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Optimising Terung Asam (*Solanum lasiocarpum* Dunal.) Sauce for Enhanced Phenolics, Flavonoids, and Antioxidant Capacity with Physicochemical Properties and Storage Stability Analysis

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Abstract *Solanum lasiocarpum* Dunal*,* commonly referred to as ‘terung asam’, is a native plant of Borneo Island that is widely utilised in Sarawak, Malaysia, for its culinary and medicinal applications. While terung asam has been commercialised into products such as sauces, research on optimising its sauce formulations remains limited. This study utilised a D-optimal mixture design, a method from response surface methodology, to optimise terung asam sauce (TAS) formulations using terung asam (TA) purée, virgin coconut oil (VCO), and garlic powder (GP). The focus was on three key responses: total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The physicochemical properties, including pH, colour, water activity, and storage stability analysis, were evaluated for the optimal TAS formulation. The optimal TAS formulation comprised 98.46%, 0.73%, and 0.81% for TA, VCO, and GP, respectively, with both predicted and actual values aligning and meeting acceptance error criteria. The physicochemical parameters for the optimal TAS formulation were within the optimal ranges, while TPC, TFC, and DPPH showed a slight decline over the storage period of 28 days. In conclusion, optimising TAS formulations offers an innovative approach to developing a healthy food product with high antioxidant properties and extended shelf life.

Keywords: Terung asam sauce, D-optimal mixture design, physicochemical, storage stability.

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Introduction

Current sauce products often include unhealthy ingredients such as high fructose corn syrup or glucose, while lacking essential nutrients like protein, fibre, vitamins, and minerals. This combination can contribute to health issues, including elevated blood sugar levels, obesity, diabetes, heart disease, and a weakened immune system [1]. A sauce, typically liquid or semi-liquid, is defined as a relish or gravy that accompanies food, enhancing meals by adding flavour, moistness, texture, and body [2]. The growing significance of fruit-based sauces lies in their ability to enhance the functional properties of foods, such as sweet-and-sour sauce with açai and unconventional food plants, offering a healthy alternative for fruit and vegetable consumption as the food industry increasingly focuses on plant-based products [3]. Incorporating plant ingredients into sauces and other products enhances nutritional benefits through phytochemicals and antioxidants, while meeting the growing demand for plant-based options and supporting sustainable food production [2].

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Solanum lasiocarpum Dunal, known as 'terung asam', is a wild vegetable native to Borneo, particularly Sarawak, where it is used as a sour flavouring agent and served as a vegetable dish [4]. This golden-yellow sour eggplant, belonging to the Solanaceae family, which also includes peppers and tomatoes, is widely found throughout Southeast Asia. In addition to Borneo, terung asam grows across Southeast and South Asia, including Malaysia, Indonesia, Indochina, the Philippines, Thailand, Southern China, Bangladesh, and India [5]. The Intellectual Property Corporation of Malaysia (MyIPO) awarded Geographical Indication status (No. GI2010-00002) to 'terung asam Sarawak' to safeguard its uniqueness in 2011 [6]. Ethnobotanical records indicate its medicinal uses for conditions such as fever, vomiting, sore throat, and gonorrhoea. Fruits and vegetables from the *Solanum* genus are recognised for their flavonoids and antioxidant properties. Hot water extracts of terung asam fruit are noted for their high phenolic and flavonoid content, making them a good source of antioxidant compounds with significant antioxidant potential [7]. Moreover, Shing *et al*. [8] studied the optimised conditions for maximum extraction of phenolics and antioxidant activity from terung asam, which were found to be high, indicating a significant impact on antioxidant activity.

Response surface methodology (RSM) is a widely used tool for model development, experimental design, and evaluating the effects of independent factors. It is particularly valuable for minimising the number of trials required in multi-factor experiments [9]. Mixture design, a specific type of RSM, is widely applied to optimise processing conditions, develop formulations, and create novel products, offering valuable applications for the food, beverage, and pharmaceutical industries. Specifically, it identifies the optimal proportions of ingredients in a mixture to achieve desired properties. Ingredients are typically represented as percentages or fractions that sum to one, influencing outcomes such as material strength, liquid viscosity, or food product flavour [10]. D-optimal designs are among the most commonly utilised mixture designs and are often employed to investigate the effects of extrusion conditions on the functional and physical properties of extruded products, particularly food-based formulations derived from fruits and vegetables, including tomato sauce [11].

