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Factorial Design to Optimize Dextran Production by the Native Strain *Leuconostoc mesenteroides* SF3

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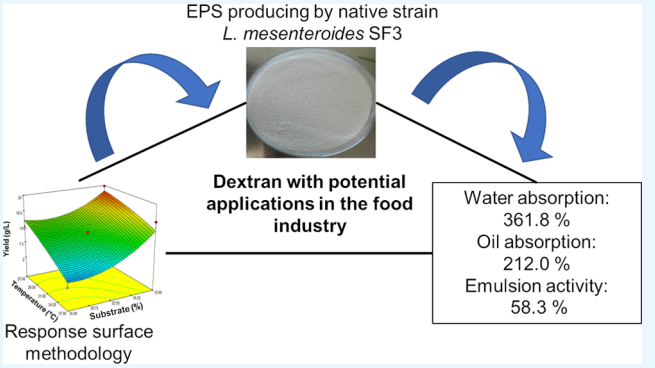
ABSTRACT: Dextran is an extracellular bacterial polysaccharide for which industrial applications have been found in different areas. Several researchers have optimized the fermentation conditions to maximize dextran production. This study aimed to characterize the dextran of *Leuconostoc mesenteroides* SF3, which was isolated from the aguamiel of *Agave salmiana*. To maximize the yield of dextran, the effects of sucrose concentration, temperature, and incubation time were studied. The experiments were conducted using a factorial design and a response surface methodology. *L. mesenteroides* SF3 produced a maximum yield of dextran (23.8 g/L ± 4) after 16 h of incubation at 25 °C with 10% sucrose. The functional properties such as water absorption capacity, oil absorption capacity, and emulsion activity of this unique dextran were 361.8% ± 3.1, 212.0% ± 6.7, and 58.3% ± 0.7, respectively. These properties indicate that the dextran produced by *L. mesenteroides* SF3 is a high-quality polysaccharide with potential applications in the food industry, and the optimized conditions for its production could be used for the commercial production of this dextran, which have significant industrial perspectives.



1. INTRODUCTION

Dextran is an exopolysaccharide (EPS) formed by α-D-glucopyranosyl residues with α-(1,6) links with either α-(1,2), α-(1,3), or α-(1,4) branching.[1,2] This EPS is a secondary metabolite of the sucrose fermentation by extracellular enzyme dextransucrase from different strains of the genera *Weissella*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, and *Lactobacillus*. Each strain produces a unique dextran, which is differentiated by its physicochemical characteristics and its glycosidic linkages.[2−5] Dextran has different applications in the food, pharmaceutical, and chemical industries.[6] Due to the many potential applications of this EPS, it is important to optimize the fermentation processes to obtain a higher yield of dextran production. Several studies have reported that both the yield and molecular weight of dextran depend on the fermentation conditions (sucrose concentration, temperature, and incubation time).[2,7] *Leuconostoc mesenteroides* NRRL B-512F produces the highest yield of commercial dextran; it contains 95% α-(1,6) linkages in the main chain and few branches (5%) in α-(1,3) and α-(1,4).[8] Keeping in mind the significance and multiple uses of different dextrans, the aim of this paper was to optimize the dextran yield produced by *L. mesenteroides* SF3, a strain isolated from aguamiel (edible sweet sap obtained from *Agave salmiana*, a traditional Mexican drink).[9] This dextran possesses convenient rheological properties for industrial applications, which were previously studied.[2] Therefore, the goal of this work was to optimize the dextran synthesis as a function of parameters such as the sucrose concentration, temperature, and incubation time. The experimental design chosen was a factorial design, and the optimization was accomplished following a response surface methodology (RSM).

2. RESULTS AND DISCUSSION

L. mesenteroides SF3, isolated from the aguamiel of *A. salmiana*, has been reported for its potentially probiotic characteristics, especially its greater pH and bile tolerance, in vitro adhesion to intestinal mucus, and its suppressed pathogen growth under in vitro conditions.[9] Another of the remarkable characteristics of this strain has been its maximum dextran production compared with three other strains isolated from the same aguamiel of *A.*

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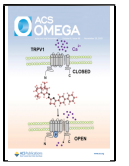

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Table 1. Dextran Yield and Sucrose Consumption by *L. mesenteroides* SF3 and NRRL B-512F for Each Experiment[a]

