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Optimized enzyme-assisted microwave extraction and potential inhibitory action against α-glucosidase of polysaccharides from sweet corncobs

Extracción optimizada con microondas asistida por enzimas y acción inhibitoria potencial de la α-glucosidasa de polisacáridos obtenidos de marlos de maíz dulce

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Abstract. The conditions for extracting polysaccharides from sweet corncobs (SCP) were studied. Four parameters (ratio of water to raw material, compound enzyme concentration, temperature of enzymolysis and duration of enzymolysis) affecting the extraction of SCP were optimized using response surface methodology (RSM). Under the optimized conditions, the yield of SCP was 17.58 %. SCP had inhibitory effects on α-amylase and α-glucosidase activities, and the IC_{50} was 20.91 mg/mL and 12.47 mg/mL. SCP may therefore have prevention and treatment effects on postprandial hyperglycemia in diabetes. The inhibitory effects of SCP were improved after fractionation, and were strongest in the fraction SCP80. The fraction of SCP belong to β-glycosides heteropolysaccharides with a pyran group.

Keywords: Sweet corncobs; Polysaccharides; α-Glucosidase inhibition.

Resumen. Se estudiaron las condiciones para extraer polisacáridos de marlos de maíz dulce (SCP). Se optimizaron cuatro parámetros [relación de agua al material inicial (materia prima), concentración de enzima compuesta, temperatura de ruptura de enzimas, y duración de la ruptura de enzimas) que afectan la extracción de SCP usando metodología de superficie de respuesta (RSM). Bajo condiciones óptimas, el rendimiento de SCP fue 17,58%. SCP tuvo efectos inhibitorios en las actividades de las enzimas α-amilasa y α-glucosidasa, y el IC_{50} fue de 20,91 y 12,47 mg/L. SPC puede tener efectos de prevención y tratamiento de los altos niveles de glucosa en sangre luego de las comidas en la diabetes. Los efectos inhibitorios de SCP mejoraron luego del fraccionamiento, y fueron los más fuertes en la fracción SCP80. La fracción de SCP pertenece a heteropolisacáridos β-glucósidos con un grupo piran.

Palabras clave: Marlos de maíz dulce; Polisacáridos; Inhibición de la α-glucosidasa.

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INTRODUCTION

Sweet corn is popular among consumers because of its nutritional value, flavor, and texture. As a result it is one of the most important food products in the world. Sweet corncobs are a by-product of sweet corn production, which accounts for 20-30 % of the total weight of the sweet corn crop (Wang et al., 2005). Corn cob material can instead be used for the preparation of caramel, xylitol, glucose, furfural, activated carbon, particle board, adhesive and more. Additionally, it has useful qualities such as water imbibition and resistance to abrasion, strong toughness, homogeneity, moderate hardness, and ease of recycling (Shi & Dai, 1995; Liu, 2009).

The composition of natural active polysaccharides has been the focus of much research in recent years. Extraction yield and content of polysaccharides have significantly improved with the development of the compound enzyme method (Zhao et al., 2007; Yang & Yang, 2012). Yield increased by 3.41% with ultrasound-assisted enzymatic extraction of cellulose, relative to single enzyme hydrolysis (Zhang & Qin, 2011). Microwave technology has also been applied to polysaccharide extraction in recent years. This method can significantly shorten extraction time and increase efficiency due to the microwaves' strong penetrating power and capacity to heat the substrate quickly (Huang et al., 2007).

Research has shown that polysaccharides *in vivo* do not only provide energy and fulfill a structural role, but also have a variety of other biological functions. Many plant polysaccharides can scavenge free radicals, improve antioxidant enzyme activity, inhibit lipid peroxidation, protect the biological membrane, and provide anti-aging effects (Huang et al., 2010). Specifically, polysaccharides can decrease blood glucose, decrease the production of malondialdehyde (MDA) in lipid peroxidation, and increase the activity of the antioxidant enzymes SOD and GSH-Px. Therefore polysaccharides can play an important role in herbal pharmacology (Liang et al., 2006).