Despite the potential biological properties, research on product development to harness the beneficial phytochemicals and biological activities in terung asam remains limited. This study aims to optimise the formulation of terung asam sauce (TAS), focusing on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, through a D-optimal mixture design. Additionally, the study evaluates the impact of physicochemical properties and storage stability on these factors, highlighting the potential of TAS in food product development due to its diverse nutraceutical properties and health benefits.

Materials and Methods

Raw Materials

Fresh ripe terung asam (TA), golden-yellow in colour, sourced from Bintulu, Sarawak, Malaysia, was processed into a purée for further use. Other ingredients, including virgin coconut oil (VCO), garlic powder (GP), chili powder, salt, and sugar, were sourced from a local supermarket in Kota Kinabalu, Sabah, Malaysia.

Experimental Design

The TAS formulation was developed using Design Expert software (Version 13), applying the mixture design method with modifications based on Teangpook *et al*. [12]. This study evaluated the effects of three component variables—TA (X_1), VCO (X_2), and GP (X_3)—on three response variables: TPC, TFC, and DPPH. The component ranges were established as follows: for X_1, between 37.5 g (75%) and 50 g (100%); for X_2, between 0 g (0%) and 10 g (20%); and for X_3, between 0 g (0%) and 2.5 g (5%). A total of 14 formulations (F1–F14) were generated by the Design Expert software, with the mixture design output for the TAS formulation shown in Table 1. Constraints were applied to ensure the total weight of the TAS was 50 g, as determined by Eq. 1.

$$X_1 + X_2 + X_3 = 50 \text{ g } (100\%) \qquad (1)$$

The preparation of TAS (Figure 1) involved determining the quantities of each ingredient (g/50 g) based on the total weight of the three main components (TA, VCO, and GP), as outlined in Table 1. In addition, 2.5 g of chili powder, 1.0 g of salt, and 7.5 g of sugar were incorporated. The mixture was blended for 30 s on low speed, followed by 30 s on high speed, and then heated in a pan for 5 min until it reached 80 °C. After cooling to room temperature, the sample was transferred to sealed glass jars and stored at 27.5 °C for 24 h with minimal light exposure and limited oxidation before the extraction process.

Figure 1. Visual representation of the prepared TAS formulation

Table 1. Experimental runs for the TAS formulation based on the mixture design

Run	Factor (pseudo)			Factor (actual)		
	X_1	X_2	X_3	X_1	X_2	X_3
1	100.00	0.00	0.00	50.00	0.00	0.00
2	89.84	10.16	0.00	44.97	5.03	0.00
3	75.00	20.00	5.00	37.50	10.00	2.50
4	87.50	10.00	2.50	43.75	5.00	1.25
5	97.80	0.00	2.20	48.90	0.00	1.10
6	87.50	10.00	2.50	43.75	5.00	1.25
7	80.00	20.00	0.00	40.00	10.00	0.00
8	100.00	0.00	0.00	50.00	0.00	0.00
9	84.63	15.37	0.00	42.32	7.68	0.00
10	87.50	10.00	2.50	43.75	5.00	1.25
11	77.89	20.00	2.11	38.95	10.00	1.05
12	90.34	4.66	5.00	45.17	2.33	2.50
13	80.33	14.67	5.00	40.17	7.34	2.50
14	95.00	0.00	5.00	47.50	0.00	2.50

Response Variable Analysis

The extraction of TAS was carried out using the method outlined by Hanis Mastura *et al*. [13], which involved mixing 2 g of the sample with 50 mL of 70% methanol, followed by incubation for 2 h at 150 rpm and 70 °C. The samples were then filtered and dried at 40 °C to obtain the crude extract, followed by the evaluation of TPC, TFC, and DPPH activity. TPC was assessed using the Folin-Ciocalteu reagent [14], TFC by aluminium chloride [15], and antioxidant capacity by the DPPH assay [16], with results expressed as mg GAE/g extract, mg QE/g extract, and % DPPH scavenging activity, respectively.

Physicochemical Analysis

The pH of the optimal TAS sample was measured using a digital pH meter (Mettler Toledo, Columbus, OH, USA). The colour of the optimal TAS sample was assessed using a colorimeter (HunterLab, Reston, VA, USA) with parameters L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) for characterisation. The water activity (a_w) of the optimal TAS sample was measured using an a_w meter (Aqualab, Pullman, WA, USA).