	SF3		NRRL B-512F	
experiment	dextran yield (g/L)	sucrose consumption (%)	dextran yield (g/L)	sucrose consumption (%)
1	18.1 ± 0.9^{b}	$8.1 \pm 0.4^{C,D}$	$25.1 \pm 5.9^{i,j}$	11.1 ± 2.6^{H}
2	$8.7 \pm 0.8^{d,e,f}$	$3.8 \pm 0.4^{E,F}$	$9.0 \pm 5.9^{k,l}$	$4.0 \pm 2.6^{J,K,L}$
3	13.0 ± 1.0^{c}	$5.8 \pm 0.5^{D,E}$	7.1 ± 0.3^{l}	$3.2 \pm 0.1^{J,K,L}$
4	0.4 ± 0.0^{h}	0.2 ± 0.0^{F}	1.7 ± 1.3^{l}	0.7 ± 0.6^{L}
5	23.8 ± 4.0^{a}	23.8 ± 4.0^{A}	$19.5 \pm 1.4^{j,k}$	19.5 ± 1.4^{G}
6	$12.6 \pm 1.0^{c,d}$	$3.6 \pm 0.3^{E,F}$	5.5 ± 0.9^{l}	$1.6 \pm 0.3^{K,L}$
7	19.0 ± 1.1^{b}	19.0 ± 1.1^{B}	5.4 ± 0.7^{l}	$5.4 \pm 0.7^{I,J,K}$
8	$11.2 \pm 1.0^{c,d,e}$	$5.0 \pm 0.4^{D,E}$	6.3 ± 3.4^{l}	$2.8 \pm 1.5^{J,K,L}$
9	$11.2 \pm 1.0^{c,d,e}$	$5.0 \pm 0.5^{D,E}$	7.8 ± 1.5^{l}	$3.5 \pm 0.7^{J,K,L}$
10	$5.8 \pm 0.3^{f,g}$	$2.6 \pm 0.1^{E,F}$	0.3 ± 0.2^{l}	0.1 ± 0.1^{L}
11	$10.9 \pm 0.6^{c,d,e}$	$4.9 \pm 0.3^{D,E}$	7.6 ± 2.3^{l}	$3.4 \pm 1.0^{J,K,L}$
12	$2.3 \pm 0.7^{g,h}$	0.7 ± 0.2^{F}	1.0 ± 0.8^{l}	0.3 ± 0.2^{L}
13	$7.8 \pm 1.0^{e,f}$	$2.2 \pm 0.3^{E,F}$	6.3 ± 0.4^{l}	$1.8 \pm 0.1^{K,L}$
14	18.2 ± 2.7^{b}	18.2 ± 2.7^{B}	0.2 ± 0.1^{l}	0.2 ± 0.1^{L}
15	$10.0 \pm 0.5^{c,d,e,f}$	10.0 ± 0.5^{C}	6.2 ± 0.9^{l}	$6.2 \pm 0.9^{I,J}$
16	$12.5 \pm 0.5^{c,d}$	$3.6 \pm 0.2^{E,F}$	31.8 ± 10.7^{i}	$9.1 \pm 3.1^{H,I}$
17	$11.2 \pm 1.0^{c,d,e}$	$5.0 \pm 0.4^{D,E}$	4.8 ± 0.6^{l}	$2.1 \pm 0.3^{J,K,L}$

[a]Results expressed with the mean ± standard deviation for each sample ($n = 3$). Different letters in each column indicate a significant difference ($p < 0.05$) according to Tukey's test. Lowercase letters are used for dextran yield (g/L) and capital letters for sucrose consumption.

Table 2. Analysis of Variance for Dextran Yield (g/L)[a]

	L. mesenteroides SF3					*L. mesenteroides* NRRL B-512F				
source	SS	DF	MS	*F*-value	*p*-value	SS	DF	MS	*F*-value	*p*-value
model	515.8	9	57.3	8.2	<0.05	1128.2	9	125.4	8.7	<0.05
A	160.5	1	160.5	23.1	<0.05	22.9	1	22.9	1.6	0.2
B	164.7	1	164.7	23.7	<0.05	845.0	1	845.0	58.9	<0.05
C	102.8	1	102.8	14.8	<0.05	21.7	1	21.7	1.5	0.3
residual	48.7	7	7.0			100.4	7	14.3		
pure error	2.8	4	0.7			5.9	4	1.5		
CV			22.8%					44.2		
R^2			91.4%					91.8		
R^2 (adj)			80.3%					81.3		

