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Optimization of Pomegranate and Sweet Orange Mixed Juice for Quality Wine Preparation Using Response Surface Methodology

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Abstract

Pomegranate and sweet orange juice have been rigorously examined in the present study as a substrate in the context of distinctive wine production research. An optimization investigation was conducted utilizing the Box-Behnken Response Surface Method (RSM) design. The influences of fermentation parameters, including the ratio of sweet orange in the mixed fruit juice must (SW), fermentation temperature (TF), inoculum size (IS), and ˚Brix (BX), on total phenolic concentration (TPC), antioxidant activity (AA), total anthocyanin concentration (ACN), and ascorbic acid equivalent (AAE) were systematically assessed. The findings of the study indicated that a 20% proportion of sweet oranges, with a ˚Brix of 23, a fermentation temperature of 26˚C, and an inoculum size of 10%, constituted the optimal conditions for the production of high-quality pomegranate-sweet orange mixed fruit juice wine. The results obtained underscore the potential of sweet orange fruit in the advancement of premium pomegranate-sweet orange wine.

Keywords

Pomegranate, Sweet Orange, RSM, Fruit Juice, Wine

1. Introduction

Fruits constitute a fundamental element of a nutritionally well-adjusted diet and represent a significant source of various essential nutrients, including minerals,

vitamins, and antioxidants such as polyphenols. The phytochemicals present in fruits are currently the subject of extensive investigation concerning their prospective health benefits. The burgeoning interest in nutritionally beneficial foods has catalyzed the exploration of technical innovations that may influence or enhance the antioxidant content within wine [1]. Wide-ranging studies have established that restrained wine ingesting is associated with a myriad of health benefits, which predominantly encompass cardioprotective, anticancer, antimutagenic, antiproliferative, vasodilatory, and antimicrobial effects [2]. A majority of the investigations have focused on red wine derived from grapes. Prior research findings have indicated that red wine exhibits more pronounced health-promoting effects compared to other alcoholic beverages, primarily attributable to its more abundant phenolic compound content [3]. Consequently, there has been a heightened interest in the nutraceutical properties of wines. Wines are recognized for their antioxidative capabilities, which are primarily ascribed to the occurrence of phenolic compounds. According to the annual report issued by the National Pomegranate Research Institute, Maharashtra, India [4], the overall quality of the fruit from the pomegranate variety "Bhagwa" was found to be significantly superior to that of the "Ganesh" variety when harvested in February. The nucellar variety of sweet orange, commonly cultivated in Maharashtra (India) within the Aurangabad region, exhibits a higher total soluble solids (TSS) content compared to the Sathgudi variety; the nucellar variety was selected for the experimental procedure [5]. Beyond its nutritional merits, the pomegranate fruit demonstrates efficacy against a diverse array of diseases, including cancer, cardiac conditions, diabetes, male infertility, Alzheimer's disease, aging, and HIV-AIDS. Numerous researchers have identified antioxidant, anticarcinogenic, and anti-inflammatory attributes [6]. Sweet orange, botanically designated as Citrus sinensis (L.), is commonly referred to as "Mosambi" within the Indian subcontinent and utilized in the treatment of various diseases. Due to the presence of distinct flavonoid compounds, it has been substantiated for its anti-cancer, anti-gastroesophageal reflux, anti-diabetic, and anti-ulcer effects, as well as in the mitigation of arteriosclerosis, kidney stones, stomach ulcers, also in the reduction of cholesterol levels and high blood pressure, thereby promoting human health. Additionally, the fruit is acknowledged for its role in combating urinary disorders, and various dermatological conditions, and in enhancing immunity and facilitating weight balancing [7].

According to the global market analysis, the worldwide wine industry is projected to expand at a compound yearly growth rate (CAGR) of 5.8% throughout the estimated period from 2020 to 2032 [8]. While grape wines maintain a higher popularity compared to fruit wines, the consumer demand for novel wine varieties is incentivizing producers to continuously innovate and develop new fruit wine flavors, thereby stimulating market growth. The principal factors driving the increased consumption of various fruit wines include the health advantages associated with these wines, aesthetic and brand appeal, delightful fragrance, compatibility with diverse cuisines, and a refreshing palate. The implementation of novel,

cost-effective technologies for the production of fruit wines is likely to further enhance the global fruit wine market. Nonetheless, challenges such as acid stability and the need for adjustments in sugar concentrations may pose constraints to the global fruit-wine sector. Furthermore, fruit wine producers may encounter challenges similar to those faced by traditional wine manufacturers, including the cultivation of high-quality fruit, the critical nature of the fermentation process, and necessary pH evaluations. The Asia-Pacific region is expected to experience significant growth within the forecast period, attributed to an increase in the number of wine-producing enterprises in nations such as China, India, and Japan. Given the rising consumer preference for celebratory wines, the sparkling wine segment is anticipated to experience accelerated growth relative to other wine categories. Consequently, efforts have been directed towards the advancement of a fermented beverage derived from pomegranate juice, as pomegranate wine has been identified as lacking in flavor quality attributes; this initiative aims to enhance consumer preference, marketability, and the nutritional profile of pomegranate wine [9]. Over the past decade, a multitude of studies have been conducted that investigate the health benefits of pomegranate, which have elucidated its antioxidant, anticarcinogenic, and anti-inflammatory properties, which demonstrating its potential efficacy in the dealing with prevention of cancer, cardiovascular ailments, diabetes, infant brain ischemia, male infertility, and Alzheimer's disease [10]. In light of these health benefits associated with pomegranate and sweet orange juice, it is imperative to preserve the phenolic compounds through fermentation, thereby offering enhanced health benefits.

During the vinification process, variables such as pH, temperature, inoculum size, and degrees Brix constitute critical parameters that necessitate optimization to ensure the production of quality wine. This study represents a pioneering effort to amalgamate pomegranate and sweet orange juice to preserve the health-promoting phenolic compounds inherent to both juices; notably, sweet orange, which is frequently underutilized in enology due to its elevated pectin content, is utilized alongside pomegranate juice to create an innovative wine with prospective health advantages. A variety of statistical methodologies have been employed to optimize process variables aimed at enhancing product yield. Response Surface Methodology (RSM) is among the most prevalent approaches utilized in bioprocess technology [11]. This technique minimizes the requisite number of observations, thereby yielding results that are both more precise and accurate. Our previous research on wine production indicated that the blended fruit wine composed of Pomegranate and sweet orange was highly regarded concerning taste, flavor, aroma, and various nutritional parameters [12]. The current study intends to optimize the fermentation conditions to attain high-quality wine from the mixed juice of pomegranate and sweet orange, which is characterized by elevated phenolic content, antioxidant activity, anthocyanin, and ascorbic acid levels. The RSM statistical approach is employed to assess the collective effects of independent variables like temperature, the pomegranate to sweet orange ratio, and inocu-

lum size Brix, on phenolic content, antioxidant properties, anthocyanin, and ascorbic acid concentrations. The design of the RSM was informed by the findings derived from our preliminary investigations [13].

2. Method

2.1. Fruit Collection and Juice Extraction

The entirely ripened Pomegranate (*Punica granatum* L.) specimens of the variety Bhagwa were cultivated during the designated season of Hastabahar, renowned for producing high-quality mature fruits, and were subsequently harvested in March 2018 from the agricultural premises of the ICAR-National Research Centre on Pomegranate, Solapur, India. The sweet orange fruit, botanically known as Citrus sinensis L., and locally named as Mosambi of the nucellar variety, was secured from a farmer's field situated in Aurangabad, India. Both the pomegranate and sweet orange were simultaneously ecstatic to the laboratory and stored at a temperature of 5˚C until they were utilized for experimental trials. The fruits underwent a washing process utilizing chlorine water (200 ppm sodium hypochlorite) and were then bisected. The juice was extracted by commissioning a hydraulic press (manufactured by Johnston) from the halved fruits. The extracted juice was upheld at a temperature of 5˚C for 24 hours to facilitate sedimentation and was subsequently filtered through muslin cloth. All reagents necessary for the experiments were procured from Hi Media, Mumbai, India.

2.2. Inoculum Preparation

The *Saccharomyces cerevisiae* NCIM 3218 wine yeast strain was secured from the National Collection of Industrial Microorganisms (NCIM) located at NCL, Pune, India, and was preserved on malt extract, peptone, yeast extract, and dextrose (MPYD) agar slants, framed with malt extract at 0.3%, peptone at 0.5%, yeast extract at 0.3%, dextrose at 2%, and agar at 2%, maintained at a temperature of 4˚C. Before the fermentation process, the yeast cells were revitalized by inoculating the slant culture onto a sterile MPYD agar medium, followed by incubation at 25˚C for 24 hours. Subsequently, the activated cells were transferred to 100 ml of pasteurized pomegranate and sweet orange juice in a ratio of 70:30, respectively, and incubated for 48 hours at 25˚C until the cell density reached 3×10^6 cfu/ml.

2.3. Juice Fermentation

Sweet orange and pomegranate juice were combined in varying proportions and adjusted to different ˚Brix levels, Afterward, it was pasteurized at 80˚C for 10 minutes. Juice with a pH of 3.5 was inoculated with a 48-hour-old inoculum in various proportions of 10%, 20%, and 30%, with total soluble solids (TSS) at 16, 20, and 24 ˚Brix, and incubated at different temperatures of 25˚C, 30˚C, and 35˚C until the TSS reduction reached at a stable value. The fermented broths were supplemented with 0.1% bentonite and sifted utilizing Whatman filter paper no. 4. The prepared wine samples suffered by pasteurization at 50˚C for 10 minutes. The

pasteurized wine samples were subsequently bottled and stored at a temperature of 5˚C for aging. All mixed juice wine samples were analyzed for bioactive components, including total phenolic concentration (TPC), antioxidant activity (AA), anthocyanin content (TAC), and ascorbic acid concentration (AA).

2.4. Response Surface Optimization Experimental Design

A multivariate statistical optimization technique, specifically response surface methodology (RSM), was employed to explore the influence of four independent fermentation constraints on the fluctuations of bioactive compounds present in wine during the process of alcoholic fermentation. A Box-Behnken design was chosen to establish the optimal conditions for mixed fruit wine fermentation. In light of our preliminary investigation and reference to prior optimization studies concerning various fruit wines, the values of the independent variables like sweet orange percentage, inoculum, ˚Brix, and temperature were encoded utilizing the following equation.

