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Antifungal Activities of Anthocyanins from Purple Sweet Potato in the Presence of Food Preservatives

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Abstract Effects of anthocyanins from purple sweet potato (APSP) against the fungal growth of *Geotrichum candidum* and *Candida albicans* were assessed. Response surface methodology was applied for optimization of proportions of sodium benzoate, potassium sorbate, and APSP. Optimum concentrations against *G. candidum* were 0.300 mg/mL of sodium benzoate, 0.290 mg/mL of potassium sorbate, and 13.9 mg/mL of APSP. Optimum concentrations against *C. albicans* were 0.380 mg/mL of sodium benzoate, 0.240 mg/mL of potassium sorbate, and 3.56 mg/mL of APSP. APSP exhibited enhanced antifungal properties in the presence of food preservatives.

Keywords: anthocyanins from purple sweet potato (APSP), food preservative, antifungal compound, antifungal activity, response surface methodology (RSM)

Introduction

Anthocyanins, the largest group of water-soluble pigments in the plant kingdom (1), are flavonoids with strong antioxidant properties (2,3). In foods, anthocyanins have been used as substitutes for synthetic pigments due to appealing colors and bioactive functions (4,5). Besides antioxidant properties, anthocyanins have pharmacological and antifungal properties (6). Several studies have shown that anthocyanins protect against a myriad of human diseases, including liver dysfunction, hypertension, vision disorders, microbial infections, and diarrhea (7-9). Recently, anthocyanins have been reported to have antibacterial activities (10,11).

As antimicrobial agents in foods, anthocyanins have several advantages, including a low level of toxic side effects and non-residual and non-resistance properties. Therefore, anthocyanins can be used as natural food preservatives. Several studies (12,13) have shown that when anthocyanins are present together with food preservatives, they enhance antimicrobial properties, compared with additive use alone, due to synergistic effects.

Purple sweet potato, a dicotyledonous plant of Family Convolvulaceae, is rich in anthocyanins. Anthocyanins from purple sweet potato (APSP) are highly stable due to complex chemical structures (1,14). Studies focused on antibacterial properties of APSP have revealed that APSP in the presence of food preservatives exerts antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*. However, few reports so far have focused on antifungal properties of APSP in the presence of food preservatives.

Response surface methodology (RSM) is an effective statistical technique for optimization of complex processes (15-18). RSM is widely used for optimization of process variables. The main advantage of RSM is a low number of experiments required for evaluation of multiple parameters and interactions. In this study, RSM was used to determine the optimum concentrations and proportions of APSP, sodium benzoate, and potassium sorbate for growth inhibition of *Geotrichum candidum* and *Candida albicans*.

Materials and Methods

Materials and chemicals Purple sweet potatoes were supplied by the National Sweet Potato Research Institute (Xuzhou, Jiangsu Province, China), washed in tap water, cut into 0.5 cm pieces, and dried in an air dryer at 50°C (DHG-9140; Shanghai Yiheng Technology Co., Ltd., Shanghai, China). Subsequently, potato pieces were ground in a disintegrator (FSD-100A; Taizhou Woliang Foodstuffs Equipment Co., Ltd., Taizhou, China), passed through an 80 mesh sieve (QD9; Westernized Instrument Technology Co., Ltd., Beijing, China), and stored at 4°C. The resulting powder was mixed with acidulated alcohol (1.5 mol/L HCl, ethanol 95% (v/v)=15:85), centrifuged (TG16-WS; Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Hunan, China) at 10,625×*g* for 20 min, purified through a AB-8 macroporous resin (Sinopharm Chemical Reagent Co., Ltd., Shanghai,

China), concentrated under reduced pressure to remove ethanol at 40°C (RE-52AA; Shanghai Yarong Biochemical Instrument Factory, Shanghai, China), and dried under vacuum (DZF-6020; Shanghai Yiheng Technology Co., Ltd.), thereby obtaining APSP (19-21).

Sodium benzoate and potassium sorbate were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical grade. *G. candidum* and *C. albicans* were obtained from the State Key Laboratory of Food Science and Technology of Nanchang University (Nanchang, Jiangxi Province, China).

