, physalis and red pitahaya fruit vinegars have demonstrated inhibitory effects on a range of bacteria, including Gram-positive (*Bacillus cereus*, *Listeria monocytogenes*, and *S. aureus*) and Gram-negative (*Salmonella enterica*) strains [37]. These findings suggest that BLFV has the potential to inhibit a broad spectrum of bacteria, including both Gram-positive and Gram-negative strains.

Table 6 Antimicrobial activity of optimised BLFV using the disc diffusion method

Bacterial strain	BLFV (mm)	Ampicillin [1] (mm)	Hexane [2] (mm)
E. faecalis	8.03 ± 0.01	17.23 ± 0.02	0.00 ± 0.00
S. aureus	9.06 ± 0.04	16.93 ± 0.02	0.00 ± 0.00
A. baumannii	7.06 ± 0.01	17.03 ± 0.02	0.00 ± 0.00
K. pneumoniae	7.20 ± 0.01	16.10 ± 0.01	0.00 ± 0.00

The values indicate the mean ± SD from three replicates. [1] Ampicillin served as a positive control. [2] Hexane served as a negative control.

4.0 CONCLUSION

The optimised BLFV process, involving a 2-month FT, 20% SC, and 1:2 g/mL SLR, produced notable results for TPC (438.070 ± 0.131 mg GAE/L vinegar), TFC (10.466 ± 0.130 mg CE/L vinegar), and DPPH (83.212 ± 0.134%). The antimicrobial evaluation confirmed the effectiveness of BLFV against both Gram-positive and Gram-negative bacteria. These results highlight the potential of BLFV as a nutraceutical and functional food product with significant antioxidant and antimicrobial properties, meriting further investigation within the food and health industries.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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