[a]SS = Sum of square; DF = degree of freedom; MS = mean square; CV = coefficient of variation; A = sucrose concentration (%); B = temperature (°C); C = incubation time (h).

salmiana and with the commercial strain *L. mesenteroides* NRRL B-512F.[2] The EPS produced by this strain is dextran. The FTIR and 1H and 13C NMR spectral analyses confirmed that the polysaccharide produced by *L. mesenteroides* SF3 contains both linear dextran with α (1 → 6) linkages and with α (1 → 3) branching. The rheological behavior of dextran solutions of different concentrations exhibited typical shear thinning and weak gel properties.[2] The rheological properties of this dextran give it a great potential as a thickener or a stabilizer.

2.1. Factorial Design to Optimize Dextran Production. The combined effect of sucrose concentration, temperature, and incubation time was studied and optimized through a factorial design with 17 experiments. The dextran yield and sucrose consumption in each run are given in Table 1. The following second-order regression equations were obtained to explain the production of dextran by *L. mesenteroides* SF3 and NRRL B-512F in terms of their initial values

For SF3

$$Y = -6.74 - 0.70A + 1.12B + 2.62C - 0.02AB - 0.01AC - 0.02BC + 0.02A^2 - 0.02B^2 - 0.04C^2$$

For NRRL B-512F

$$Y = 104.56 + 0.86A - 7.63B + 4.26C - 0.04AB - 5 \times 10^{-5}AC - 0.09BC + 0.01A^2 + 0.13B^2 - 0.04C^2$$

where Y is the dextran yield (as a response) and sucrose (A), temperature (B), and incubation time (C) are independent factors influencing the dextran production. The models calculated with the three variables A, B, and C feature different explanatory capacities. When the model was designed, the R^2 coefficient of the linear regression (considering A, B, and C) was 0.7580. However, when the model was reformulated with interaction components (namely, $A*B$, $A*C$, and $B*C$), the predictive capacity of the model improved, with $R^2 = 0.7821$. Finally, the best fit was obtained considering a regression with linear, cross-product, and quadratic components, this time featuring an $R^2 = 0.9137$. The regression-based R^2 coefficient was similar for both strains SF3 ($R^2 = 0.9137$) and NRRL B-512F ($R^2 = 0.9183$). It shows that the model explains about 91% of the variability on dextran production in both strains and defines the significance of the model and also that the