Experimental design

***Minimum inhibitory concentrations of APSP, sodium benzoate, and potassium sorbate*:** A double dilution method (22) was used for calculation of minimum inhibitory concentrations (MIC) of APSP, sodium benzoate, and potassium sorbate against *G. candidum* and *C. albicans* (10). APSP, sodium benzoate, and potassium sorbate were diluted with double dilution method from 40 to 0.3125 mg/mL. Then, 2 mL of resulting dilutions was added to different Petri dishes containing 20 mL of a solid culture medium (beef extract peptone agar). After dishes cooled and solidified, 0.2 mL of a *G. candidum* or *C. albicans* fungal suspension (1×10^7 to 2×10^7 CFU/mL) was evenly spread on the Petri dish surface using an SS spreader (53800-008; Shanghai Huake Experiment Equipment Co., Ltd., Shanghai, China). Petri dishes were incubated at 37°C (DNP-9052; Shanghai Sanfa Scientific Instrument Co., Ltd., Shanghai, China) for 24 h and the MIC value of each compound was calculated.

***MIC of optimum combined compounds*:** Three combined compound sets of sodium benzoate and potassium sorbate, sodium benzoate and APSP, and potassium sorbate and APSP were all prepared in proportions of 1:9, 3:7, 5:5, 7:3, and 9:1. The optimum compound combination and proportion were determined using the paper filtering method (23,24). A criss-cross method (25) was performed for measurement of inhibition zone diameters, which was done 3x. MIC values of combined compounds were calculated.

Antifungal activities of APSP, sodium benzoate, and potassium sorbate using RSM A 3-level, 3-variable Box-Behnken factorial design (26) (BBD) (Design Expert software 8.0.5.0) was used for determination of optimum antifungal compound concentrations. Independent variables were the sodium benzoate concentration (X_1, mg/mL), potassium sorbate concentration (X_2, mg/mL), and APSP concentration (X_3, mg/mL). Values of the 3 independent variables were determined based on single factor analysis. Average values of inhibition zone diameters based on the paper filtering method for *G. candidum* and *C. albicans* were interpreted as response (dependent) variables, Y_1 and Y_2, respectively. The experimental design consisting of 5 zero points and 17 experimental points with 3 replicates performed in randomized order for calculation of the sum of squares for error is shown in Table 1.

Experimental data were fitted to the non-linear computer-generated quadratic polynomial model:

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}X_i^2+\sum_{i>j}^{k}\beta_{ij}X_iX_j$$

where Y is the response variable, β_0, β_i, β_{ii}, and β_{ij} are regression coefficients of independent variables, X_i and X_j are independent variables, and k is the number of tested factors.

Design Expert software was used for determination of the optimum proportions of APSP, sodium benzoate, and potassium sorbate. Data were expressed as mean±standard error (SE). Data obtained from the experimental design were subjected to multiple non-linear regression analysis. Equations were validated using an analysis of variance (ANOVA). The fit of the quadratic polynomial model was assessed based on the coefficient of determination (R^2). Significance

Table 1. Box-Behnken experimental design for *G. candidum* and *C. albicans*

	G. candidum				*C. albicans*			
Run	X_1	X_2	X_3	Y_1 (mm)	X_1	X_2	X_3	Y_2 (mm)
1	0 (0.443)	0 (0.251)	0 (15.0)	20.0	0 (0.443)	1 (0.335)	1 (5.00)	16.0
2	-1 (0.295)	1 (0.335)	0	19.0	0	0 (0.251)	0 (3.75)	21.8
3	0	-1 (0.167)	1 (20.0)	15.7	0	1	-1 (2.50)	14.5
4	-1	0	1	16.8	-1 (0.295)	1	0	18.6
5	0	0	0	19.9	-1	-1 (0.167)	0	20.0
6	0	0	0	20.1	0	-1	-1	16.9
7	0	1	1	10.9	1 (0.590)	0	1	17.2
8	1 (0.590)	0	-1 (10.0)	15.8	1	-1	0	18.0
9	-1	0	-1	17.3	0	-1	1	15.7
10	-1	-1	0	15.4	0	0	0	22.4
11	0	1	-1	17.3	0	0	0	22.0
12	1	0	1	12.9	-1	0	1	14.9
13	1	1	0	11.6	0	0	0	21.9
14	0	0	0	19.8	0	0	0	22.6
15	0	0	0	20.8	1	1	0	19.3
16	0	-1	-1	12.9	-1	0	-1	20.2
17	1	-1	0	19.0	1	0	-1	15.0

of the regression coefficient was assessed based on the F test and $p<0.05$ (27).