At present only lentinan, pachyman, polystictusversicolor polysaccharides and polyporus polysaccharides have been explored for use in clinical practice, and their biological activity shows promising development prospects. However, in-depth research on aspects such as quality and safety standards, separation and extraction technologies, synthesis and structure is still needed.

The objectives of this study therefore include: (i) investigating the main extraction variables (microwave power, microwave time, ratio of water to raw material, compound enzyme concentration, and the temperature and duration of enzymolysis) and optimizing conditions for extraction of polysaccharides from sweet corn cobs (SCP) using response surface methodology (RSM) and a three-level, four-variable Box-Behnken design (BBD); and (ii) describing the hypoglycemic activity of polysaccharides extracted from sweet corn cobs.

MATERIALS AND METHODS

Chemicals. The sweet corn cobs were gathered from a cornfield in SuiHua, Heilongjiang Province, China in September 2014. The dried materials were pulverized to a fine powder in a grinder (FW177, Taisite Instrument Co. Ltd.), and then screened though an 80-mesh sieve. The powder was extracted using a Soxhlet apparatus with petroleum ether at 60-90 °C. The powder was then dried and stored in a desiccator at room temperature. All other reagents were analytically pure. Three replicates of all experiments were run in parallel, and all reported data are averages of the three replicates.

Preparation of crude polysaccharides

Water extraction of polysaccharides. A pretreated sample (10.0 g) was extracted for 2 h in water, at a ratio of 1 g raw material to 20 mL water, at a temperature of 100 °C. The suspension was centrifuged and this procedure was repeated two more times with the insoluble residue. The absorbance of the suspension was measured at 490 nm using an ultraviolet spectrophotometer.

Enzyme-assisted microwave extraction of crude polysaccharides. A pretreated sample (10.0 g) was soaked in 200 mL sodium hydroxide-citric acid-hydrochloric acid buffer and was then extracted with a compound enzyme (cellulase and pectinase at a ratio of 3:2). The ratio of water to raw material, compound enzyme concentration, and temperature and duration of enzymolysis were fixed. Enzymolysis followed microwave treatment for 8 min at 25 °C and 600 W. RSM extractions were conducted on the basis of a one-factor experimental design (Table 1). Thereafter the procedure followed was as for section of water extraction of polysaccharides (Pan & Wu, 2014).

Ultrasonic wave-assisted extraction of crude polysaccharides. Pretreated samples (10.0 g) were extracted in distilled water in a beaker, which was put in the Ultrasonic Cell Disruption System. Duration, power and temperature were varied across samples. Thereafter the procedure followed was as for section of water extraction of polysaccharides (You et al., 2013).

Analytical methods. The content of the polysaccharides was determined using the phenol-sulfuric method (Zhang, 1999). The yield of SCP was calculated as follows:

$$(1)\ \text{Polysaccharide yield (\%)} = \frac{\text{Extracted polysacharide content (g)}}{\text{Sweet corncob (g)}} \times 100$$

RSM experimental design. Based on our preliminary experiments, the four independent experimental variables we considered were: ratio of water toraw material (x_1, A); compound enzyme concentration (x_2, B); temperature of enzymolysis (x_3, C); and duration of enzymolysis (x_4, D). The extraction parameters were optimized using RSM. The results of

Table 1. Independent and response variables in the Box-Behnken experimental design.
Tabla 1. Variables independientes y de respuesta en el diseño experimental Box-Behnken.