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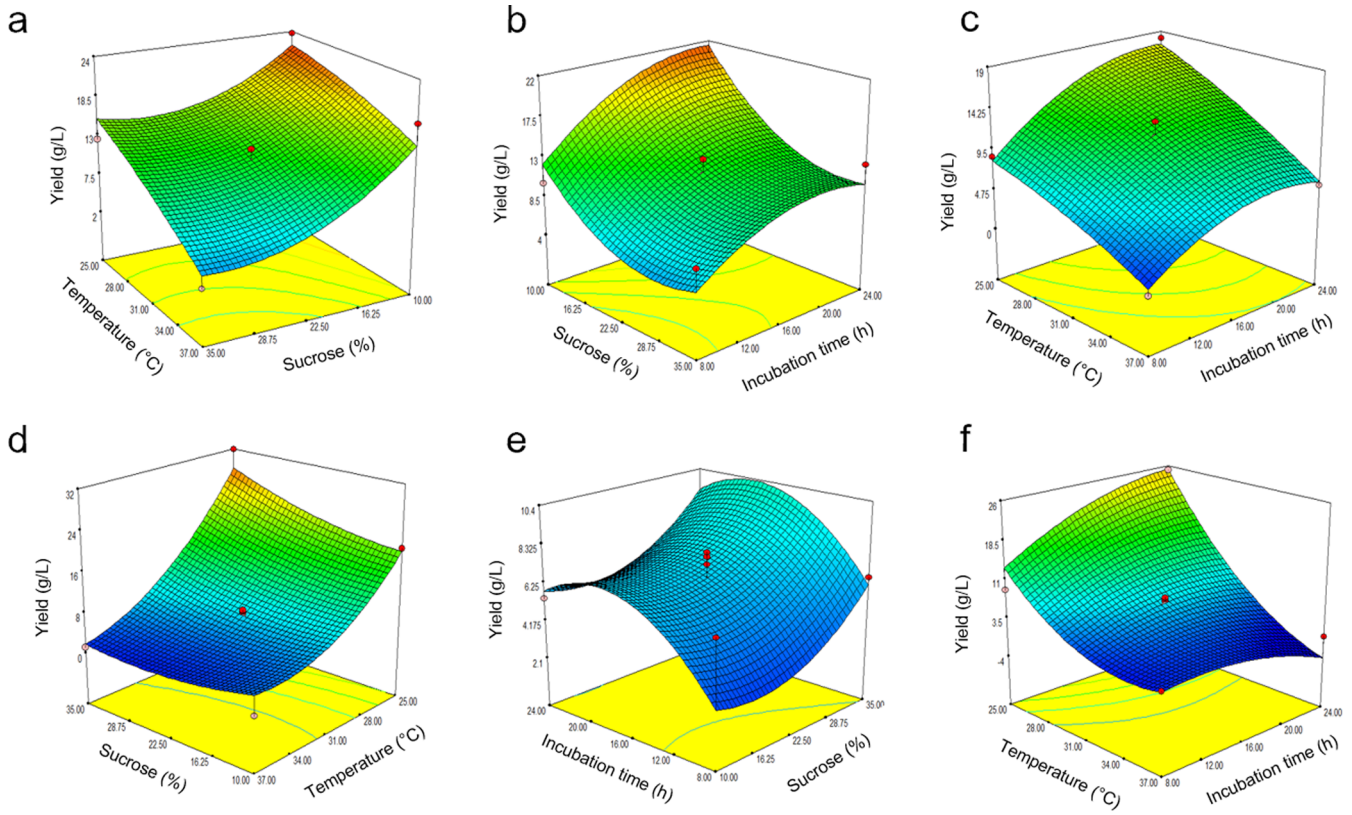

Figure 1. Surface plots showing the interplay of all the possible pairs of independent variables on dextran yield by *L. mesenteroides* SF3. (a) Temperature vs sucrose concentration. (b) Sucrose concentration vs incubation time. (c) Temperature vs incubation time and by *L. mesenteroides* NRRL B-512F. (d) Sucrose concentration vs temperature. (e) Incubation time vs sucrose concentration. (f) Temperature vs incubation time.

predicted model is more accurate than the models that include fewer terms (regression equations not shown).

A highly significant quadratic regression model was obtained with a similar *F*-value in both strains SF3 ($F = 8.2$) and NRRL B-512F ($F = 8.7$), suggesting that the model is significant (Table 2). The combined effects of three independent variables (sucrose concentration, temperature, and incubation time) significantly contributed to maximize the dextran production for strain *L. mesenteroides* SF3. In strain *L. mesenteroides* NRRL B-512F, only the temperature significantly contributed to optimize the dextran production.

The graphical representation of the interaction of three independent variables on dextran yield was investigated via surface plots (Figure 1a–f). These plots show the relationships between sucrose concentration, temperature, and incubation time, whose effect on the yield was more significant in the case of the strain SF3 than in the case of the strain NRRL B-512F. *L. mesenteroides* SF3 produced a maximum dextran yield after 16 h of incubation at 25 °C with 10% sucrose. The strain SF3 featured the largest amount of dextran (23.8 g/L) compared to the commercial strain NRRL B-512F (19.5 g/L) under the same conditions (16 h of incubation at 25 °C with 10% sucrose). Also, the amount of dextran produced by this strain (SF3) was higher than that by some other strains reported in the literature, which used sucrose concentrations higher than 20% w/v[3].[10,11] It is important to highlight that the strain isolated from the aguamiel of *A. salmiana* (*L. mesenteroides* SF3) optimized the production of dextran with the minimum amount of sucrose (10%); however, the commercial strain NRRL B-512F produced its highest yield of dextran when it used the highest amount of sucrose (35%).