Results and Discussion

MIC values for APSP, sodium benzoate, potassium sorbate, and optimum combined compounds MIC values for APSP, sodium benzoate, and potassium sorbate for *G. candidum* were 10.00, 4.72, and 5.36, respectively, and for *C. albicans* were 2.50, 0.59, and 1.34, respectively. MIC values of APSP were close to values for sodium benzoate and potassium sorbate, respectively, against *G. candidum* and *C. albicans*, and APSP exhibited antifungal activities against *G. candidum* and *C. albicans*.

Sodium benzoate-APSP (5:5) and sodium benzoate-APSP (7:3) exhibited optimum antifungal activities against *G. candidum* and *C. albicans*, respectively (Table 2). Calculated MIC values for sodium benzoate-APSP (5:5) and sodium benzoate-APSP (7:3) were 0.500 mg/mL and 0.300 mg/mL, respectively.

Application of RSM

***Fitting the model*:** Response variables Y_1 and Y_2 indicating average inhibition zone diameters for *G. candidum* and *C. albicans*, respectively, are shown in Table 1. Regression analysis was performed to fit data to mathematical models for identification of optimal regions for response variables. Predicted values for response variables Y_1 and Y_2 were calculated using the second-order polynomial equations:

$$Y_1=20.12-1.15X_1-0.53X_2-0.87X_3-2.25X_1X_2-0.60X_1X_3-2.30X_2X_3 -1.18X_1^2-2.68X_2^2-3.23X_3^2$$

$$Y_2=22.14-0.52X_1-0.27X_2-0.35X_3-0.67X_1X_2-1.88X_1X_3-0.67X_2X_3 -1.06X_1^2-2.11X_2^2-4.26X_3^2$$

In general, optimization of a fitted response variable can lead to poor or misleading results unless the model has a good fit (28). The F value depends on the number of degrees of freedom (DF) and on the *p* value for a 95% confidence level. Effects at $p<0.05$ were considered to be significant (29,30).

ANOVA results for the fitted quadratic polynomial model of antifungal effects against *G. candidum* and *C. albicans*, respectively, are shown in Table 3. High F values of 51.88 and 36.72 and $p<0.05$ revealed that the model was significant. *F* and *p* values for lack of fit for *G. candidum* were 3.76 and 0.1168, respectively; *F* and *p* values for lack of fit for *C. albicans* were 6.40 and 0.0524, respectively (Table 3). Therefore, models were adequate for prediction of antifungal effects of compounds.

R^2_{adj} (adjusted coefficient of determination) is the correlation coefficient of the goodness-of-fit of a regression equation. High R^2_{adj} values indicate a high degree of correlation between observed and

Table 2. Antifungal activities of combined compounds against *G. candidum* and *C. albicans*

Average inhibition zone diameter for combined compounds	Proportions					Optimal inhibition zone diameter (mm)
	1:9	3:7	5:5	7:3	9:1	
G. candidum						
Sodium benzoate-Potassium sorbate	10.2	12.1	14.8	16.8	17.4	17.4
Sodium benzoate-APSP	10.1	12.6	18.1	16.5	13.7	18.1
Potassium sorbate-APSP	17.4	16.8	13.5	12.8	9.1	17.4
C. albicans						
Sodium benzoate-Potassium sorbate	19.6	20.3	22.4	17.4	16.8	22.4
Sodium benzoate-APSP	16.5	17.4	19.8	23.9	23.1	23.9
Potassium sorbate-APSP	17.4	19.4	22.4	20.8	18.2	22.4

Table 3. A quadratic polynomial model of antifungal activities against *G. candidum* and *C. albicans*

Source	SS	DF	MS	F value	*p* value
G. candidum					
Model	159.95	9	17.77	51.88	<0.0001
Residual	2.40	7	0.34		
Lack of fit	1.77	3	0.59	3.76	0.1168
Pure Error	0.63	4	0.16		
Cor Total	162.35	16			
	R^2=0.9852	R^2_{adj} =0.9662	CV=3.49%		
C. albicans					
Model	129.21	9	14.36	36.72	<0.0001
Residual	2.74	7	0.39		
Lack of fit	2.27	3	0.76	6.40	0.0524
Pure Error	0.47	4	0.12		
Cor Total	131.94	16			
	R^2=0.9793	R^2_{adj} =0.9526	CV=3.35%		