Run	x_1(mL:g)	x_2 (%)	x_3 (°C)	x_4 (h)	Actual values Y (%)	Predicted values Y (%)
1	0 (20:1)	0 (1.0)	–1 (40)	–1 (1.5)	7.38	7.64
2	1 (25:1)	0	0 (50)	1 (2.5)	13.45	13.73
3	0	0	–1	1	7.90	7.39
4	1	0	–1	0 (2.0)	11.97	10.47
5	1	0	1 (60)	0	8.56	8.02
6	–1 (15:1)	1 (1.5)	0	0	9.23	9.88
7	1	0	0	–1	5.50	6.99
8	0	0	1	1	12.14	12.21
9	0	0	0	0	16.45	17.22
10	0	–1 (0.5)	0	1	8.20	8.41
11	–1	0	–1	0	6.36	5.96
12	0	0	0	0	17.47	17.22
13	1	–1	0	0	8.55	8.23
14	–1	–1	0	0	7.18	6.92
15	0	1	0	–1	8.69	7.54
16	0	0	0	0	17.96	17.22
17	0	–1	1	0	7.81	8.11
18	0	1	1	0	8.34	9.11
19	0	1	0	1	14.4	13.23
20	0	0	1	–1	8.37	7.20
21	0	1	–1	0	9.13	9.44
22	–1	0	0	1	10.39	9.51
23	1	1	0	0	9.72	10.31
24	–1	0	1	0	10.23	10.79
25	0	–1	–1	0	5.57	5.41
26	–1	0	0	–1	9.15	9.48
27	0	–1	0	–1	7.11	7.34
28	0	0	0	0	16.85	17.22
29	0	0	0	0	17.36	17.22

Note: The x variables represent the ratio of water to raw material (x_1), compound enzyme concentration (x_2), temperature of enzymolysis (x_3) and duration of enzymolysis (x_4). Y is the response variable. Figures in parentheses show what the actual parameter value is for a Box-Behnken value of –1, 0 or 1.
Nota: Las variables x representan la relación de agua al material de inicio (x_1), concentración de la enzima compuesta (x_2), temperatura de ruptura enzimática (x_3) y duración de la ruptura enzimática (x_4). Y es la variable respuesta. Los números entre paréntesis muestran que el valor real del parámetro es para un valor de Box-Behnken de -1, 0 ó 1.

the polysaccharide extraction were modeled by the following second degree polynomial equation (Shiet al., 1996; Fei, 2001; Vahid & Amir, 2013):

$$(2) \qquad Y = \sum \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i<j=2}^{4} \beta_{ij} X_i X_j$$

where Y is the response variable, β_0 is a constant, and β_i,β_{ii} and β_{ij} are coefficients of the linear, quadratic and interaction terms, respectively. X_i and X_j represent the coded independent variables. Based on an analysis of variance (ANOVA), the effect and regression coefficients of the individual linear, quadratic and interaction terms were measured. The regression

coefficients were used in the calculations necessary to generate three-dimensional plots and contour maps of polysaccharide yield (Fig. 1) (Cui, 2014).

Fractionation and FTIR analysis. The protein in the crude polysaccharide was removed using the Sevag method and the polysaccharide was decolored using resin. The protein and nucleic acid residue was detected at 190-400 nm and the presence of an absorption peak at 260-280 nm was assessed. The solution of polysaccharides was precipitated by placing it in a 10% concentration of alcohol, allowing it to settle overnight and collecting the precipitate the following day. This fraction was designated SCP10. This was placed in 20% alcohol and the procedure was repeated. This second fraction was designated SCP20. This was repeated to produce fractions SCP30 through to SCP90 (da Costa Gonzaga et al., 2005). The Fourier transform infrared spectroscopy (FTIR) were used to identify different fractionations.

Inhibition of α-amylase activity. In this experiment, we added 100 μL samples of different concentrations and fractions of SCP and α-amylase to 1 mL phosphate buffer (25 mM, pH 6.8). This reaction mixture was incubated for 20 min in a water bath at 37 ˚C. One hundred μL of soluble starch (1%) was added to the sample and the mixture was incubated for 5 min in a water bath at 37 ˚C. We then added 2 mL of DNS and incubated the mixture for 5 min in a water bath at 100 ˚C. This reaction mixture was diluted with 2 mL distilled water in an ice-water bath. The absorbance of the resultant sample and α-amylase at 540 nm was read using a spectrophotometer (Jiao, 2012).