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i}, \ i = 1, 2, 3, 4, \cdots, K \qquad \text{Equation (1)}$$

where x_i is the coded value of an independent variable i; X_i is the uncoded real value of an independent variable; X_{cp} is the value of X_i at the center point, and ΔX_i is the step change between the levels 0 and 1. The values of Sweet orange % (A), inoculum (B), TSS (C), and Temperature(D) are listed in **Table 1**.

Y =¼ b0 þX3 i¼1 biXi þX3 i¼1 biiX2 i þX2 i¼1 X3 j¼iþ1 bijXiXj ð1Þ
Equation (2)

where Y is the predicted response, b0, bi, bii, and bij are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and Xi and Xj are the independent variables [14]. The regression coefficients for the intercept, quadratic, linearity, and interaction of the model, and Xi and Xj represent the independent variables

A multifactorial analysis of variance (ANOVA) was employed to assess the robustness of the models formulated utilizing the statistical software suite Design-Expert version 7 (Stat-Ease Inc., Minneapolis, MN, USA). Furthermore, the adequacy of the models was scrutinized through numerous metrics, including the Absolute Average Deviation (AAD), R-squared (R^2), adjusted R-squared (adj-R^2), predicted R-squared (pre-R^2), suitability of precision, lack of fit, and the coefficient of variation (C.V.). The coefficients of determination (R^2) alongside the absolute average deviation (AAD) were instrumental in evaluating the comprehensive prognostic efficacy of the models constructed with this software. The statistical significance of the linear interactions involving sweet orange percentage (A), inoculum (B), Brix (C), and temperature (D), as well as their pairwise interactions such as sweet orange percentage with inoculum (AB), sweet orange percentage with Brix (AC), sweet orange percentage with temperature (AD), inoculum with total soluble solids (BC), inoculum with temperature (BD), and total soluble solids

with temperature (CD), were examined through the application of p-values ($p < 0.05$, $p < 0.001$, and $p < 0.0001$); additionally, quadratic effects concerning sweet orange percentage (A^2), inoculum (B^2), Brix (C^2), and temperature (D^2) on total phenolic concentration (TPC), antioxidant activity (AA), total anthocyanin concentration (ANC), and ascorbic acid content (AAE) were also evaluated. The influence of the independent variables, specifically inoculum, temperature, Brix, and juice proportion, was assessed across three varying levels: 10%, 20%, and 30% inoculum; incubation temperatures of 25˚C, 30˚C, and 35˚C; Brix values of 16, 20, and 24; and sweet orange concentrations of 20%, 30%, and 40% in pomegranate juice throughout the optimization procedure. A total of twenty-eight experimental configurations were generated utilizing the Design Expert® software (Version 7, Stat-Ease Inc., Minneapolis, USA) to analyze the resultant response patterns and to draw foundational conclusions [15]. The methodology included four experimental trials conducted at the center point to ascertain the reproducibility of the results.

2.5. Bioactive Compound Assessment

The quantification of ascorbic acid in fruit juices was executed by utilizing a methodology predicated on the dropping down of 2,6-dichlorophenol indophenol by L-ascorbic acid, as delineated by previous studies [16]. The entire concentration of phenolic compounds was ascertained through the Folin-Ciocalteu (FC) colorimetric assay at a wavelength of 765 nm, as articulated by previous studies [17]. The results were represented in terms of milligrams of Gallic acid equivalent (GAE) per liter of the analyzed sample. The total anthocyanin concentrations were evaluated by employing a pH-differential procedure utilizing two distinct buffer systems, specifically a 0.025 M potassium chloride buffer at pH 1.0 and a 0.4 M sodium acetate buffer at pH 4.5 while employing spectrophotometric techniques at wavelengths of 510 and 700 nm, as described by preceding investigations [18]. The assessment of antioxidant activity was conducted via the FRAP assay, as reported by standard method [19], with minor amendments applied.

2.6. Statistical Analysis

All experimentations and assays were carried out in triplicate, and results were expressed as a mean of three readings. Design Expert® software (Version 7, Stat. EaseInc., Minneapolis, USA) was castoff for the statistical analysis.

3. Results and discussion

3.1. Suitable for the Response Surface Models

To investigate the statistical implication of the contributing aspects and the corresponding model, analysis of variance (ANOVA) and relapsing analysis were executed, as delineated in **Table 2** and **Table 3**. Additionally, **Table 4** illustrates the estimated regression coefficients associated with the quadratic polynomial models for the response variables, accompanied by the relevant coefficients of determina-

tion (R^2). The pre-R^2, competence of accuracy, PRESS, and adjusted R^2 are elaborated upon in Table 4. In assessing the model's accuracy in forecasting the pertinent replies of the fermentation process, the lack of fit has been identified as a critical predictor of response. A model exhibiting an insignificant lack of fit, as indicated by a high probability value, suggests a reliable representation of the experimental data, whereas a significant lack of fit, denoted by a low probability value, was excluded from consideration. As evidenced in Table 2 and Table 3, none of the response predictors revealed a significant p-value for the selected variables, thereby indicating that all models do not exhibit a significant lack of fit. In light of these findings, the models were deemed accurate for predicting the pertinent responses of the fermentation process. As presented in Table 3, the developed model Equations (3), (4), (5), and (6) yield F values of 5.58, 4.29, 12.49, and 26.31, respectively, which signifies that the models are highly significant ($p < 0.01$) with only a 0.18%, 0.63%, 0.01%, and 0.01% probability that such large F values could arise due to random noise. This observation underscores that the established model equations are reasonably precise in forecasting the quality of predictive responses in the context of the fermentation optimization study. The coefficient of determination (R^2) quantifies the proportion of variation in the response attributed to the model as opposed to random error, reflecting the accuracy of the predicted values about actual values, with a threshold of not less than 80% necessary to substantiate the model's accuracy. The experimental model achieves a satisfactory fit to the actual data when R^2 values approach unity [20]. The R^2 values obtained for all responses are also delineated in Table 4, with all models exhibiting R^2 values exceeding 80% [21]. Given that the R^2 index serves as a metric for the extent of variability reduction in response attributable to the inclusion of predictor variables in the model, it is noteworthy that the addition of a variable to the model will invariably enhance R^2, irrespective of the statistical significance of the supplementary variable. In the present study, the non-significant lack of fit value, along with the adjusted and predicted R^2 values approximating unity, corroborates the model's validity.

3.2. Final Equation in Terms of Coded Factors

$$\begin{aligned}\text{Total phenolics} = {} & +2316.69 - 298.33A + 81.94B - 1.22C - 241.06D \\ & + 86.33AB + 27.33AC + 264.67AD - 252.33BC \\ & - 381.50BD + 220.00CD - 172.21A^2 - 265.29B^2 \\ & - 68.21C^2 - 304.29D^2\end{aligned} \quad \text{Equation (3)}$$

$$\begin{aligned}\text{Antioxidant activity} = {} & +15.33 - 1.44A + 0.44B - 0.41C - 3.54D - 0.23AB \\ & + 0.22AC + 0.65AD + 0.18BC + 0.76BD + 1.37CD \\ & - 3.84A^2 - 1.35B^2 + 0.28C^2 + 4.55D^2\end{aligned} \quad \text{Equation (4)}$$

$$\begin{aligned}\text{Anthocyanin} = {} & +14.90 - 1.68A - 1.37B + 1.38C + 1.89D - 1.82AB \\ & + 0.80AC - 0.76AD - 0.33BC - 0.90BD + 0.33CD \\ & + 1.21A^2 + 0.69B^2 - 0.72C^2 - 3.24D^2\end{aligned} \quad \text{Equation (5)}$$

$$\text{Ascorbic acid} = +14.93 + 0.17A + 0.100B - 0.26C - 3.02D - 0.32AB - 0.88AC - 0.3AD + 0.000BC + 0.48BD - 1.50CD - 0.43A^2 - 0.62B^2 - 0.83C^2 - 3.35D^2 \quad \text{Equation (6)}$$

where A, Sweet orange, B Inoculum, C Brix, D Temperature

3.3. Fermentation Conditions Effect on Responses

The linear, interactive, and quadratic impacts of independent fermentation process variables of the Fermentation process on the total phenolic content, antioxidant activity, anthocyanin levels, and ascorbic acid concentration of the resultant wine samples were assessed through the implication of each coefficient at p values (refer to Table 2 and Table 3). In a similar vein, the interdependencies among the examined variables were evaluated based on p values. Model terms that exhibited a non-significant effect ($p > 0.1$) on the response variables, which could potentially compromise the integrity of the model equations (Equations (3), (4), (5), and (6)), were subsequently eliminated. Response surface and contour plots, illustrated in Figures 1-3, were produced utilizing Design-Expert software to elucidate the synergistic effects of two factors on any given response. The visualization of each fitted model was executed as a function of two independent variables while maintaining the other variable at its central value. As evidenced in Table 2 and Table 3, the interaction effect of fermentation temperature, Brix, inoculum, and juice proportion demonstrated a statistically insignificant effect ($p > 0.1$) on total phenolic content (TPC), anthocyanin levels, ascorbic acid concentration, and antioxidant activity.

Table 1. Experimental design for process variables and values of experimental data for optimization of pomegranate: sweet orange mix juice fermentation studies.