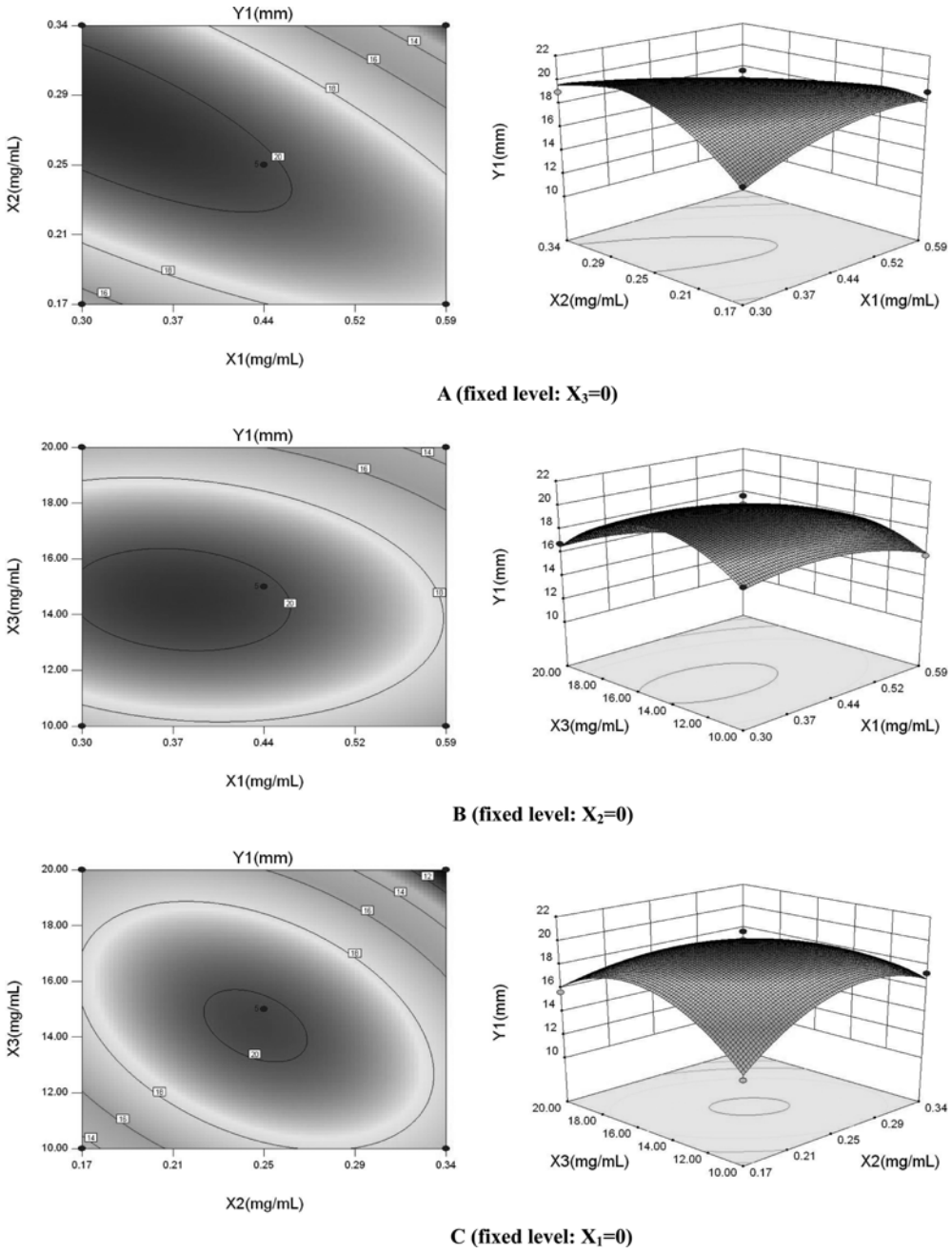


Fig. 1. Two-dimensional contour plots and 3-dimensional response surface plots of antifungal activities of compounds against *G. candidum.*

predicted values (31). R^2_{adj} values were 0.9662 and 0.9526 (Table 3), indicating that <5.0% of total variation was not explained by models. Additionally, obtained R^2_{adj} values confirmed that models were significant with a high degree of correlation between observed and predicted values. The coefficient of variation (CV) indicates the degree of precision at which experiments were compared. Relatively low CV values of 3.49 and 3.35 revealed high precision and reliability of experiments.