Storage Stability Analysis

The optimal TAS sample was stored at room temperature (27.5 °C) for four weeks (28 days). Analyses of TPC, TFC, and DPPH were conducted at five intervals during the storage period, using the methods previously described [14–16].

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics software (Version 29), with physicochemical and storage stability data presented as the mean ± standard deviation (SD) from triplicate measurements. One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test was performed to identify significant differences at $p < 0.05$.

Results and Discussion

Optimisation of TAS Formulation

The optimisation of TA, VCO, and GP focused on TPC, TFC, and DPPH in the TAS formulation. Data were fitted to regression models, and the goodness of fit was assessed, as shown in Table 2. The results showed that the quadratic model was the best fit for the three responses, as indicated by a lack of fit *p*-value greater than the significance level ($p > 0.05$) and a sequential *p*-value below the threshold ($p < 0.05$). Consequently, the quadratic model was chosen to explain the relationship between the components and response variables.

Table 2. Model fitting summary of TPC, TFC, and DPPH

Resp.	Source	Linear	Quadratic	Special cubic	Cubic	Special quartic vs quadratic	Quartic vs cubic	Quartic vs special quartic
TPC	Sequential *p*-value	< 0.0001	0.0009	0.1063	0.3212	0.2116	0.6736	0.5254
	Lack of fit *p*-value	0.0492	0.3892	0.5133	0.6736	0.5254		
	R^2adj	0.7787	0.9569	0.9670	0.9738	0.9699	0.9674	0.9674
	R^2pred	0.7166	0.9134	0.8841		0.0303		
			Suggested				Aliased	Aliased
TFC	Sequential *p*-value	0.0120	< 0.0001	0.9881	0.4893	0.8522	0.9367	0.5499
	Lack of fit *p*-value	0.0224	0.8028	0.7169	0.9367	0.5499		
	R^2adj	0.4708	0.9706	0.9664	0.9659	0.9593	0.9547	0.9547
	R^2pred	0.2801	0.9507	0.9395		–0.2561		
			Suggested				Aliased	Aliased
DPPH	Sequential *p*-value	0.1673	< 0.0001	0.5033	0.1437	0.4041	0.3183	0.1781
	Lack of fit *p*-value	0.0051	0.2268	0.1944	0.3183	0.1781		
	R^2adj	0.1462	0.9455	0.9418	0.9702	0.9490	0.9731	0.9731
	R^2pred	–0.1429	0.8461	0.7877		–2.0475		
			Suggested				Aliased	Aliased

The significance of the model was assessed using ANOVA, with the statistical parameters for regression and residuals for TPC, TFC, and DPPH presented in Table 3. The ANOVA results confirmed the significance of the model, with $p < 0.0001$ for all assessments (TPC, TFC, and DPPH), indicating its ability to sufficiently explain data variability. Significant interactions among parameters (AB, AC, and BC) were identified, highlighting the complexity of these relationships. The lack of fit *p*-values for TPC (0.3892), TFC (0.8028), and DPPH (0.2268) showed that lack of fit was not significantly different from pure error, reinforcing the validity of the model. The R^2 values for TPC (0.9735), TFC (0.9819), and DPPH (0.9665) were close to unity, indicating minimal fitting error. The close alignment of the R^2 and R^2adj values suggest minimal non-significant terms, demonstrating strong quadratic model significance. These findings emphasise the robustness of the statistical model in effectively capturing relationships between variables.