RSM RUN	Sweet orange % (A)	Inoculum % (B)	Brix (C)	Temperature ˚C (D)	Total Phenolics mg/L GAE	Antioxidants mg/L AAE	Anthocyanin mg/L cyanidin	Ascorbic acid mg/100 ml AAE
1	30	20	16	25	2488.00 ± 6.9	12.34 ± 16	9.60 ± 5.3	0.866 ± 0.05
2	30	20	24	25	1765.33 ± 23.1	1121.67 ± 11.21	9.91 ± 5.5	0.866 ± 0.05
3	20	20	20	25	1786.67 ± 23.1	1121.67 ± 11.22	14.23 ± 8.00	0.866 ± 0.05
4	20	20	20	25	2217.33 ± 12.9	1050.33 ± 10.50	10.24 ± 5.85	0.8 ± 0.51
5	30	10	20	25	1254.67 ± 37.0	1151 ± 11.51	10.63 ± 6.11	0.833 ± 0.05
6	30	30	20	25	2578.67 ± 12.2	1150 ± 11.50	11.96 ± 6.88	0.9 ± 0.57
7	20	10	20	30	2176.00 ± 6.9	674 ± 6.74	19.93 ± 11.60	0.76 ± 0.05
8	40	10	20	30	1753.33 ± 23.1	591 ± 5.91	9.83 ± 5.65	0.733 ± 0.05
9	40	30	20	30	1890.67 ± 4.6	690.67 ± 6.91	18.25 ± 10.42	0.7 ± 0.44
10	20	30	20	30	2313.33 ± 23.1	5588.33 ± 5.5.8	10.82 ± 6.25	0.766 ± 0.05
11	30	10	16	30	1733.33 ± 32.3	823.67 ± 8.24	12.36 ± 7.01	0.7 ± 0.44
12	30	30	16	30	2281.33 ± 12.2	352.33 ± 3.52	11.81 ± 7.02	0.7 ± 0.44
13	30	10	24	30	2380.00 ± 0.0	572.33 ± 5.72	19.29 ± 11.20	0.766 ± 0.05

Continued

14	30	30	24	30	1918.67 ± 23.1	642.67 ± 6.43	15.92 ± 9.24	0.733 ± 0.05
15	40	20	16	30	2353.33 ± 16.1	635 ± 6.35	11.02 ± 6.47	0.766 ± 0.05
16	40	20	16	30	1698.67 ± 18.5	644.33 ± 6.44	11.62 ± 6.77	0.866 ± 0.05
17	20	20	24	30	2750.67 ± 18.5	561.67 ± 5.62	17.38 ± 9.74	0.966 ± 0.05
18	20	20	24	30	1805.33 ± 18.5	465.67 ± 4.66	9.44 ± 5.16	0.7 ± 0.44
19	30	20	20	30	2353.33 ± 16.2	694.67 ± 6.95	12.15 ± 6.97	0.766 ± 0.05
20	30	20	20	30	2082.67±18.5	859.67 ± 8.6	12.20 ± 6.98	0.866 ± 0.05
21	30	20	20	30	2565.33 ± 18.5	592.33 ± 5.92	14.32 ± 8.28	1.266 ± 0.05
22	30	20	20	30	1712 ± 20.8	622.33 ± 6.22	14.53 ± 8.52	0.8 ± 0.51
23	30	20	16	35	1041.33 ± 23.1	933.67 ± 9.23	12.26 ± 7.17	0.766 ± 0.05
24	30	20	24	35	1589 ± 34.6	1132.33 ± 11.32	10.48 ± 5.83	0.866 ± 0.05
25	40	20	20	35	1324 ± 00	668.0 ± 6.68	18.27 ± 10.58	0.766 ± 0.05
26	20	20	20	35	1613.33 ± 18.5	476.33 ± 4.76	14.40 ± 8.35	0.966 ± 0.05
27	30	10	20	35	1200 ± 20.5	629.67 ± 6.30	16.34 ± 9.39	0.833 ± 0.05
28	30	30	20	35	1428 ± 00	813.00 ± 8.13	12.35 ± 6.88	1 ± 0.64

[a]Central point (used to determine experimental error, GAE Gallic acid equivalent, AAE Ascorbic acid equivalent).

Table 2. ANOVA evaluation of linear, interaction, and quadratic terms for total phenol, antioxidant activity variables, and coefficients of the model prediction.

	Total Phenolic Content (mg/L GAE)					Antioxidant activity (mg/L AAE)				
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	4340818	14	310058.5	3.077902	0.0251	531.066	14	37.93329	7.018084	0.0006
A-A sweet orange	64533.33	1	64533.33	0.640612	0.4379	10.67853	1	10.67853	1.975649	0.1833
B-B Inoculum	305070.4	1	305070.4	3.028386	0.1054	8.840833	1	8.840833	1.635654	0.2233
C-C Brix	28551.26	1	28551.26	0.283424	0.6034	8.134533	1	8.134533	1.50498	0.2417
D-D Temp	1282276	1	1282276	12.72896	0.0034	165.0208	1	165.0208	30.53071	<0.0001
AB	178647.1	1	178647.1	1.773402	0.2058	0.2209	1	0.2209	0.040869	0.8429
AC	21121.78	1	21121.78	0.209673	0.6546	0.3136	1	0.3136	0.05802	0.8134
AD	4993.778	1	4993.778	0.049572	0.8273	0.5041	1	0.5041	0.093264	0.7649
BC	254688.4	1	254688.4	2.528253	0.1358	1.3225	1	1.3225	0.244677	0.6291
BD	300304	1	300304	2.981071	0.1079	6.4009	1	6.4009	1.184238	0.2963
CD	409600	1	409600	4.066035	0.0649	7.4529	1	7.4529	1.37887	0.2614
A^2	23530.93	1	23530.93	0.233588	0.6369	35.23527	1	35.23527	6.51892	0.0240
B^2	152560.7	1	152560.7	1.514446	0.2403	0.022204	1	0.022204	0.004108	0.9499
C^2	12764.79	1	12764.79	0.126714	0.7276	14.82082	1	14.82082	2.742018	0.1217
D^2	1416930	1	1416930	14.06564	0.0024	213.4277	1	213.4277	39.48652	<0.0001
Residual	1309580	13	100736.9			70.266	13	5.405077		

Continued

Lack of Fit	1138519	10	113851.9	1.996689	0.3099	54.3049	10	5.43049	1.020698	0.5594
Pure Error	171061.1	3	57020.35			15.9611	3	5.320367		
Cor Total	5650399	27				601.332	27			

Temp., DF, and SS represent temperature, degree of freedom, and the sum of squares respectively.

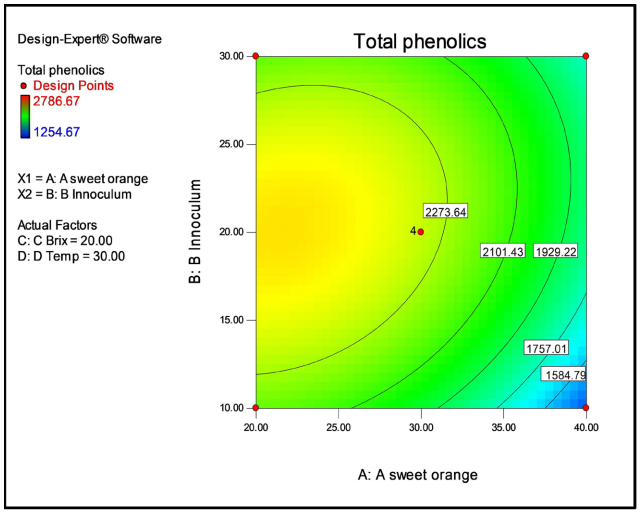
Table 3. ANOVA evaluation of linear, interaction, and quadratic terms for TPC and antioxidant activity.

	Anthocyanin mg/L Cyanidin					Ascorbic acid mg/100 ml AAE)				
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	264.8101	14	18.915	12.48843	<0.0001	192.6994	14	13.76424	26.30755	<0.0001
A-A sweet orange	175.9417	1	175.9417	116.1636	<0.0001	0.3675	1	0.3675	0.702401	0.4171
B-B Inoculum	0.022433	1	0.022433	0.014811	0.9050	0.12	1	0.12	0.229356	0.6400
C-C Brix	2.248013	1	2.248013	1.484227	0.2448	0.800833	1	0.800833	1.53063	0.2379
D-D Temp	2.639734	1	2.639734	1.742857	0.2096	109.2033	1	109.2033	208.7199	<0.0001
AB	1.829553	1	1.829553	1.207943	0.2917	0.4225	1	0.4225	0.807523	0.3852
AC	0.051105	1	0.051105	0.033742	0.8571	3.0625	1	3.0625	5.853345	0.0309
AD	3.900491	1	3.900491	2.575258	0.1326	0.4225	1	0.4225	0.807523	0.3852
BC	0.028868	1	0.028868	0.01906	0.8923	0	1	0	0	1.0000
BD	0.038237	1	0.038237	0.025246	0.8762	0.9025	1	0.9025	1.724945	0.2118
CD	1.807036	1	1.807036	1.193076	0.2945	9	1	9	17.20167	0.0011
A^2	64.0064	1	64.0064	42.25955	<0.0001	1.126667	1	1.126667	2.153394	0.1660
B^2	2.119521	1	2.119521	1.399392	0.2580	2.312604	1	2.312604	4.420072	0.0556
C^2	0.1581	1	0.1581	0.104384	0.7518	4.166667	1	4.166667	7.963734	0.0144
D^2	0.658982	1	0.658982	0.435086	0.5210	67.1676	1	67.1676	128.3772	<0.0001
Residual	19.68983	13	1.514602			6.801667	13	0.523205		
Lack of Fit	18.19352	10	1.819352	3.647676	0.1573	6.534167	10	0.653417	7.328037	0.0640
Pure Error	1.49631	3	0.49877			0.2675	3	0.089167		
Cor Total	284.4999	27				199.5011	27			

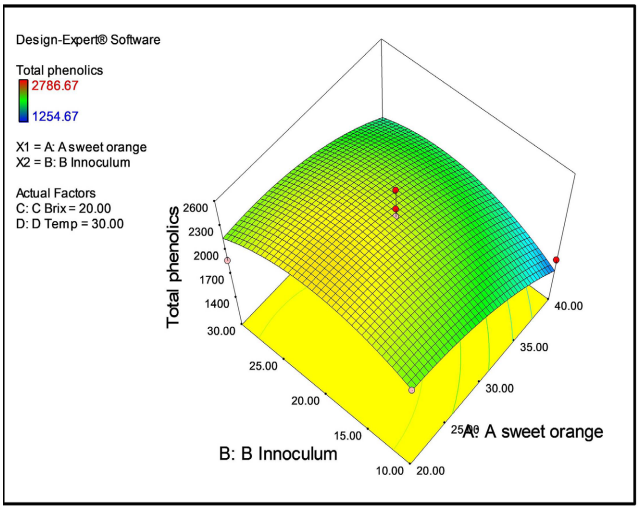
3.4. Total Phenolic Content (TPC)

Three-dimensional response surface plots (**Figures 1(a)-(h)**) were developed conferring to the equations prediction (Equation (3)) about interactions among the different variables, and their corresponding effect on the total phenolic content response variables can be easily made.