Inhibition of α-glucosidase activity. A 1 mL sample was added to 2 mL phosphate buffer (67 mM, pH 6.8), and then added to 0.1 mL α-glucosidase (1 U/mL). This reaction mixture was incubated for 10 min at 37 ˚C. We then added 0.1 mL GSH (3 mmol/L) to the solution and initiated the reaction with 0.25 mL PNPG (10 mmol/L). The reaction was terminated with 2 mL $NaCO_3$ (0.1 mol/L) after 20 min at 37 ˚C. The absorbance of the resultant sample and α-glucosidase at 400 nm was read using a spectrophotometer. Inhibition of α-amylase and α-glucosidase activities was calculated using the following equation:

$$\text{Inhibition ratio} = 1 - \frac{A_{\text{sample}} - A_{\text{Buffer instead of enzyme}}}{A_{\text{Buffer instead of sample}} - A_{\text{control}}} \times 100 \quad (3)$$

RESULTS

ANOVA of the response surface quadratic model. Our Box-Behnken design consisted of 29 pairs of trials, 25 of which were factorial experiments. The independent variable values appeared in the four vertices and five zero trials were in the center of the area. The designed matrix, as well as the measured and predicted values for the response variable Y (SCP yield), are given in Table 1. The three levels of the four experimental variables (ratio of water to raw material, compound enzyme concentration, and temperature and duration of enzymolysis) were coded +1, 0 and –1 for the high, medium and low values respectively. Multiple regression analysis of the experimental data yielded the following second order polynomial equation giving the response variable Y:

$$Y=17.22+0.43x_1+1.26x_2+0.59x_3+1.69x_4-0.22x_1x_2-1.82x_1x_3+1.68x_1x_4-0.76x_2x_3+1.16x_2x_4+0.81x_3x_4-3.79x_{12}-4.59x_{22}-4.61x_{32}-3.5x_{42} \quad (4)$$

The Box-Behnken design was analyzed using ANOVA (Table 2). The model that was selected for testing was statistically significant ($P<0.0001$), and the coefficient of determination indicated an approximate linear fit ($R^2=0.9606$). The model explained about 92.12% of the change in response values (Chen et al., 2012).

The correlation coefficient ($R=0.9801$) showed that the experimental variance was small and that the linear model had a strong positive correlation. The model was highly significant ($P<0.0001$) and could be used to design a procedure for extracting SCP with maximum efficiency. The linear coefficients x_2 and x_4, the quadratic coefficients x_{12}, x_{22}, x_{32} and x_{42}, and the interaction coefficients x_1x_3, x_1x_4 and x_2x_4 were significant at $P<0.05$; the remaining coefficients (x_1, x_3, x_1x_2, x_2x_3 and x_3x_4) were not significant.

Optimization of extraction conditions. The temperature and duration of enzymolysis were significantly correlated with SCP yield. The three-dimensional (3D) response surface and two-dimensional contour plots simulated by Design-Expert software using Eq. (4) predicted well the relationships between the independent and dependent factors (Fig.1) (You et al., 2013). In these plots we investigated the effects on SCP yield of varying two of the continuous variables while keeping the other two constant at their respective intermediate levels (i.e. zero in the Box-Behnken design) (Shen et al., 2014).

When temperature and duration of enzymolysis were held constant, SCP yield increased with the ratio of water to raw material up to 20:1 and compound enzyme concentration up to 1.25% (Fig.1a). Above these levels, however, yield decreased slightly. When compound enzyme concentration and duration were held constant, the yield increased slightly with an increasing ratio of water to raw material (up to 22.5:1), concentration, and temperature and duration of enzymolysis on SCP yield, and with temperature up to 55 ˚C (Fig.1b). Increasing the ratio and duration up to 20:1 and 2 h resulted in a strong increase in yield (Fig.1c), as did increasing the compound enzyme concentration up to 1.25% and temperature up to 40 ˚C (Fig. 1d). Increasing the temperature together with compound enzyme concentration caused yield to increase up to a concentration of 1%, after which it decreased again

Table 2. Analysis of variance and significance test of the regression equation coefficients.
Tabla 2. Análisis de varianza y prueba de significancia de los coeficientes de la ecuación de regresión.