Table 3. ANOVA summary of TPC, TFC, and DPPH

Resp.	Source	Model	Linear mixture	X_1X_2	X_1X_3	X_2X_3	Residual	Lack of fit	Pure error	Cor total	R^2	R^2pred	R^2adj
TPC	Sum of square	242.20	202.38	11.28	16.55	21.75	6.60	4.73	1.87	249.00	0.9735	0.9134	0.9569
	dF	5	2	1	1	1	8	5	3	13			
	Mean square	48.48	101.19	11.28	16.55	21.75	0.8256	0.9640	0.6248				
	F-value	58.72	122.57	13.67	20.04	26.35		1.51					
	p-value	< 0.0001	< 0.0001	0.0061	0.0021	0.0009		0.3892					
		Significant						Not significant					
TFC	Sum of square	0.0438	0.0246	0.0154	0.0062	0.0056	0.0008	0.0003	0.0005	0.0446	0.9819	0.9507	0.9706
	dF	5	2	1	1	1	8	5	3	13			
	Mean square	0.0088	0.0123	0.0154	0.0062	0.0056	0.0001	0.0001	0.0002				
	F-value	86.76	121.99	152.27	61.53	55.67		0.44					
	p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.8028					
		Significant						Not significant					
DPPH	Sum of square	162.30	46.61	38.48	46.10	57.39	5.63	4.59	1.04	167.93	0.9665	0.8641	0.9455
	dF	5	2	1	1	1	8	5	3	13			
	Mean square	32.41	23.31	38.48	46.10	57.39	0.70	0.92	0.35				
	F-value	46.11	33.10	54.66	65.47	81.51		2.64					
	p-value	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001		0.2268					
		Significant						Not significant					

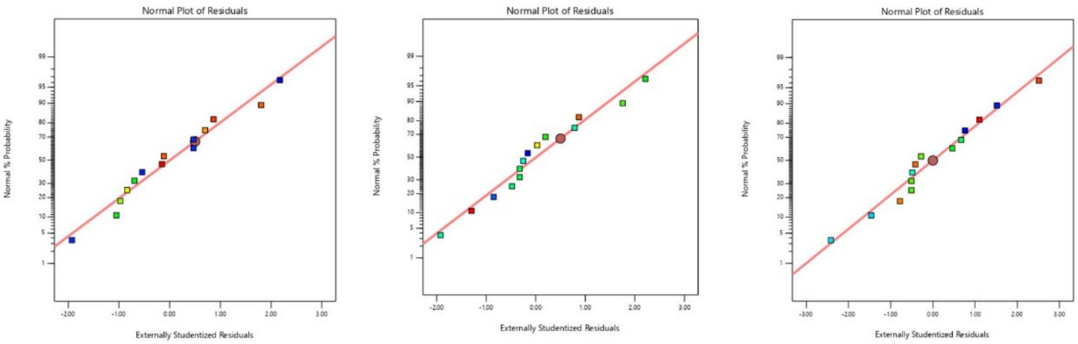
Using multiple regression analysis, Eqs. 2, 3, and 4 illustrate the relationships between the component variables and TPC, TFC, and DPPH, respectively. The statistical results confirm that these models accurately predict the responses within the design parameters, with $p < 0.0001$, indicating that the model terms significantly affect the corresponding outcomes.

$$\text{TPC} = 0.503311\,X_1 - 1.22593\,X_2 - 34.04571\,X_3 + 0.020879\,X_1X_2 + 0.379502\,X_1X_3 + 0.436207\,X_2X_3 \quad (2)$$

$$\text{TFC} = 0.005926\,X_1 + 0.072190\,X_2 - 0.706366\,X_3 - 0.000771\,X_1X_2 + 0.007356\,X_1X_3 + 0.007015\,X_2X_3 \quad (3)$$

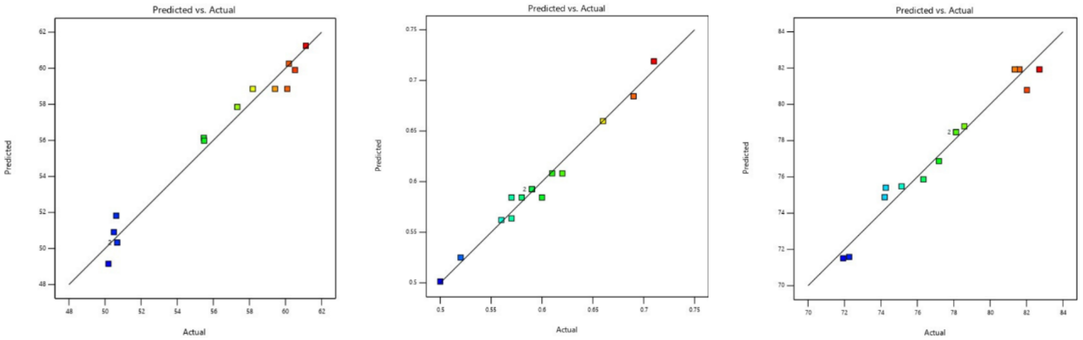
$$\text{DPPH} = 0.784669\,X_1 - 2.64769\,X_2 - 60.10575\,X_3 + 0.038558\,X_1X_2 + 0.633404\,X_1X_3 + 0.708537\,X_2X_3 \quad (4)$$