The strain SF3 exhibited a substrate inhibitory effect when the sucrose concentration was higher.[2] These results were similar to those found in other studies. Sarwat et al. reported that *L. mesenteroides* CMG713 produced a maximum dextran yield after 20 h of incubation at 30 °C with 15% sucrose at pH 7.0, and the strain exhibited a substrate inhibitory effect when the sucrose concentration was higher.[12] Kanimozhi et al. reported that the strain *Weissella cibaria* NITCSK4 produced a higher amount of dextran with 15.5% sucrose, noting that a further increase in sucrose concentration decreases the dextran production by NITCSK4.[13] Temperature, sucrose concentration, and incubation time had a negative effect on the dextran production as shown in Figure 1a–f. The dextran yield was affected by high temperatures due to the slow growth of *L. mesenteroides* SF3 and the loss of enzyme activity of dextransucrase.[14]

The influence of the independent variables stated above on the dependent responses (dextran yield) can be better understood by examining the contour plots (Figure 2a–d), which indicate the response of the dextran yield to sucrose concentration and temperature with a constant incubation time of 16 h. Figure 2a,b shows an optimized dextran yield (prediction value of 22.1 g/L) at a low sucrose concentration (10%) and temperature (25 °C) for the strain SF3. However, for the commercial strain NRRL B-512F, the predicted value is 18.8 g/L under the same culture conditions (Figure 2c,d).

Several studies have reported that physical and chemical conditions, such as temperature and sucrose concentration, play an important role not only in the production of dextran but also in its properties.[3−5] According to Aman et al., both a 5° increase in temperature (20–25 °C) and twice the amount

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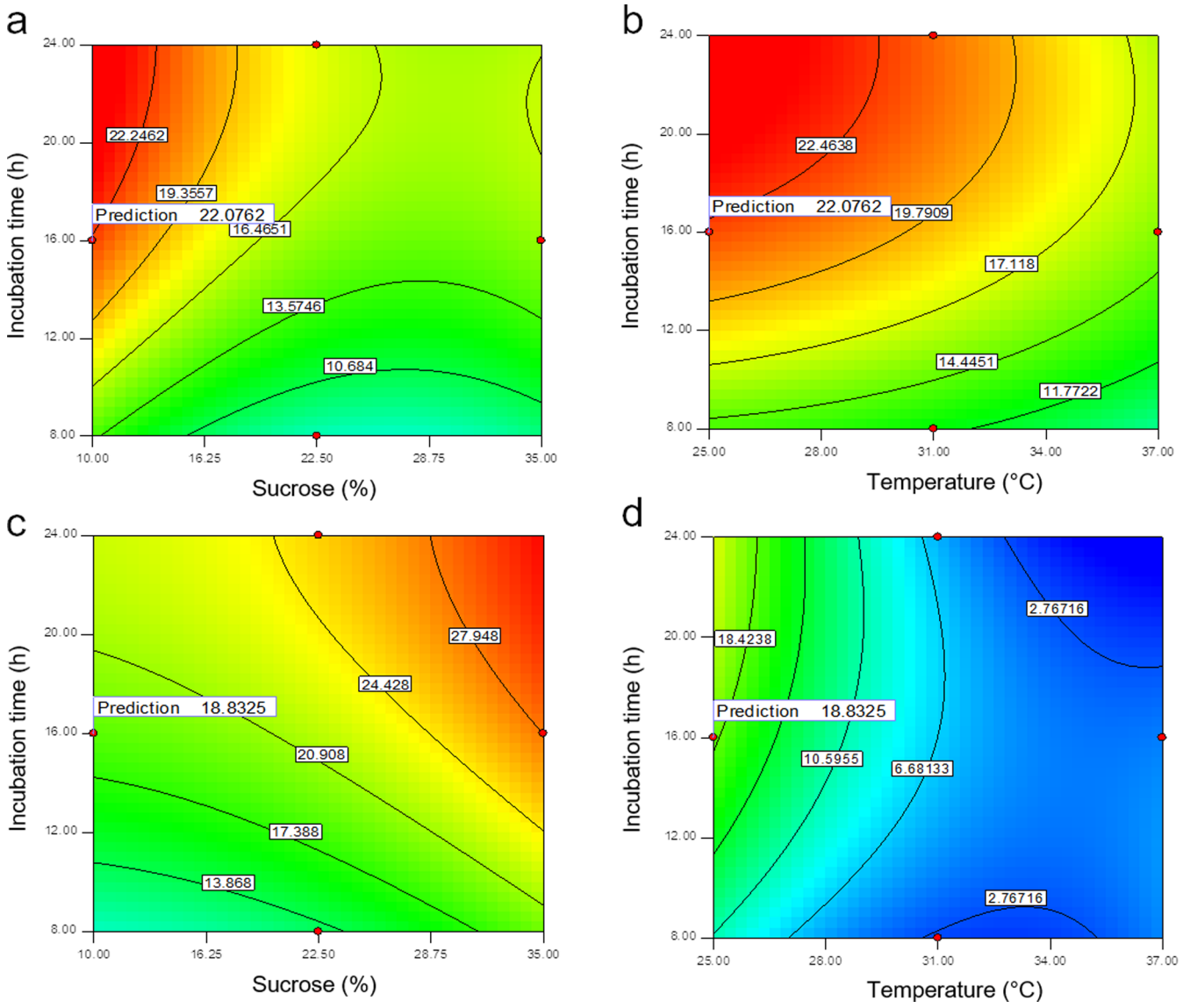