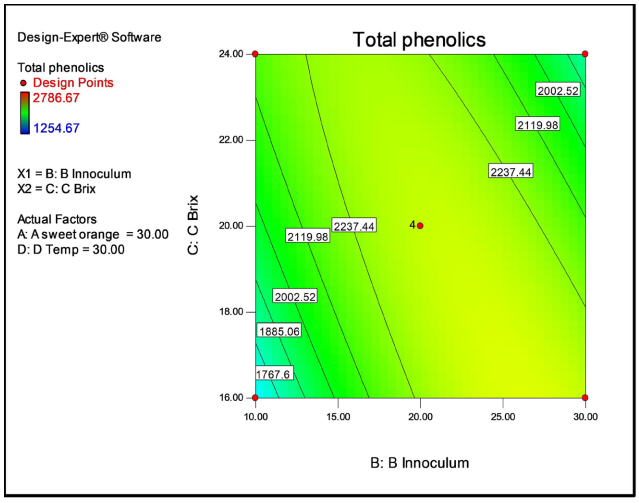
The findings regarding the influence of fermentation parameters presented in **Table 2** suggest that both linear and interaction effects of fermentation temperature and sweet orange fruit juice concentration were statistically significant ($p < 0.1$). Furthermore, the linear and interaction effects of inoculum concentration in


Design-Expert® Software
Total phenolics
Design Points
2786.67
1254.67
X1 = A: A sweet orange
X2 = B: B Innoculum
Actual Factors
C: C Brix = 20.00
D: D Temp = 30.00
Total phenolics
2273.64
2101.43
1929.22
1757.01
1584.79
B: B Innoculum
A: A sweet orange

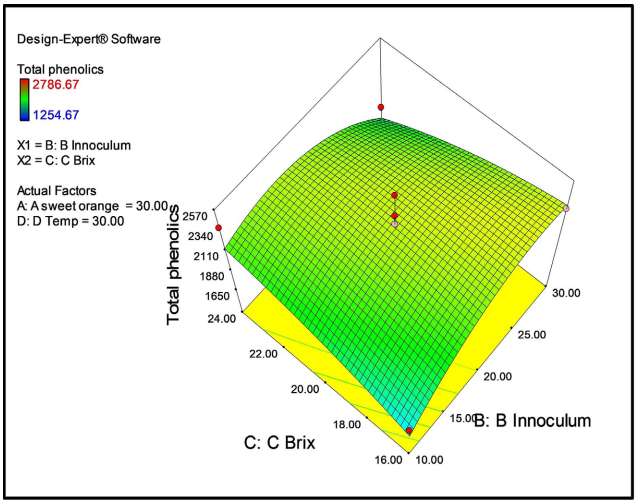
(a)


Design-Expert® Software
Total phenolics
2786.67
1254.67
X1 = A: A sweet orange
X2 = B: B Innoculum
Actual Factors
C: C Brix = 20.00
D: D Temp = 30.00
Total phenolics
B: B Innoculum
A: A sweet orange

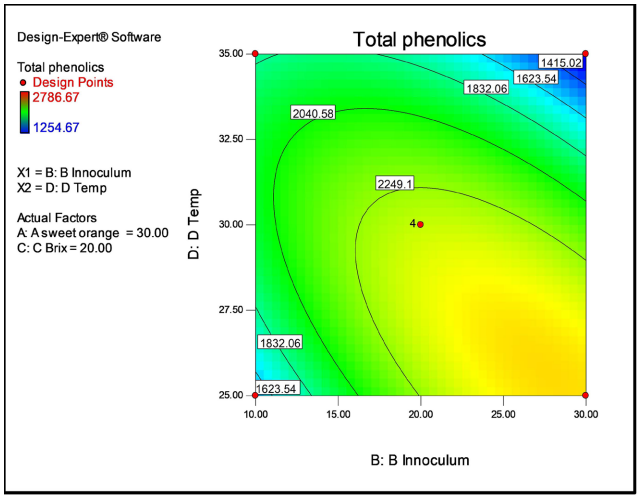
(b)


Design-Expert® Software
Total phenolics
Design Points
2786.67
1254.67
X1 = B: B Innoculum
X2 = C: C Brix
Actual Factors
A: A sweet orange = 30.00
D: D Temp = 30.00
Total phenolics
2237.44
2119.98
2002.52
1885.06
1767.6
C: C Brix
B: B Innoculum

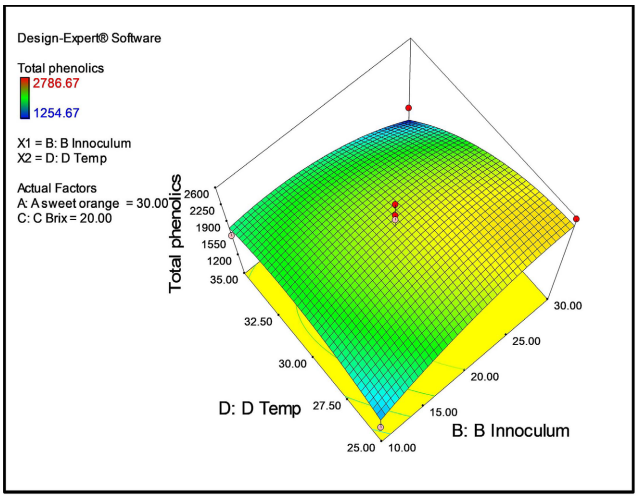
(c)


Design-Expert® Software
Total phenolics
2786.67
1254.67
X1 = B: B Innoculum
X2 = C: C Brix
Actual Factors
A: A sweet orange = 30.00
D: D Temp = 30.00
Total phenolics
C: C Brix
B: B Innoculum

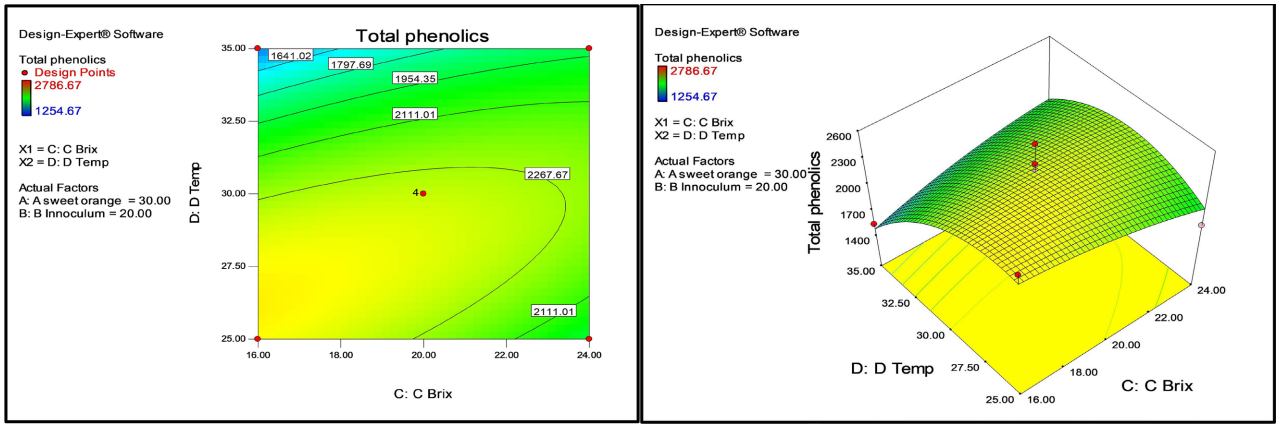
(d)


Design-Expert® Software
Total phenolics
Design Points
2786.67
1254.67
X1 = B: B Innoculum
X2 = D: D Temp
Actual Factors
A: A sweet orange = 30.00
C: C Brix = 20.00
Total phenolics
2249.1
2040.58
1832.06
1623.54
1415.02
D: D Temp
B: B Innoculum

(e)


Design-Expert® Software
Total phenolics
2786.67
1254.67
X1 = B: B Innoculum
X2 = D: D Temp
Actual Factors
A: A sweet orange = 30.00
C: C Brix = 20.00
Total phenolics
D: D Temp
B: B Innoculum

(f)



(g) (h)

Figure 1. (a)-(h) Response surface and counter plots for the effect of sweet orange% %, inoculum, ˚Brix, and Temperature on the phenolic content of the pomegranate-sweet orange wine. TPC: Total phenolic content, temp; temperature. (a) Contour plots for the outcome of sweet orange% and inoculum on TPC concentration; (b) Response surface plot for the effect of sweet orange% and inoculum on TPC concentration; (c) Contour plots for the effect of inoculum and ˚brix on TPC concentration; (d) Response surface plot for the effect of inoculum and ˚brix on TPC concentration; (e) Contour plots for the effect of inoculum and temperature on TPC concentration; (f) Response surface plot for the effect of inoculum and temperature on TPC concentration; (g) Contour plots for the effect of brix and temperature on TPC concentration; (h) Response surface plot for the effect of brix and temperature on TPC concentration.