The analysis was conducted by examining the normal probability plots of residuals, shown in Figure 2(i) for TPC, Figure 2(ii) for TFC, and Figure 2(iii) for DPPH. The residuals were normally distributed along a straight line, indicating statistical insignificance. Consequently, the proposed model was deemed appropriate, with no violations of the assumptions of constant variance or independence. Figures 3(i), 3(ii), and 3(iii) illustrate a strong correlation between the predicted and actual values for TPC, TFC, and DPPH, respectively, demonstrating the effectiveness of the model in prediction. Additionally, the R^2 values close to unity further validate the accuracy of the model equations. Figures 4(i), 4(ii), and 4(iii) depict the relationship between residuals and experimental runs for TPC, TFC, and DPPH, respectively. The red lines represent the range of SD (±4.2989) for the total experimental runs, with all data points falling within this range, confirming the acceptability and significance of the model, with no outliers identified.



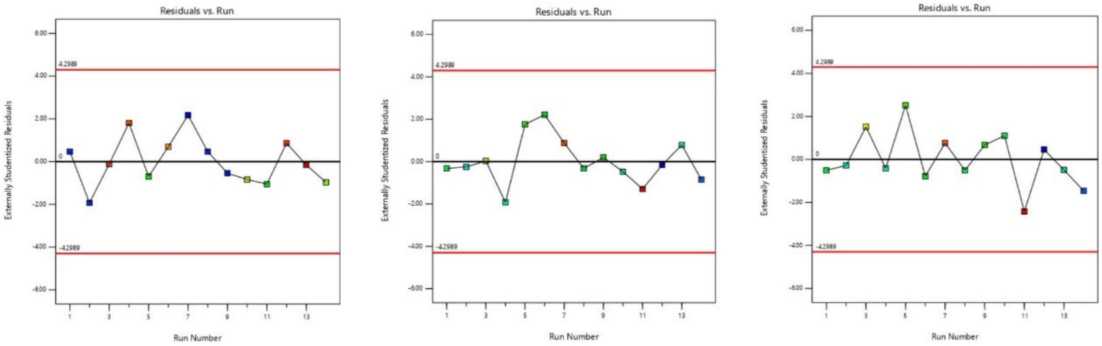
(i) (ii) (iii)

Figure 2. Normal probability plots of residuals for (i) TPC, (ii) TFC, and (iii) DPPH



(i) (ii) (iii)

Figure 3. Predicted versus actual values for (i) TPC, (ii) TFC, and (iii) DPPH



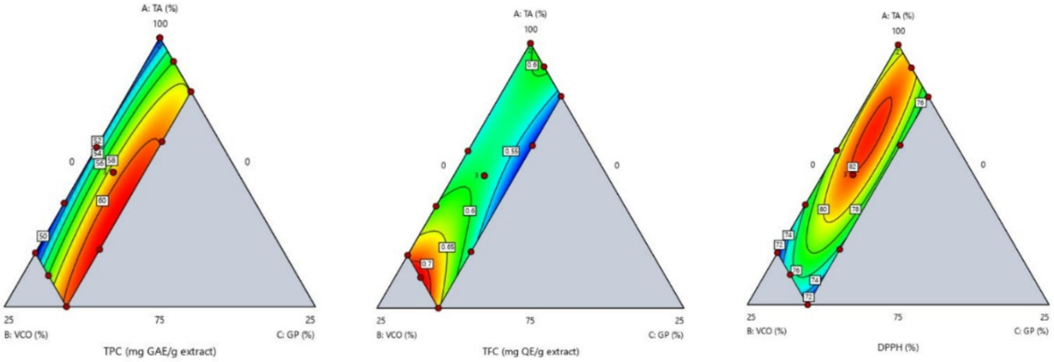
(i) (ii) (iii)

Figure 4. Residuals plotted against experimental runs for (i) TPC, (ii) TFC, and (iii) DPPH