Figure 2. Contour plots of dextran yield for *L. mesenteroides* SF3. (a) Incubation time vs sucrose concentration. (b) Incubation time vs temperature and for *L. mesenteroides* B512F. (c) Incubation time vs sucrose concentration. (d) Incubation time vs temperature.

of sucrose (5−10% w/w) double the viscosity and slightly increase the density of the dextran.[3] However, the goal of this work was to evaluate the yield of dextran production as a function of the culture conditions (sucrose concentration, temperature, and incubation time).

The differences in the amount of dextran produced could be due to the composition of the medium and the growth conditions.[15−18] The production of this type of EPS depends on the carbon sources, sucrose concentrations, nitrogen sources, inorganic salts, pH, temperature, and fermentation time. According to Majumder et al., the production of dextran is strongly influenced by the presence of sucrose, peptone, and beef extract. They report in their study that the presence of higher sucrose concentrations (50 g/L) and the requirement of higher nitrogenous sources (peptone and beef extract each at 25 g/L) promote increased dextran production. On the other hand, they report that K_2HPO_4 acts as a buffering agent for the culture medium and thus promotes microbial growth and dextransucrase release from *L. mesenteroides* NRRL B-640, but it did not affect the dextran production.[19] However, the production of dextran is a biotechnological process affected by multiple variables, whose optimization is a time-consuming task, which entails a complex analysis. In this work, the process was optimized by using only three variables: sucrose concentration, temperature, and incubation time.

2.2. Physicochemical Properties. The dextran produced under optimum conditions (16 h of incubation at 25 °C with 10% sucrose) by the strain SF3 isolated from aguamiel had a moisture of 8.8% and a water activity of 0.3 (Table 3). These are directly related to the shelf-life of food products.[20] The content of moisture in the powders should generally be lower than 5%, so they can be stored for a long time. In addition to this, products with a water activity lower than 0.6 are considered stable.[20,21] The hygroscopicity of dextran was of 15 g of adsorbed moisture per 100 g of dry solids, which means that dextran has little ability to take up water from the environment and consequently to not change its physical properties.[22,23] The solubility, defined as the chemical property of a solid to be dissolved in a solvent (e.g., water), is an important parameter in the development of new powder-type

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Table 3. Physicochemical Properties of Dextran Produced by *L. mesenteroides* SF3[a]

parameter	results
moisture content (%)	8.8 ± 0.3
water activity	0.3 ± 0.0
hygroscopicity (g 100 g^{-1})	15.1 ± 3.4
solubility in water (%)	56.7 ± 2.4
absorption capacity in water (%)	361.8 ± 3.1
absorption capacity in oil (%)	212.0 ± 6.7
emulsion activity (%)	58.3 ± 0.7
apparent density (g/cm^3)	0.7 ± 0.0
packing density (g/cm^3)	0.7 ± 0.0

[a]Results expressed as the mean ± standard deviation for each sample (n = 3).

products. This dextran displayed a solubility of 56.7% and a water absorption capacity of 361.8%. Dextran is water-soluble and features a good water-holding capacity due to the absorptive structure of the EPS, which can contain large amounts of water through hydrogen bonds.[2,24] This dextran could be used in the food industry as a stabilizing, an emulsifying, and a water-binding agent.[12]