***Analysis of response surfaces*:** Three-dimensional response surface plots and two-dimensional contour plots are graphical representations of regression equations. These plots provide visual representations of relationships between response variables and independent variables and types of interactions between 2 independent variables. Circular or elliptical shapes of contour plots indicate whether interactions between 2 independent variables are significant or not. Circular contour plots indicate that interactions between independent variables are not significant, whereas elliptical contour plots indicate that interactions between independent variables are significant (32-34). Relationships between independent and dependent variables were illustrated using 3-dimensional plots of response surfaces and 2-dimensional contour plots generated from models for compound antifungal activities (Fig. 1 and 2). Two variables were depicted in a

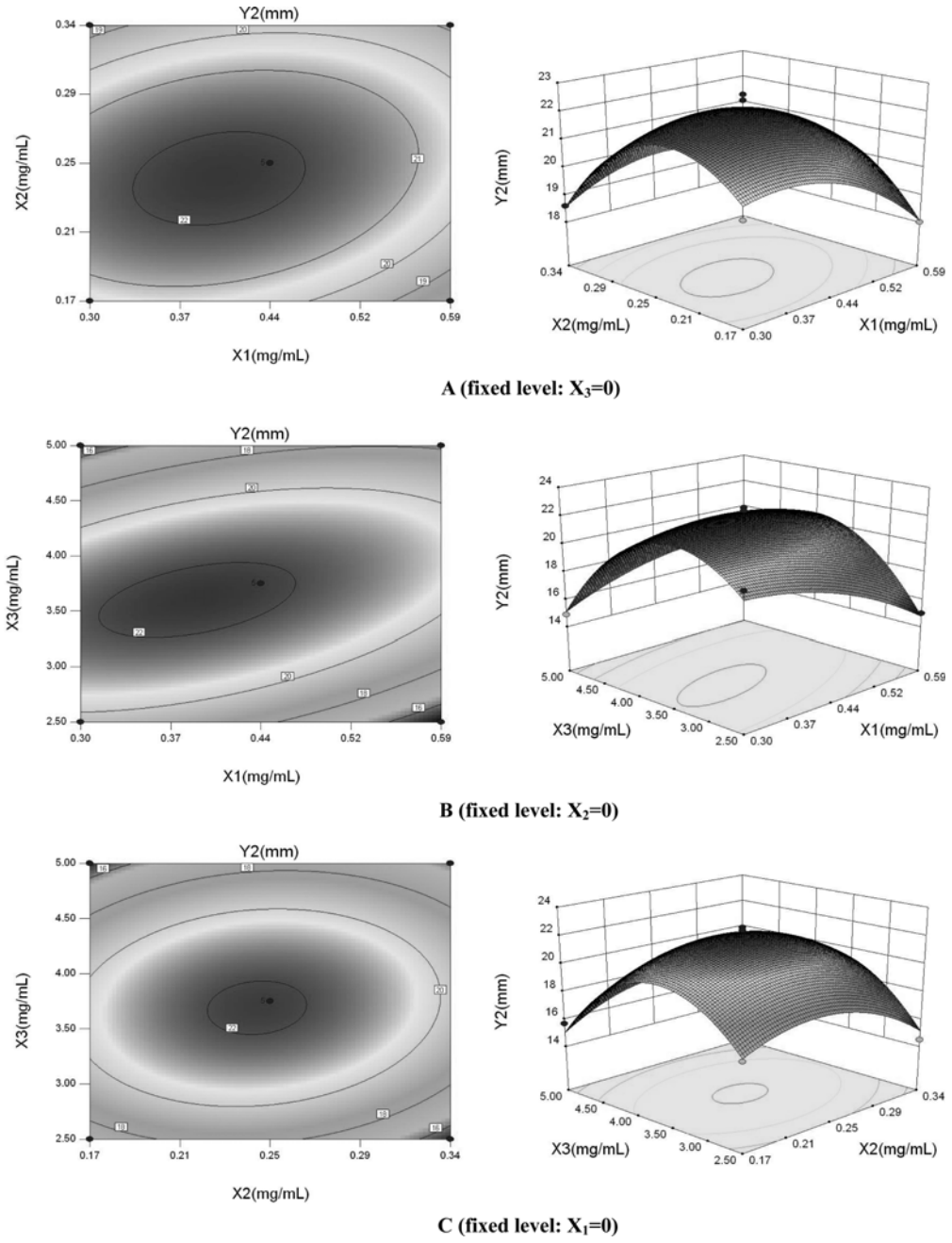


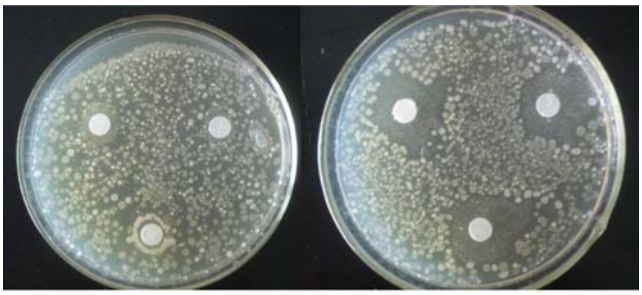
Fig. 2. Two-dimensional contour plots and 3-dimensional response surface plots of antifungal activities of compounds against *C. albicans*.

single 3-dimensional plot while another variable was held at 0.

When X_1=0.44 mg/mL and X_2=0.25 mg/mL, the inhibition zone diameter of *G. candidum* reached a maximum value (Fig. 1A). Similarly, when X_1=0.44 mg/mL and X_3=15.0 mg/mL (Fig. 1B) and when X_2=0.25 mg/mL and X_3=15.0 mg/mL (Fig. 1C), inhibition zone diameters of *G. candidum* reached maximum values. Interaction of X_1-X_3 was not significant (p>0.1), while interactions of X_1-X_2 and X_2-X_3 were both significant (p<0.05).