Model	Coefficient estimate	DF	SD	Sum of squares	F	P
Model	17.22	14	1.06	383.74	24.38	< 0.0001
x_1	0.43	1	0.31	2.26	2.01	0.1779
x_2	1.26	1	0.31	18.98	16.88	0.0011
x_3	0.59	1	0.31	4.25	3.78	0.0723
x_4	1.69	1	0.31	34.27	30.49	< 0.0001
x_1^2	–3.79	1	0.42	93.41	83.09	< 0.0001
x_2^2	–4.59	1	0.42	136.65	121.56	< 0.0001
x_3^2	–4.61	1	0.42	137.92	122.68	< 0.0001
x_4^2	–3.50	1	0.42	79.28	70.53	< 0.0001
x_1x_2	–0.22	1	0.53	0.19	0.17	0.6844
x_1x_3	–1.82	1	0.53	13.25	11.79	0.0040
x_1x_4	1.68	1	0.53	11.26	10.01	0.0069
x_2x_3	–0.76	1	0.53	2.30	2.04	0.1750
x_2x_4	1.16	1	0.53	5.34	4.75	0.0469
x_3x_4	0.81	1	0.53	2.64	2.35	0.1476
Lack of fit		10		14.38	4.23	0.0885
Error		4		1.36		
Total		28		399.48		
$R = 0.9801, R^2 = 0.9606, R^2_{Adj} = 0.9212$						

(Fig.1e). Finally, when duration and temperature were varied, yield increased as they increased, and was maximized at 2.15 h and 53 ˚C (Fig. 1f).

Optimum extraction conditions (X_1 = 20.1:1, X_2 = 1.17%, X_3 = 50.06 ˚C, X_4 = 2.3 h) for SCP extraction were estimated using the model equation by solving the regression equation and analyzing the response surface contour plots. The theoretical polysaccharides yield predicted under these conditions was 17.62%.

In each row, two of these variables are held constant at their intermediate levels (zero in the Box-Behnken design) while the remaining two are varied. Colors indicate relative yield, which increases from blue through to orange-red.

Verification of the predictive model. To test the predictive model we ran three verification experiments. In these we set the four experimental variables to rounded-off values close to the values identified as optimal above. The extraction conditions in these experiments were thus: microwave power 600 W, microwave time 8 min, ratio of water to raw material 20:1, compound enzyme concentration 1.2%, temperature of enzymolysis 50 ˚C, and duration of enzymolysis 2.3 h. The yield was 17.58%, which was close to the predicted value of 17.62%. There was no significant difference between the experimental and predicted yields, which confirmed that our response model was accurate and appropriate for SCP extraction.

Fractionation and FTIR analysis. Alcohol concentration affected the quantity of polysaccharides precipitated. In general, the lower the ethanol concentration, the greater the polysaccharides yield. The proportion of polysaccharides in the precipitate was greatest, however, at ethanol concentrations of 30%, 50% and 80% (Fig. 2). We therefore used these concentrations (designated SCP30, SCP50 and SCP80, respectively) to perform a step-by-step analysis of polysaccharides precipitation in alcohol. We therefore analyzed the organic functional groups and investigated the hypoglycemic activity of these three fractions (Figs. 3, 4, 5 and 6). Figure 3 shows the FTIR spectra of the three polysaccharides from sweet corncobs. Stretching vibration of O-H, saturated C-H was observed at 3400–3500/cm and 2929–2989/cm. A stretching peak appeared at around 1636/cm with a weak peak at around 1385/cm, suggesting the presence of carboxyl groups. The absorption band at 1000–1120/cm suggested that the three polysaccharides contained C-O-C or C-O-H in their structures. An absorbance at approximately 890/cm strongly structure of the three polysaccharides. In conclusion, no significant difference was observed in the FTIR of the three polysaccharides and