The oil absorption capacity of this dextran was 212%, making it useful in products in which fat absorption is desired and those that require flavor retention and improved palatability, such as bakery products.[25] The emulsion activity of this dextran was 58.3%, and it is illustrated in Figure 3. This value was higher than those reported by Chandra et al., who recorded that the emulsion activity for several flours ranged between 41.49 and 44.69%.[26] This value of the EPS produced by SF3 is also a good indicator to be used in the food industry, mainly in comminuted meat products, salad dressing, frozen desserts, and mayonnaise.[26] On the other hand, the apparent and packing densities of dextran were 0.7 and 0.7 g/cm^3, respectively. These values were similar to those reported by Chandra et al., who recorded that the apparent density for several flours ranged between 0.762 and 0.820 g/cm^3.[26] Therefore, the availability of low-density dextran would be advantageous in the formulation of complementary foods.[27]

The dextran produced by the strain isolated from aguamiel had an average molecular weight of 1 455 072 Da, as shown in Figure 4. The molecular weight of homopolysaccharides, such as dextran, usually changes depending on the producing strain and polymer type.[28] The average molecular weights reported in the literature for homopolysaccharides range between 1×10^6 and 21×10^6 Da for LAB.[28,29] This result suggested that the dextran produced by *L. mesenteroides* SF3 is of a high molecular weight and can be used in the food and pharmaceutical industries; it can also be hydrolyzed into smaller molecular weight fractions.[30]

Sarwat et al. evaluated the physicochemical properties of the dextran produced by *L. mesenteroides* CMG713, finding a higher percentage of moisture (10.2%), higher molecular weight (5000−20,000 kDa), and lower percentage of solubility (5%) compared to the dextran produced by the strain SF3.[12] These physical variations between one dextran and the other may be due to differences in the molecular structure of the two strains, particularly differences in the type of branching they feature.[2,12] The physicochemical properties determine the possible applications that these types of dextrans may have but are needed to evaluate the bioavailability as a novel ingredient in some processed foods and pharmaceutical industries.

3. CONCLUSIONS

In this study, the following findings can be highlighted: the central composite design based on the RSM was used to predict the optimum conditions (16 h of incubation at 25 °C with 10% sucrose) to produce the highest quantity of dextran (23.8 g/L). The native strain *L. mesenteroides* SF3 produced a dextran of 1.4 MDa, which can be used in the food industry due to its high molecular weight. Furthermore, the characterization of this dextran is the first step to take before pursuing any technological application. In this way, knowing the chemical and physical properties of dextran such as solubility, moisture content, water activity, hygroscopicity, absorption capacity, emulsion activity, and density will allow us to know if this dextran can used as a thickener, a carrier for drug delivery, an emulsifier, an additive in the food industry, or as part of a starter culture. The findings in this study lay the foundation for proposing large-scale production of SF3 dextran and they open the possibility for other studies, for example, using dextran in combination with nanomaterials or copolymers.

4. MATERIALS AND METHODS

4.1. Production and Purification of Dextran. *L. mesenteroides* subsp. *mesenteroides* SF3 (GenBank: KR362874), previously isolated from aguamiel of *A. salmiana*,[9] was employed in the present research. In addition, one commercial strain, *L. mesenteroides* NRRL B-512F, was used as the control (Culture Collection of CINVESTAV, México). To produce dextran, the organism was grown in a medium

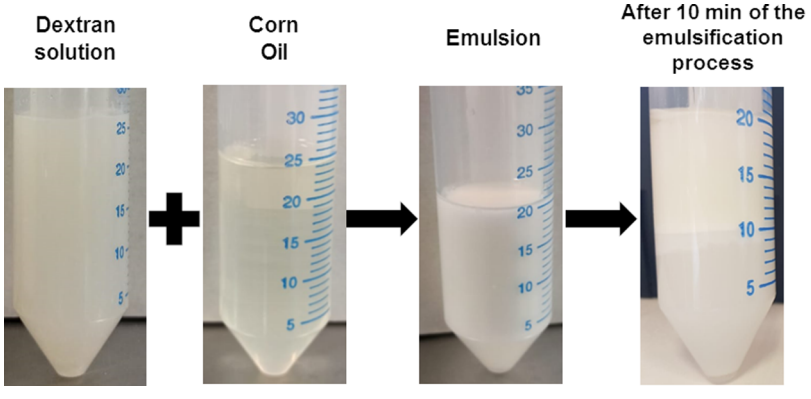

Figure 3. Emulsification process of dextran produced by SF3.