The contour plot in Figure 5(i) illustrates the effect on TPC, showing a high concentration in the lower right area, highlighted in red, within the defined component ranges (75% < X_1 < 100%, 0% < X_2 < 20%, and 0 < X_3 < 5%), forming a parallelogram shape. The highest TPC was observed in F13 (80.33% TA, 14.67% VCO, 5% GP), while the lowest was in F7 (80% TA, 20% VCO, 0% GP), underscoring the impact of GP on TPC levels. TA contains various phenolic compounds, including flavonoids, coumarins, lignans, sterols, steroidal alkaloids, steroidal saponins, and terpenes [17]. Rahman *et al*. [7] reported that the TPC in TA ranged from 2.29 to 6.01 mg GAE/g of dry extract, suggesting the presence of diverse phenolic compounds. The inclusion of a small amount of GP in the formulation significantly impacted TPC, consistent with the findings of Cavalcanti *et al*. [18], who observed an increase in TPC with the addition of dried GP in solvent mixtures. A comprehensive review on the use of garlic highlighted its ability to elevate phenolic compounds, as it contains various bioactive compounds, particularly organosulphur and phenolic compounds, which, when incorporated as an ingredient, could enhance TPC [19].

The TFC contour plot in Figure 5(ii) indicates higher concentrations in the lower centre region, highlighted in red. F12 (90.34% TA, 4.66% VCO, 5% GP) achieved the lowest TFC, while F11 (77.89% TA, 20% VCO, 2.11% GP) exhibited the highest TFC. VCO played a significant role in determining both the highest and lowest TFC levels due to its flavonoid compounds, such as quercetin-3-O-rutinoside and quercetin-3-O-glucosyl-rutinoside, which are commonly found in Solanaceae plants like TA and potatoes [20]. Recent studies have emphasised the contribution of VCO flavonoids to the antioxidant properties of plant extracts [21,22], enhancing TFC in TA despite its low percentage in the formulation. This is further supported by the use of mangosteen pericarp combined with VCO, yielding high TFC through optimisation in the range of 24.72 ± 2.53–82.72 ± 4.87 mg RE/100 g [23]. Hence, the integration of VCO as part of the ingredient provides a flavonoid-rich component that contributes to the overall nutritional value of the formulation.

Figure 5(iii) illustrates the DPPH contour plot, showing higher antioxidant capacity levels in the upper centre, marked by red shading. F10 (87.50% TA, 10% VCO, 2.50% GP) achieved the highest DPPH proportion, while F7 (80% TA, 20% VCO, 0% GP) recorded the lowest. Antioxidant capacity in TA is influenced by ingredient quantities and various compounds. The presence of TPC and TFC significantly impacts antioxidant capacity, with fruit maturity also playing a role in *Solanum melongena*, where a slight reduction in antioxidant capacity occurs upon ripening [24]. In VCO, antioxidant capacity is enhanced by unsaponifiable compounds such as polyphenols and tocotrienols [25]. Medium-chain fatty acids in VCO further contribute to its antioxidant capacity by reducing oxidative stress and supporting the stability of bioactive compounds [26]. In addition, the phenolic compounds in VCO enhance its antioxidant potential through mechanisms involving the number of hydroxyl groups bonded to the aromatic ring, their bonding sites, and the mutual positions of hydroxyl groups on the aromatic ring [23].



(i) (ii) (iii)

Figure 5. Contour plots of TAS for (i) TPC, (ii) TFC, and (iii) DPPH

To evaluate the predictive accuracy of the model, TPC, TFC, and DPPH values were determined using the optimal formulation recommended by the model. Table 4 shows a strong agreement between predicted and actual values: TPC (predicted: 53.11 mg GAE/g extract, actual: 54.38 ± 0.04 mg GAE/g extract), TFC (predicted: 0.60 mg QE/g extract, actual: 0.65 ± 0.02 mg QE/g extract), and DPPH (predicted: 80.35%, actual: 82.71 ± 0.03%). The percentage errors for TPC (2.34%), TFC (7.69%), and DPPH (2.85%) were all below 10%, confirming the reliability of the data. The optimal TAS formulation consisted of 98.46% TA, 0.73% VCO, and 0.81% GP.