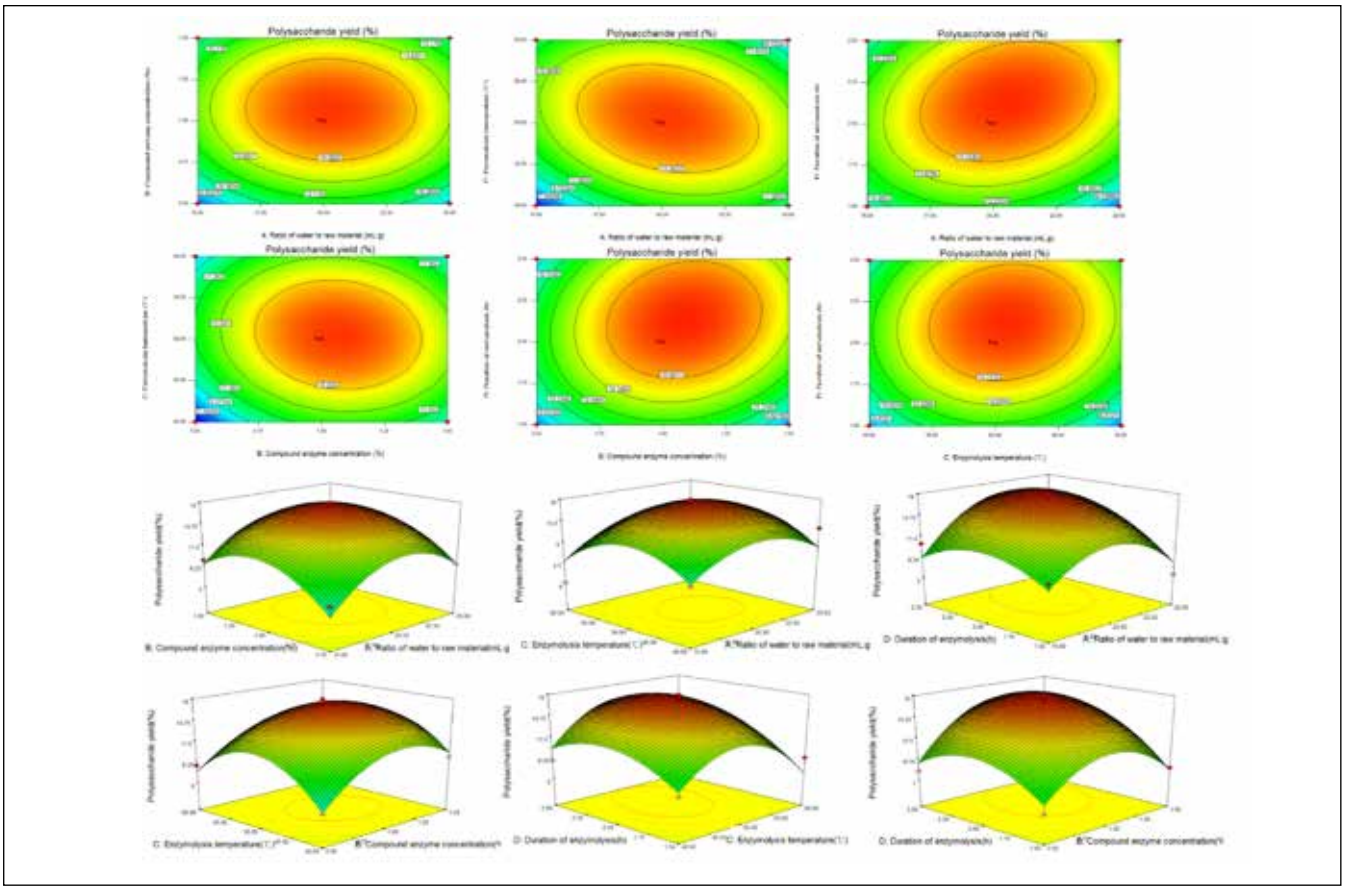

Fig. 1. Response surface plots and contour plots showing the effects of varying independent extraction procedure variables (ratio of water to raw material, compound enzyme concentration, and temperature and duration of enzymolysis) on SCP yield.
Fig. 1. Gráficos de superficie de respuesta y gráficos de contorno mostrando los efectos de variar variables de procedimientos de extracción independientes [relación de agua al material inicial (materia prima), concentración de enzima compuesta, y temperatura y duración de la ruptura enzimática] sobre el rendimiento SCP.

FTIR spectroscopy did not clearly distinguish the three polysaccharides from each other.