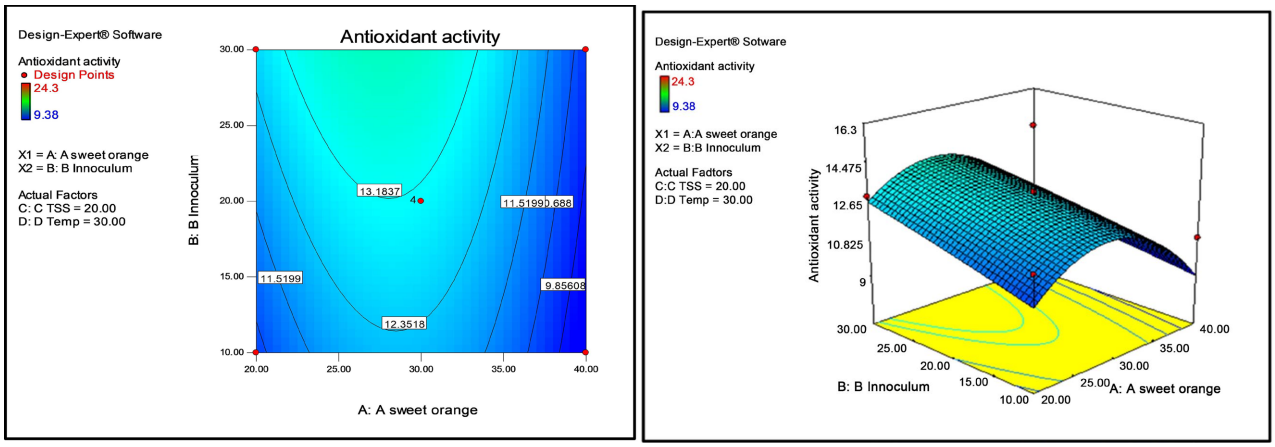
conjunction with fermentation temperature, sweet orange juice concentration, and total soluble solids (TSS) were also found to be significant ($p < 0.1$). A highly significant effect ($p < 0.0001$) attributed to all quadratic terms involved in the fermentation process was noted concerning the total phenol content of the resultant wine. The observed linear effect of fermentation temperature on the enhancement of total phenol concentration can be ascribed to the facilitation of solubility rates and diffusion of phenolic compounds during the fermentation process due to elevated fermentation temperatures. These findings corroborate the research conducted by [22]. The observed diminution in total phenolic content (TPC) at 35˚C may be indicative of a reduced fermentation rate, which is associated with the phenomenon of hot fermentation, ultimately leading to a decrease in the availability of extractable bioactive compounds [23]. Moreover, the significant effects ($p < 0.1$) of the quadratic interaction between inoculum concentration and sweet orange fruit juice suggest that total phenol concentration was enhanced due to the yeast's capacity to convert non-phenolic compounds into phenolic compounds [24]. Additionally, hydrolytic enzymes such as esterases may facilitate the release of soluble or insoluble bound phenolic acids from the cell walls of pomegranate and sweet orange fruit juices through yeast activity [22]. The fermentation process engenders the liberation of microbial enzymes, which consequently yield a more bioavailable form of phytochemicals, including flavonoids, tannins, alkaloids, and phenylpropanoids [25]. Furthermore, the literature also documents the depolymerization of high molecular weight phenolic compounds [26] [27]. **Figure 1(c)** and **Figure 1(d)** illustrate that an increase in TSS corresponds with an elevation in TPC. Typically, as TSS rises, the rate of ethanol production also increases. Phe-

nolic compounds, which exhibit greater solubility in polar organic solvents, benefit from the high solubility of phenols in ethanol, a polar solvent, resulting in elevated concentrations of TPC compounds [28]. Thus, a higher concentration of ethanol enhances solubility. It has been observed that yeast metabolism is significantly enhanced at elevated TSS levels, thereby yielding wines with the highest levels of phenolic compounds during high TSS conditions (Table no. 1, sr. no. 17).

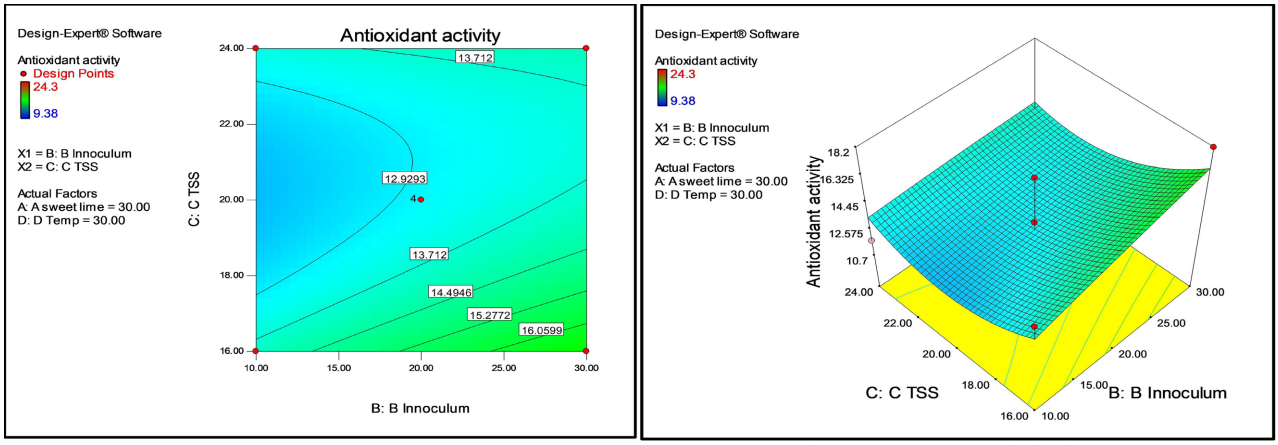
Moreover, both linear, interactive, and quadratic relationships regarding the concentration of sweet orange fruit juice have demonstrated a statistically significant influence ($p < 0.1$) on the overall phenolic content of the wine. This phenomenon can be attributed to the relatively low total phenolic concentration present in sweet orange juice, which markedly reduces the total phenolic content in the wine produced from mixed fruit juices when the concentration exceeds 30%. The incorporation of sweet orange juice at concentrations of 10% and 20% during the fermentation process has been documented to yield optimal levels of polyphenols in mixed fruit wine. Consequently, sweet oranges can be employed to boost the quality of mixed juice wine that is enriched with medicinal polyphenols, albeit within a controlled concentration range. Therefore, the inclusion of fruits such as sweet orange, which possess higher polyphenolic concentrations, during fermentation can significantly improve the quality of phenolic compounds in mixed juice wine, thereby facilitating the use of sweet orange fruit for high-quality wine production. Comparable investigations have been conducted examining the fermentative effects of apple juice in conjunction with pine needles and medicinal herbs (specifically hwanggi and mistletoe), which suggest that the total phenolic content in apple wine is enhanced through the fermentation process [29].

3.5. Changes in Antioxidant Activity

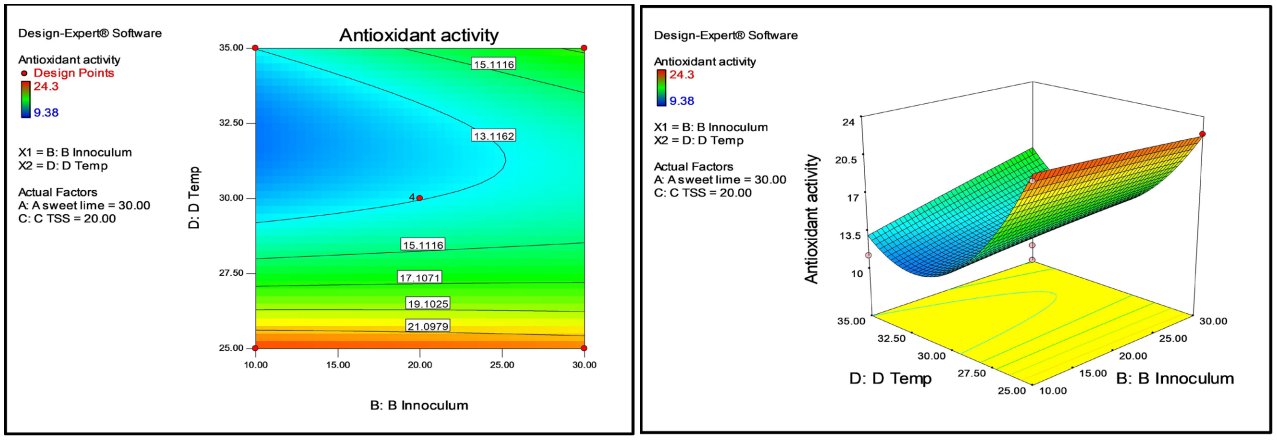
Three-dimensional response surface plots (**Figures 2(a)-(h)**) were constructed by the predictive equations (Equation (4)) to elucidate interactions among various independent variables and their respective impacts on the dependent response variables. The findings regarding the influence of fermentation parameters, as delineated in **Table 2**, reveal that the linear and interactive effects of fermentation



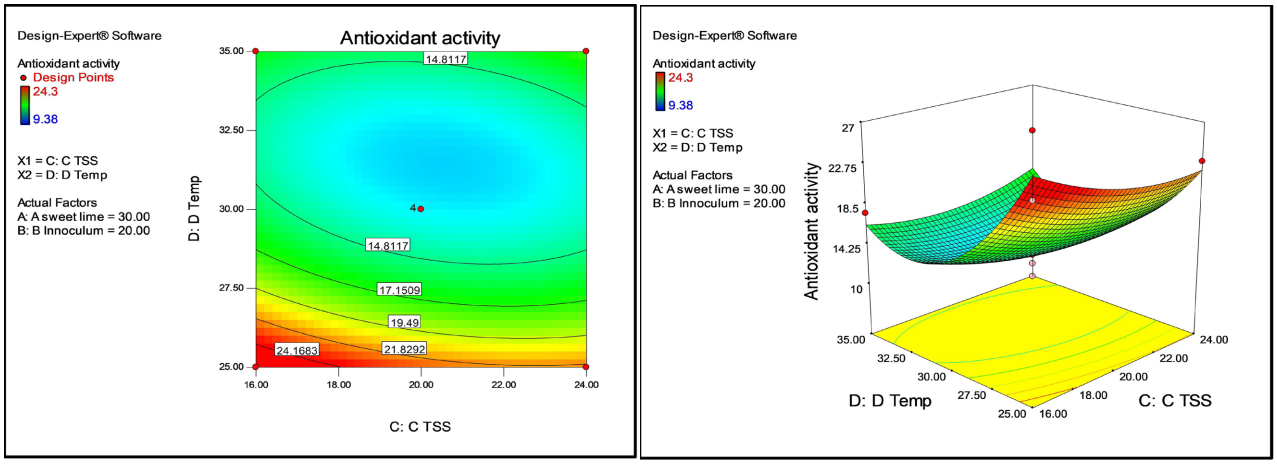
(a) (b)