Table 4. Verification of the optimal conditions for the TAS formulation

Components		TA (%)	98.46
		VCO (%)	0.73
		GP (%)	0.81
Responses	Predicted value	TPC (mg GAE/g extract)	53.11
		TFC (mg QE/g extract)	0.60
		DPPH (%)	80.35
	*Actual value	TPC (mg GAE/g extract)	54.38 ± 0.04
		TFC (mg QE/g extract)	0.65 ± 0.02
		DPPH (%)	82.71 ± 0.03
	Error (%)	TPC	2.34
		TFC	7.69
		DPPH	2.85

*Values represent the mean ± SD from three replicates.

Physical Properties of the Optimal TAS Formulation

According to Table 5, the optimal TAS formulation has a pH of 4.40 ± 0.02, indicating slight acidity. Optimal pH levels for sauces are typically below 4.60 to prevent bacterial growth [27]. A study on tomato and pumpkin pulp, as well as their integration, reported a pH range of 4.40 ± 0.06 to 5.23 ± 0.06, with pH value less than 4.40 indicating an acceptable range [28]. Nonetheless, maintaining a pH below 4.6 is recommended for effective preservation and extended shelf life [29]. Specific pH requirements vary depending on the sauce type and ingredients, with lower pH values generally ensuring safety and longevity.

The colour analysis of the optimal TAS formulation showed an L* value of 43.85 ± 0.02, indicating medium brightness. The a* value of 32.47 ± 0.06 suggests a slight red hue, while the b* value of 58.83 ± 0.27 indicates a yellowish tint. These hues are influenced by specific compounds, such as the phenolic compounds found in ripened TA, which contribute to its yellow colour. Flavonoids in various plant species are also known to enhance yellow tones in sauces, including TAS [4]. Hence, the TAS, with a dark orange or amber hue similar to fruit-based purees made from orange, demonstrates its potential to meet consumer expectations and willingness to purchase based on its colour appearance [30].

Water activity (a_w) analysis is crucial for evaluating the shelf life of food products, as it indicates the amount of available water for chemical reactions. The optimal TAS formulation recorded an a_w of 0.85 ± 0.02, reflecting an intermediate level for moist foods. Ahouagi *et al*. [31] reported that tomato and strawberry sauces, along with their complementary formulations, had an $a_w > 0.95$, indicating high susceptibility to microbial contamination and the need for refrigeration. A value closer to one suggests increased susceptibility to spoilage. Generally, an a_w of 0.85 or lower is recommended to prevent the growth of mould, yeast, and bacteria without refrigeration [32]. Some dressings and sauces, which are high in salt, sugar, and oil content, fall into the intermediate moisture food category and require precise control of a_w [27].

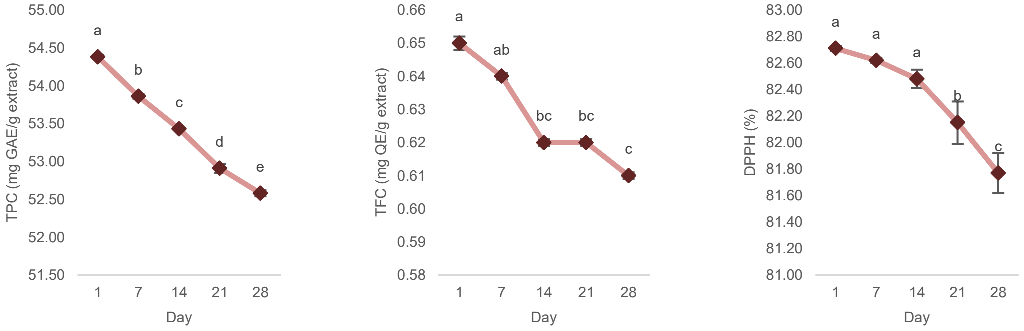
Table 5. Physicochemical parameters of the optimal TAS formulation.

Physicochemical parameters	pH	4.40 ± 0.02
	Colour	L* = 43.85 ± 0.02 a* = 32.47 ± 0.06 b* = 58.83 ± 0.27
	a_w	0.85 ± 0.02

Values represent the mean ± SD from three replicates.