The inhibitory effects of SCP on α-amylase and α-glucosidase increased with increasing SCP concentration (Fig. 4). There was a strong dose-effect relationship, and the greatest inhibition (38.3% for α-amylase and 58.7% for α-glucosidase) occurred at the highest concentration tested (16 mg/mL). The IC50 under these experimental conditions was 20.91 mg/mL for α-amylase and 12.47 mg/mL for α-glucosidase. Inhibition of α-amylase (Fig. 5) and α-glucosidase (Fig. 6) activities by the three SCP fractions was stronger than that of raw SCP at the same concentration. SCP80 had the strongest inhibitory effects, at 1.4 and 1.7 times that of raw polysaccharides for α-amylase and α-glucosidase, respectively.

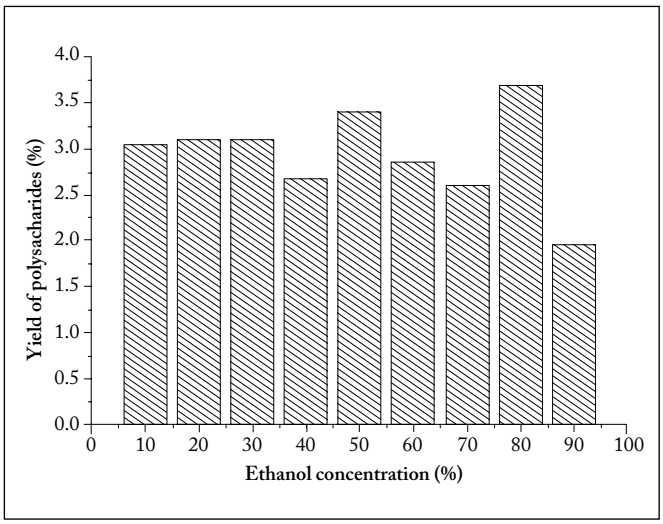

Fig. 2. Fraction of polysaccharides precipitated under different alcohol concentrations.
Fig. 2. Porcentaje de polisacáridos precipitados con diferentes concentraciones de etanol.

DISCUSSION

In this study, BBD combined with RSM is employed to optimize the enzyme-assisted microwave extraction pro-

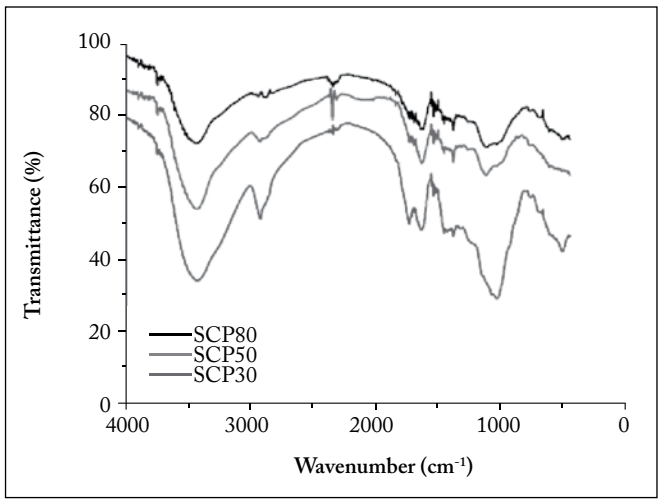

Fig. 3. FITR spectra of different fractions of SCP.
Fig. 3. Espectro FITR de diferentes fracciones de SCP.

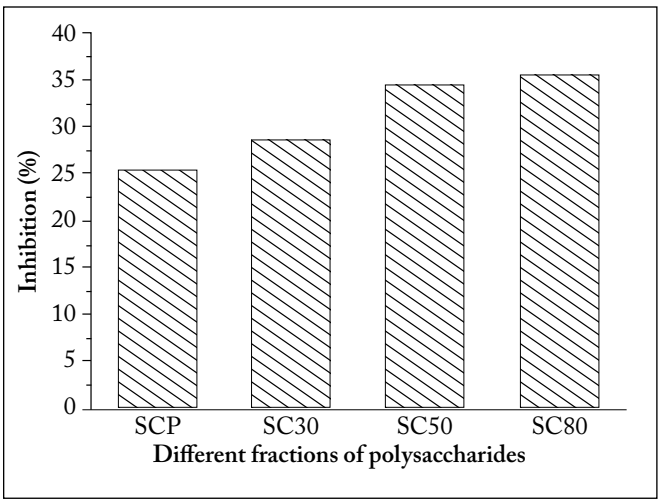

Fig. 5. Inhibitory effects of different fractions of SCP, at a concentration of 5 mg/mL, on α-amylase activity.
Fig. 5. Efectos inhibitorios de diferentes fracciones de SCP, a una concentración de 5 mg/mL; sobre la actividad de la α-amilasa.