(c) (d)



(e) (f)



(g) (h)

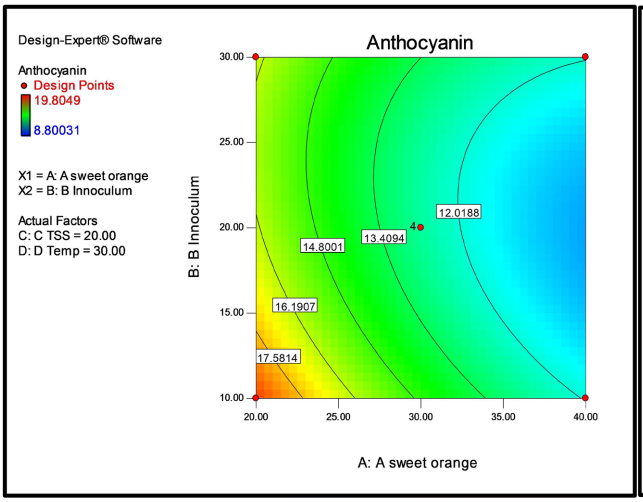
Figure 2. (a)-(h) Response surface and contour plots for the effect of sweet orange %, inoculum, ˚Brix, and temperature on the Antioxidant activity content of the pomegranate-sweet orange wine. AA: Antioxidant activity, temp; temperature. (a) Counter plots for the effect of sweet orange % and inoculum on AA activity; (b) Response surface plot for the effect of sweet orange % and inoculum on AA activity; (c) Counter plots for the effect of inoculum and ˚brix on AA activity; (d) Response surface plot for the effect of inoculum and ˚brix on AA activity; (e) Counter plots for the effect of inoculum and temperature on AA activity; (f) Response surface plot for the effect of inoculum and temperature on AA activity; (g) Contour plots for the effect of ˚Brix and temperature on AA concentration; (h) Response surface plot for the effect of ˚Brix and temperature on AA concentration.

on temperature in conjunction with the concentration of sweet orange fruit juice were statistically significant ($p < 0.1$). Conversely, the linear and interaction effects of inoculum concentration when analyzed alongside fermentation temperature, sweet orange juice concentration, and total soluble solids (TSS) were found to be statistically insignificant ($p > 0.1$). A highly significant effect ($p < 0.0001$) attributable to all quadratic terms of the fermentation process was observed concerning the total antioxidant activity of the resultant wine. Notably, the fermentation temperature emerged as the most significant variable ($p < 0.05$) influencing the antioxidant activity derived from mixed juice wine. **Figure 2(a)** and **Figure 2(b)** illustrate a positive correlation between antioxidant activity and the percentage of inoculum. Additionally, antioxidant activity exhibited an increase in response to the increment of sweet orange proportion up to 30%, beyond which a decline was noted, attributed to a significant reduction in phenolic content. The observed augmentation in antioxidant activity up to a 30% juice proportion of sweet oranges may be ascribed to an elevation in total phenolic content (TPC) and variations in their profiles [30]. The effect of TSS on antioxidant activity was deemed statistically insignificant. Remarkably, antioxidant activity was determined to be elevated at lower temperatures. It has been documented that fermentation enhances the antioxidant capacity of fruit and vegetable juices through elevated TPC and total flavonoid content (TFC) [31]. The yeast-mediated metabolic processes occurring during fermentation lead to the structural disintegration of cell walls, resulting in the liberation of various antioxidant compounds, thereby augmenting antioxidant activity. This mechanism also encompasses the exposure of microorganisms to oxidative stress during fermentation, consequently prompting the cellular evolution of protective mechanisms involving enzymatic antioxidation, which may further contribute to the antioxidative effects associated with fermentation [32]. However, as articulated by [33], different wines exhibit varying quantities and spectra of native antioxidants, hence conferring distinct health benefits. The antioxidant capacity of wine is significantly influenced by the concentration of phenolic compounds as well as other determinants such as the fruit cultivar, soil composition, nutritional factors, climatic conditions, weather patterns, vinification processes, and storage and maturation conditions. In the current investigation, the utilization of two medicinally significant fruits (pomegranate and sweet orange) for wine production is posited to yield a novel quality wine characterized by beneficial phenolic compounds and their robust antioxidant activity.

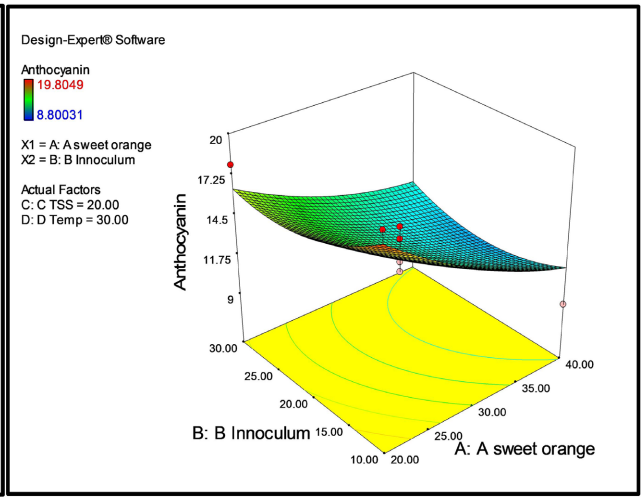
3.6. Changes in Anthocyanins

Three-dimensional response surface plots (**Figures 3(a)-(f)**) were constructed by the predictive equations (Equation (5)), thereby facilitating an analysis of the interactions among various variables and their respective effects on the response variables with relative ease.

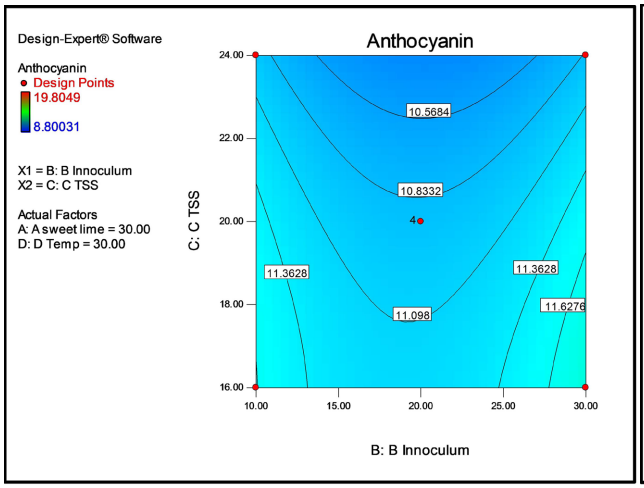
Figure 3(a) and **Figure 3(b)** illustrate that there is no statistically significant influence on the total anthocyanin content (TAC) as the percentage of inoculum increases; conversely, there is a notable decrease in anthocyanin levels in response


Design-Expert® Software
Anthocyanin
Design Points
19.8049
8.80031
X1 = A: A sweet orange
X2 = B: B Innoculum
Actual Factors
C: C TSS = 20.00
D: D Temp = 30.00
Anthocyanin
12.0188
13.4094
14.8001
16.1907
17.5814
B: B Innoculum
A: A sweet orange

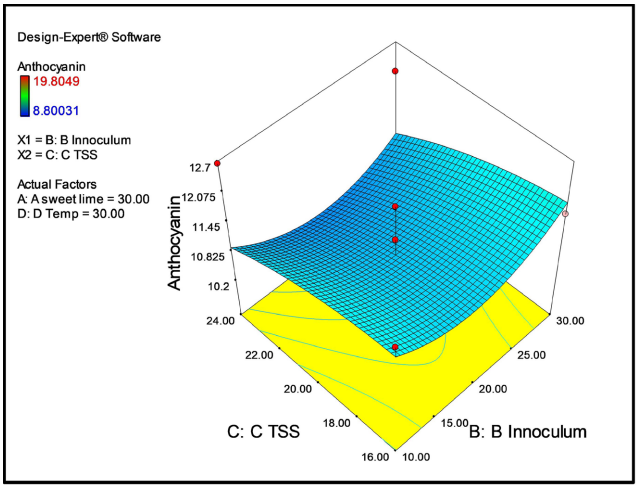
(a)


Design-Expert® Software
Anthocyanin
19.8049
8.80031
X1 = A: A sweet orange
X2 = B: B Innoculum
Actual Factors
C: C TSS = 20.00
D: D Temp = 30.00
Anthocyanin
B: B Innoculum
A: A sweet orange

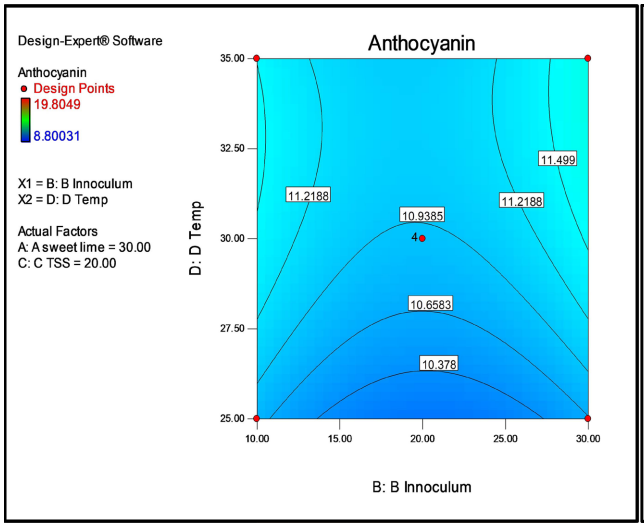
(b)


Design-Expert® Software
Anthocyanin
Design Points
19.8049
8.80031
X1 = B: B Innoculum
X2 = C: C TSS
Actual Factors
A: A sweet lime = 30.00
D: D Temp = 30.00
Anthocyanin
10.5684
10.8332
11.098
11.3628
11.3628
11.6276
C: C TSS
B: B Innoculum