Effect of Storage Stability of the Optimal TAS Formulation

Figures 6(i) for TPC, 6(ii) for TFC, and 6(iii) for DPPH illustrate the impact of storage stability on the optimal TAS formulation, showing a slight decline after 28 days. TPC decreased from 54.38 ± 0.03 to 52.58 ± 0.04 mg GAE/g extract between day 1 and day 28. TFC started at 0.65 ± 0.02 mg QE/g extract on day 1, stabilised at 0.62 ± 0.01 mg QE/g extract from days 14 to 21, and decreased to 0.61 ± 0.01 mg QE/g extract by day 28. Although there are limited studies on the use of sauces, these trends reflect a slight decline in both TPC and TFC over the storage period, consistent with findings by Lin *et al*. [33] and de Oliveira *et al*. [34] on *Momordica charantia* and xique-xique juices, respectively which reported reductions in bioactive compounds during storage. DPPH values were 82.71 ± 0.02% on day 1, 82.62 ± 0.02% on day 7, 82.48 ± 0.07% on day 14, 82.15 ± 0.16% on day 21, and 81.77 ± 0.15% on day 28. The slight decrease in DPPH may be attributed to the reduction in TPC observed during storage. Studies on meat-based sauces enriched with phenolic extracts have shown a decrease in DPPH over time [35]. Research also indicates that DPPH in bioactive sauces varies during storage, with some sauces maintaining stability [36]. Additionally, studies on stew sauces mixed with *Smilax china* extract found no significant variation in DPPH over a 5-week storage period [37]. Therefore, the effect of storage stability on DPPH in sauces depends on their specific composition and storage conditions.



(i) (ii) (iii)

Figure 6. Effect of storage stability on (i) TPC, (ii) TFC, and (iii) DPPH of the optimal TAS formulation. Values represent the mean ± SD from three replicates. Different letters within a line indicate significant differences (one-way ANOVA, Tukey's HSD test, $p < 0.05$)

Percentage losses at room temperature were 3.31% for TPC, 6.15% for TFC, and 1.14% for DPPH, likely due to polymerisation and oxidation during storage. Rodriguez-Amaya and Shahidi [38] emphasises that the oxidation of unsaturated fatty acids in oils can lead to flavour deterioration and nutrient loss. Benjamin *et al*. [39] highlight the role of temperature in accelerating the oxidation of phenolic compounds, thereby reducing their therapeutic properties. Koontz [40] discusses how light exposure can polymerise monomeric compounds in cosmetics, advocating for the use of light-protective packaging. The observed decline in phenolics, flavonoids, and antioxidant properties over the storage period emphasises the need for strategies to enhance stability. Potential approaches include incorporating the TAS formulation with natural preservatives, such as lycopene-enhanced carriers (e.g., antibodies and antimicrobial agents), which have been shown to retard oxidation, degradation, and isomerisation [41,42]. Additionally, packaging materials like polyvinyl chloride (PVC) can help preserve the bioactive properties and sensory attributes of the developed food product for up to 30 days of storage [36].

Limitations and Future Research

This study acknowledges several limitations that could affect the interpretation of its findings. The research was conducted under controlled laboratory conditions, which may not fully replicate real-world storage and environmental variations, potentially limiting the generalisability of the results. As mentioned regarding the incorporation of natural preservatives and advanced packaging materials, other future studies should focus on conducting detailed sensory analysis to evaluate the taste, texture, aroma, and overall acceptability of the TAS formulation. Additionally, investigating consumer acceptance will provide valuable insights into preferences, marketability, and potential areas for improvement to meet consumer expectations. A thorough cost reduction analysis should also be performed to identify strategies for lowering production costs without compromising the quality and stability of the formulation. These efforts will collectively contribute to optimising the stability of the TAS formulation while ensuring the prolonged retention of its antioxidant properties, enhancing its feasibility as a sustainable and consumer-friendly product.

Conclusions

This study employed a quadratic model using a D-optimal mixture design to optimise TAS formulations. The selected formulations incorporated significant proportions of TA, VCO, and GP, achieving optimal levels of TPC, TFC, and DPPH, with favourable physicochemical properties aligned with established sauce development protocols. The formulation meets consumer demand for healthier food products by using natural ingredients with high antioxidant properties, enhancing its nutritional value. Its extended shelf life and optimised production process further support sustainable food practices by reducing waste and promoting resource efficiency. Although a slight decline in quality was observed over 28 days of storage, the optimal TAS formulation demonstrates potential for commercial viability as a functional food product with appropriate natural preservatives and packaging materials. Further studies should explore the incorporation of natural preservatives, advanced packaging materials, sensory analysis, consumer acceptance, and cost reduction optimisation to enhance its shelf life, stability, market appeal, and accessibility.

Conflicts of Interest

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

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