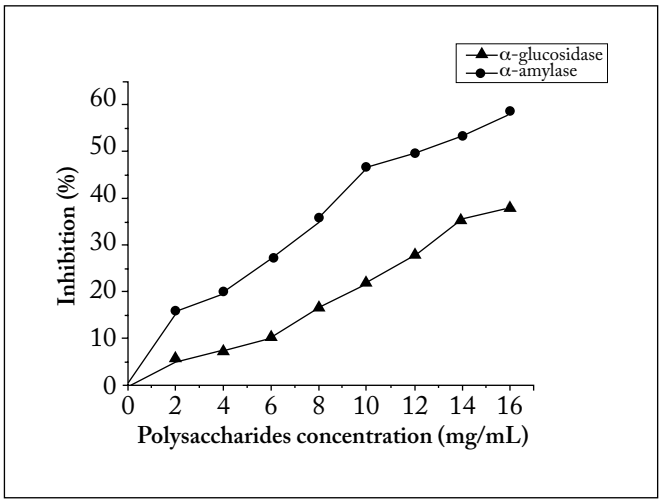

Fig. 4. Inhibition of α-amylase and α-glucosidase activities by polysaccharides at different concentrations.
Fig. 4. Inhibición de las actividades de α-amilasa y la α-glucosidasa por polisacáridos a diferentes concentraciones.

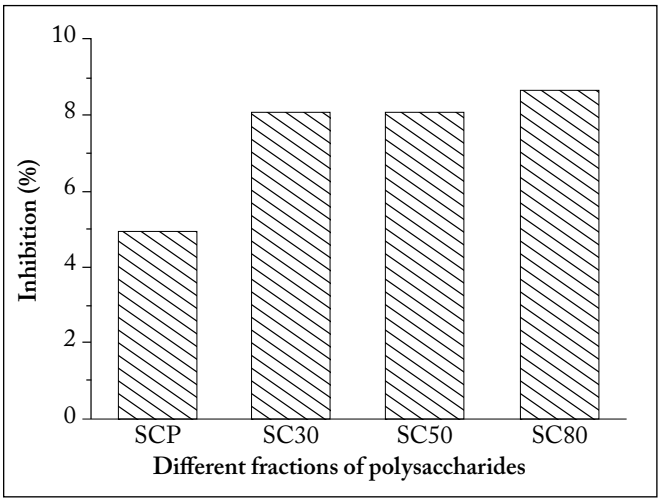

Fig. 6. Inhibitory effects of different fractions of SCP, at a concentration of 2 mg/mL, on α-glucosidase activity.
Fig. 6. Efectos inhibitorios de diferentes fracciones de SCP, a una concentración de 2 mg/mL, sobre la actividad de α-glucosidasa.

cess of polysaccharides from sweet corncob (Wan et al., 2015). The actual experimental values identified under the optimal enzyme-assisted microwave extraction conditions are closely correlated to the predicted ones. Enzyme-assisted microwave extraction is higher polysaccharides yield compared with other methods, such as water extraction or ultrasonic-assisted extraction. The results suggested the linkage of β-glycosides in the molecular structure of SCP. α-Amylase and α-glucosidase inhibitory activities tests *in vitro* indicate that SCP has strong inhibitory activities (Xi, et al., 2016). Because of its inhibitory effects on α-amylase and α-glucosidase activities, SCP may be beneficial in the prevention and treatment of postprandial hyperglycemia in diabetes patients. Furthermore, its suppression of non-enzymatic glycosylation suggests that it may have potential for improving diabetes conditions. These treatment possibilities warrant further investigation.

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