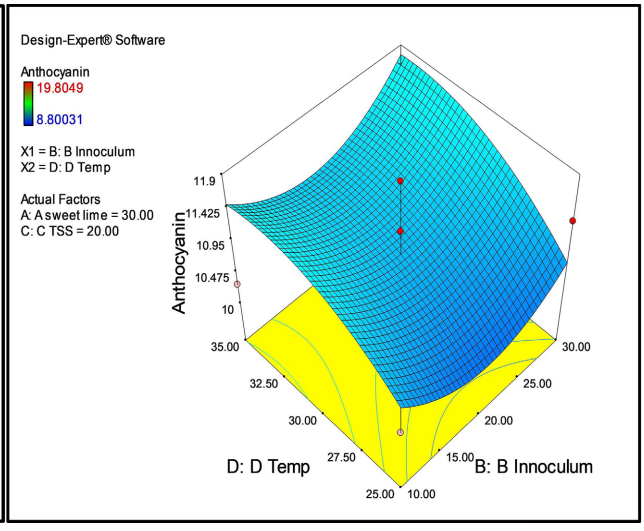
(c)


Design-Expert® Software
Anthocyanin
19.8049
8.80031
X1 = B: B Innoculum
X2 = C: C TSS
Actual Factors
A: A sweet lime = 30.00
D: D Temp = 30.00
Anthocyanin
C: C TSS
B: B Innoculum

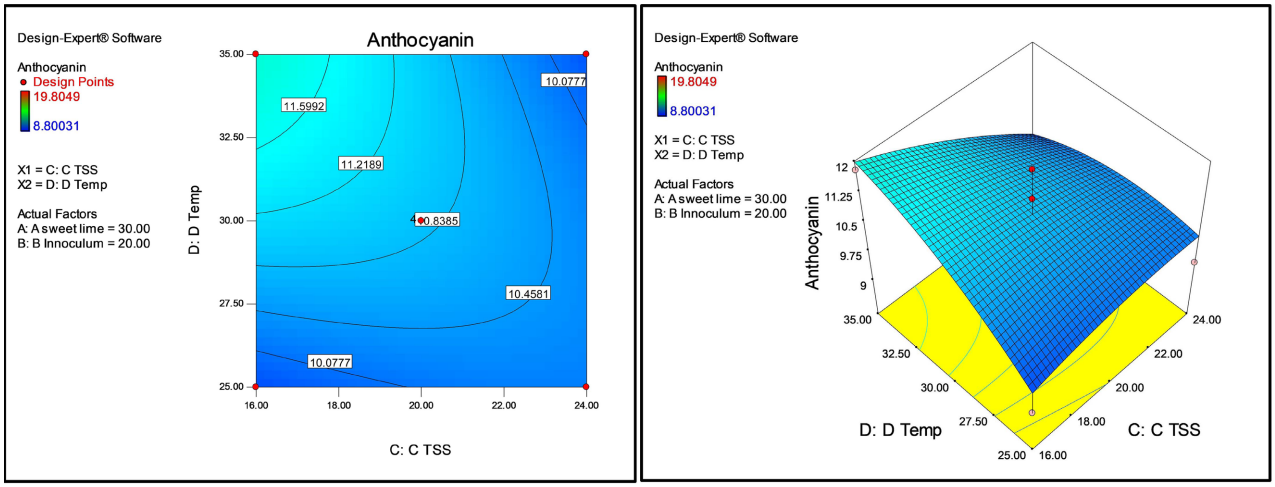
(d)


Design-Expert® Software
Anthocyanin
Design Points
19.8049
8.80031
X1 = B: B Innoculum
X2 = D: D Temp
Actual Factors
A: A sweet lime = 30.00
C: C TSS = 20.00
Anthocyanin
10.9385
11.2188
11.2188
11.499
10.6583
10.378
D: D Temp
B: B Innoculum

(e)


Design-Expert® Software
Anthocyanin
19.8049
8.80031
X1 = B: B Innoculum
X2 = D: D Temp
Actual Factors
A: A sweet lime = 30.00
C: C TSS = 20.00
Anthocyanin
D: D Temp
B: B Innoculum

(f)



(g) (h)

Figure 3. (a)-(h) Response surface and contour plots for the effect of sweet orange %, inoculum, Brix, and temperature on the Anthocyanin content of the pomegranate-sweet orange wine. temp; temperature. (a) Contour plots for the effect of sweet orange % and inoculum on anthocyanin content; (b) Response surface plot for the effect of sweet orange % and inoculum on anthocyanin content; (c) Contour plots for the effect of inoculum and Brix on anthocyanin content; (d) Response surface plot for the effect of inoculum and Brix on anthocyanin content; (e) Contour plots for the effect of inoculum and temperature on anthocyanin content; (f) Response surface plot for the effect of inoculum and temperature on anthocyanin content; (g) Contour plots for the effect of Brix and temperature of anthocyanin content; (h) Response surface plot for the effect of Brix and temperature on anthocyanin content.

to an increase in sweet orange content. The lower TAC associated with sweet orange results in a reduction of TAC content as its proportion within the mixed juice escalates. An increase in total soluble solids (TSS) and fermentation temperature correlates with an augmentation of anthocyanin content. The linear relationship observed between fermentation temperature and the enhancement of total phenol concentration is attributed to the elevation in fermentation temperature, which not only enhances the diffusion coefficient but also the solubility of analytes [34]. It was observed that ˚Brix exerts a significant positive influence on TAC, potentially attributable to the inhibition of pigment-degrading enzymes through the condensation of anthocyanins with phenolic compounds or the establishment of a partial oxygen barrier [35], which contributes to the stabilization of anthocyanins. Notably, it was determined that yeast inoculum does not exert a significant effect on TAC. According to earlier studies [36], anthocyanins exhibit pH-dependent behavior. This suggests that elevated levels of yeast cells may enhance the presence of lees yeast capable of absorbing anthocyanins. The alterations in TAC may be attributed to the absorption of specific anthocyanins by yeast cell walls and potentially due to precipitation as wine salts, as inferred from existing literature [37].

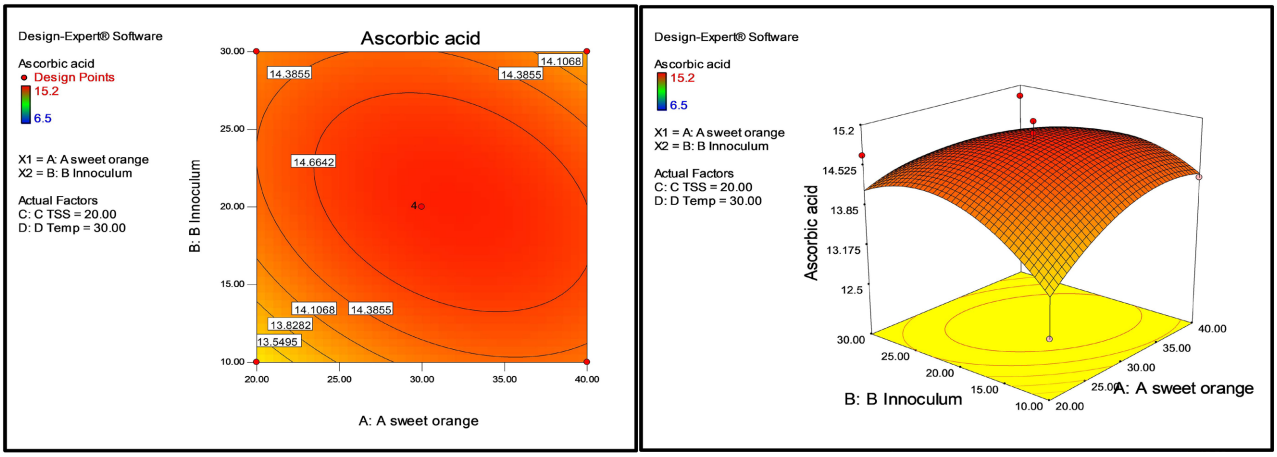
As noted by authors [38], temperature and ˚Brix significantly influence the release of yeast metabolites from *S. cerevisiae* during the fermentation process. These electrophilic metabolites possess the capability to condense with anthocyanins, leading to the formation of proanthocyanins [39]. Indeed, the elevation in temperature is correlated with increased production of yeast metabolites, subse-

quently resulting in enhanced proanthocyanin formation [39]. Furthermore, ˚Brix has been shown to facilitate the generation of proanthocyanins due to its ability to affect the stability of anthocyanin flavylium formation in the presence of pyruvic acid [40]. Additionally, research has highlighted a pronounced occurrence of pyranoanthocyanin formation when alcoholic fermentation transpires in the absence of sulfur dioxide [39]. Consequently, this phenomenon may be relevant in predicting the potential for pyranoanthocyanin formation, which could be responsible for the observed alterations in TAC. Similar findings have been reported in the optimization of mulberry wine conducted by researchers [22].

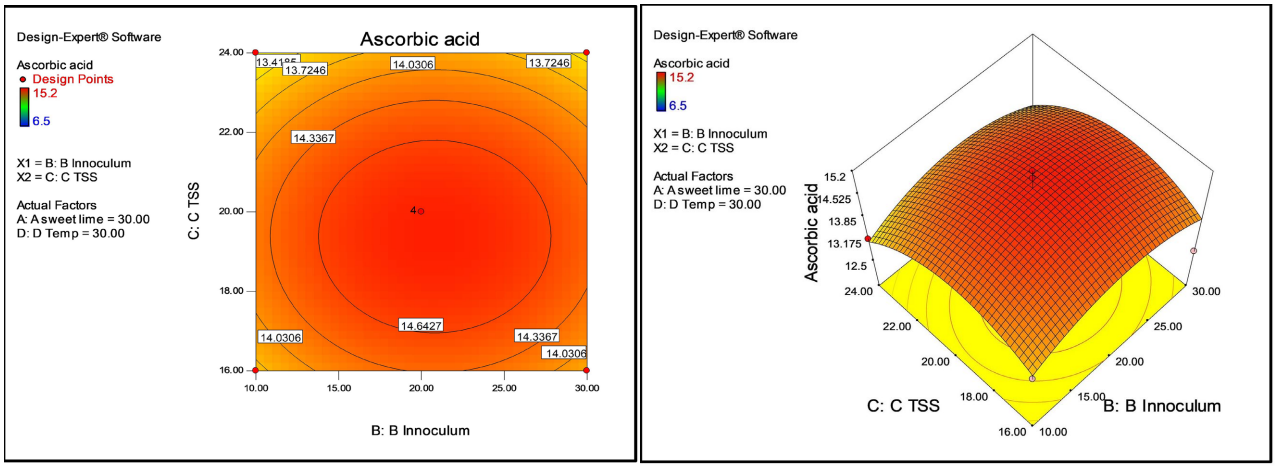
3.7. Changes in Ascorbic Acid

Three-dimensional response surface plots (**Figures 4(a)-(h)**) were developed according to equations prediction (Equation (6)) about interactions among the different variables and their corresponding effect on the response variables can be easily made.