When X_1=0.44 mg/mL and X_2=0.25 mg/mL, the inhibition zone diameter of *C. albicans* reached a maximum value (Fig. 2A). Similarly, when X_1=0.44 mg/mL and X_3=3.50 mg/mL (Fig. 2B), and when X_2=0.25 mg/mL and X_3=3.50 mg/mL (Fig. 2C), the inhibition zone diameter of *C. albicans* reached a maximum value. Interaction of X_1-X_3 was significant (p<0.05) while interactions of X_1-X_2 and X_2-X_3 were not significant (p>0.1).

Optimization of compound proportions Suitability of the model for prediction of optimum response values was tested under optimum conditions. Optimum compound concentrations against *G. candidum* were 0.300 mg/mL sodium benzoate, 0.290 mg/mL potassium sorbate, and 13.9 mg/mL APSP. Under these conditions, the predicted inhibition zone diameter was 20.7 mm while the actual inhibition



(A) *G. candidum*

(B) *C. albicans*

Fig. 3. Antifungal activities under optimum conditions against *G. candidum* and *C. albicans*.

zone diameter was 19.6 mm with an MIC value of 0.130 mg/mL (Fig. 3A). Optimum compound concentrations against *C. albicans* were 0.380 mg/mL sodium benzoate, 0.240 mg/mL potassium sorbate, and 3.56 mg/mL APSP. Under these conditions, the predicted inhibition zone diameter was 22.3 mm while the actual inhibition zone diameter was 21.8 mm with an MIC value of 0.060 mg/mL (Fig. 3B). These results, in agreement with predicted values, confirmed that RSM models were suitable and accurate.

In conclusion, APSP exhibited antifungal effects against *G. candidum* and *C. albicans* with sodium benzoate and potassium sorbate. Optimum combinations of sodium benzoate, potassium sorbate, and APSP are obtained using RSM. MIC values of combinations were lower than for sodium benzoate, potassium sorbate, and APSP alone, indicating synergic effects among the 3 compounds. Therefore, APSP can be used as a natural preservative.

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Disclosure The authors declare no conflict of interest.

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