Ascorbic acid is also utilized within the white wine sector as an antioxidant aimed at inhibiting browning [41]. The current investigation pertains to the synthesis of



(a) (b)



(c) (d)

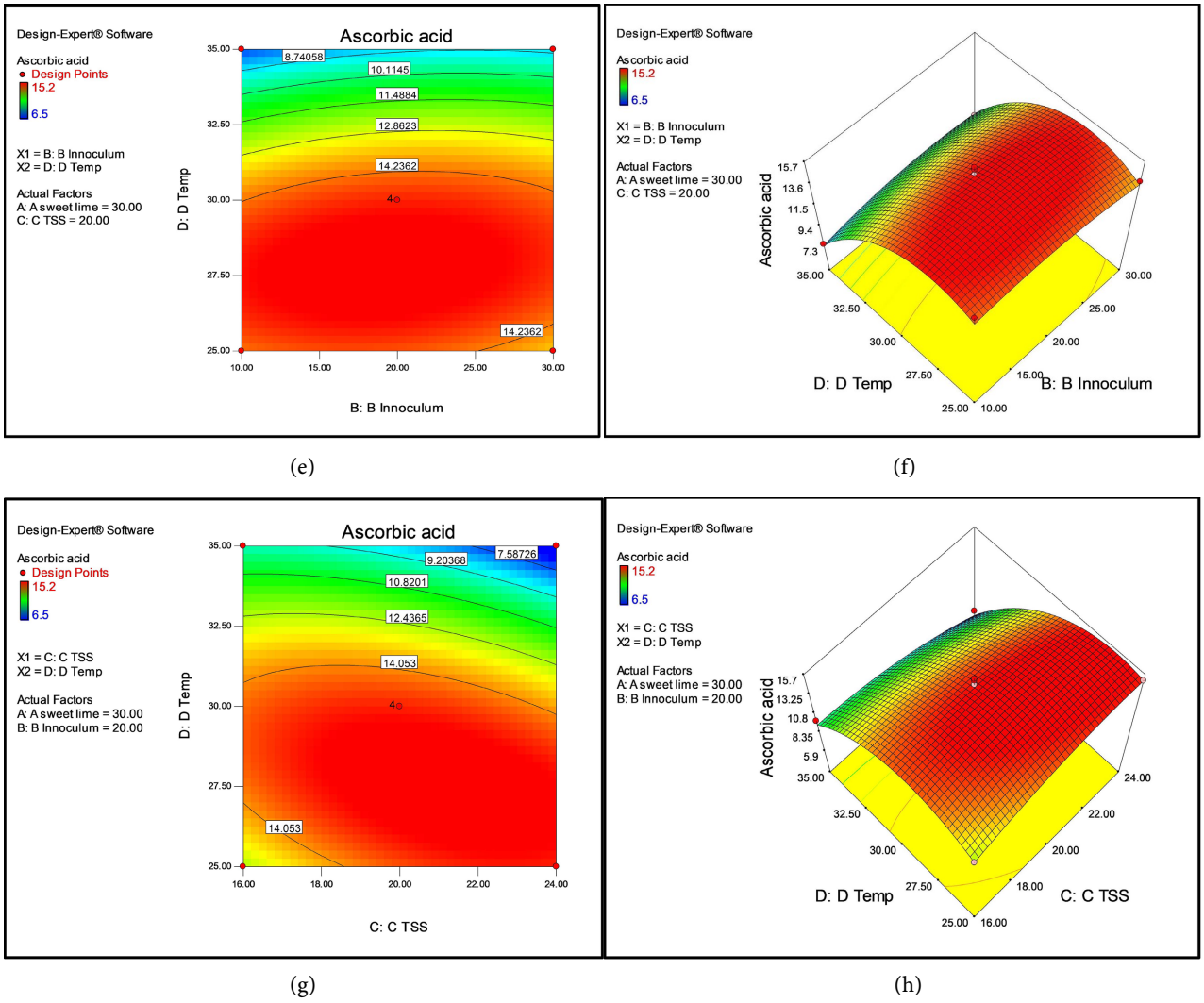


Figure 4. (a)-(h) Response surface and contour plots for the effect of sweet orange %, inoculum, ˚Brix, and temperature on the Ascorbic acid content of the pomegranate-sweet orange wine. temp; temperature. (a) Contour plots for the effect of sweet orange % and inoculum on ascorbic acid content; (b) Response surface plot for the effect of sweet orange % and inoculum on the ascorbic acid content; (c) Contour plots for the effect of inoculum and Brix on ascorbic content; (d) Response surface plot for the effect of inoculum and Brix on the ascorbic acid content; (e) Contour plots for the effect of inoculum and temperature on ascorbic acidcontent; (f) Response surface plot for the effect of inoculum and temperature on the ascorbic acid content; (g) Counter plots for the effect of ˚Brix and temperature on ascorbic acid content; (h) Response surface plot for the effect of ˚Brix and temperature on the ascorbic acid content.

mixed fruit wine derived from fruits abundant in ascorbic acid, specifically pomegranate and sweet orange; consequently, an examination of the influence of fermentation parameters on ascorbic acid concentration is of considerable importance. Figure 4(a) and Figure 4(b) illustrate the augment in ascorbic acid concentration relative to the increasing proportion of sweet oranges. The percentage of inoculum does not exert a statistically significant influence on the ascorbic acid concentration. As total soluble solids (TSS) increase, there is a concomitant rise in ascorbic acid levels, whereas elevated temperatures adversely affect ascorbic acid stability. Numerous studies have documented the degradation of ascorbic acid at-

tributable to elevated temperatures [42] [43]. Ethanol concentration serves as a critical factor that influences the Maillard reaction mechanism and the rate of browning. In alcoholic beverages, ascorbic acid and phenolic compounds are typically present in conjunction. In such conditions, ascorbic acid may undergo degradation through either its pathway or a pathway involving its interaction with phenolic compounds [43]. Chuang *et al.* reported that a heightened ethanol concentration correlates with an increased degradation rate of ascorbic acid in ascorbic acid-catechin model solutions. A higher concentration of ethanol creates a lower water activity environment, which facilitates the dehydration of the ascorbic acid degradation intermediate compound L-xylose, thereby resulting in an escalated rate of ascorbic acid degradation [43] [44]. In our investigation, although the direct-ethanol concentration was not quantified, the correlation between TSS and ethanol production, as established in previous studies [45], suggests that ascorbic acid deprivation in ethanolic solutions at a constant temperature was also observed to surge with the augmentation of ethanol concentration.

Table 4. Experimental data analysis for the predictive response model.

Statistical parameters	TPC mg/L	Antioxidants mg/L	Anthocyanin mg/L	Ascorbic acid mg/100 ml
Std. Dev.	226.70	2.86	1.23	0.72
Mean	1969.55	15.17	12.34	12.68
C.V. %	11.51	18.84	9.97	5.70
PRESS	3368161.55	433.94	107.45	38.11
R-Squared	0.86	0.82	0.93	0.97
Adj R-Squared	0.70	0.63	0.86	0.93
Pred R-Squared	0.68	0.57	0.62	0.81
Adeq Precision	8.67	8.12	11.85	17.06

Std. dev., C.V. TP, PRESS, and AAD stand for standard deviation, coefficient of variation, total phenol content, predicted regression error sum of a square, and absolute average deviation.

Table 5. Point prediction table showing optimum parameters generated after the RSM experiment.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	A sweet orange	**20.00295172**	20	40	0	Actual	
B	B Inoculum	**10.01057869**	10	30	0	Actual	
C	C TSS	**23.99998524**	16	24	0	Actual	
D	D Temp	**26.25484372**	25	35	0	Actual	
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Total phenolics	1998.472463	421.9312356	1086.945447	2909.999479	527.9800328	857.84095	3139.103976
Antioxidant activity	17.82808148	3.090638595	11.15116274	24.50500023	3.867444098	9.472976482	26.18318648
Anthocyanin	19.740213	2.999910064	13.25930133	26.22112467	3.753911728	11.63037977	27.85004623
Ascorbic acid	14.67407597	0.961575258	12.59671892	16.75143301	1.203258952	12.07459305	17.27355889

Table 5 reflects the 20% sweet orange in mixed juice proportion, 10% inoculum 23% TSS, and 26˚C temperature required to maintain throughout the fermentation process.

4. Conclusion

The evaluation of the findings derived from the RSM optimization indicated that a proportion of 80:20 pomegranate to sweet orange is essential for the production of a premium quality mixed juice wine exhibiting the highest antioxidant activity levels. Furthermore, it was indicated that wine production at reduced temperatures could effectively maintain a significant concentration of ascorbic acid. The investigation underscored that a greater quantity of anthocyanins could also be retained in this innovative wine formulation. The RSM experiment demonstrated that the 80:20 pomegranate to sweet orange ratio is requisite for achieving a quality mixed juice wine. These results further substantiate the notion that sweet orange, recognized for its medicinal properties, can be effectively utilized in conjunction with pomegranate juice, thereby ensuring the preservation of its valuable phenolic compounds during the wine-making process. Additional research employing RSM to establish optimal conditions should be conducted within a bioreactor under regulated conditions to maximize the yield of all bioactive components, thereby validating the potential of the pomegranate and sweet orange mixed juice wine.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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