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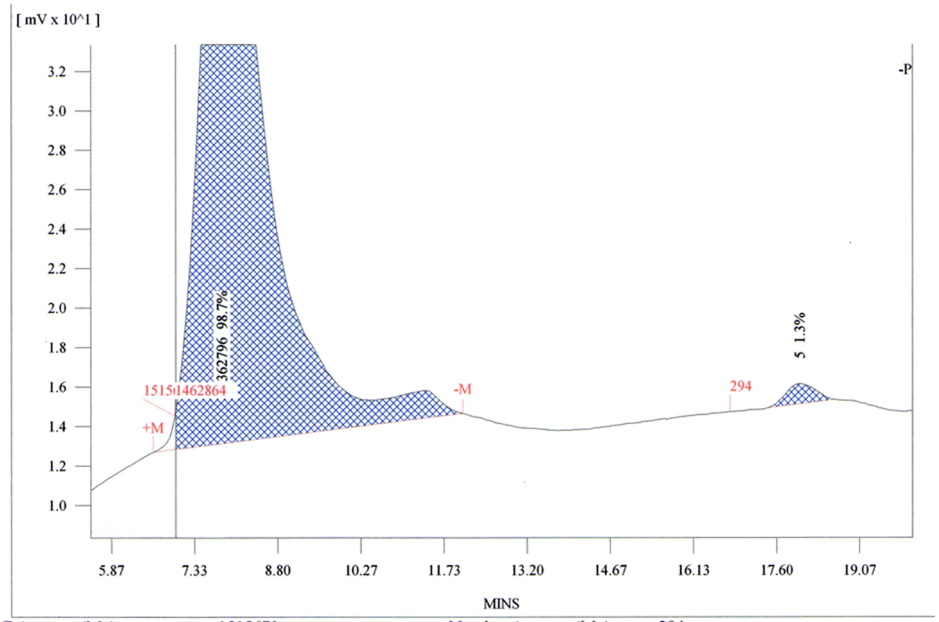

Z Average (Mz) : 1515670 Number Average (Mn) : 294
(Z + 1) Average : 1554482 Weight Average (Mw) : 1462864
PolyDispersity (Mw/Mn) 4977.068 Total Area : 2.7892433e+006 (microvolt seconds)

Peak Molecular Weight Report

Peak	Component	RT	Area	%Area	PMwt	Mw	Mn	Mw/Mn	StartMw	EndMw
1		7.819	2751842.6	98.659	1362796.7	1482745.8	1455072.3	1.0190	1087310.6	1416008.5
2		18.014	37400.6	1.341	5.4	6.6	3.9	1.6628	35.2	0.7

Figure 4. Gel permeation chromatogram of the dextran produced by SF3.

containing (g/L) different concentrations of sucrose (see Table 4); universal peptone, 10.0; meat extract, 5.0; yeast extract, 5.0; K_2HPO_4, 2.0; diammonium citrate, 2.0; sodium acetate, 5.0; $MnSO_4{\cdot}H_2O$, 0.05; and $MgSO_4{\cdot}7H_2O$, 0.1. The pH of the medium was adjusted to 6 before sterilization at 121 °C for 15 min. For producing dextran, the inoculum was cultivated at 30 °C for 24 h to a final concentration of 10^8 cells/mL. This inoculum (10% v/v) was transferred to the fermentation broth in a flask of 100 mL and incubated as per the parameters of the factorial design (temperature and incubation time). After the incubation, the cells were collected by centrifugation at 16,000*g* for 30 min at 4 °C.[31] The pellet was discarded, and absolute alcohol (1:1) was added to the supernatant, which was stored in the refrigerator (at 5 °C) for 24 h. The above mixture was centrifuged at 16,000*g* for 30 min at 4 °C to obtain the dextran sample. To purify the dextran, a solution in water of 3% w/v was prepared, and then cold ethanol (the same volume of water added) was used to precipitate the dextran. This cycle of dissolving and precipitating was repeated three times. The pH was maintained in the range of 5−5.2 in which the maximum enzyme stability was found.[32] At the end, the pellet was dried in an oven at 50 °C for 24 h to a constant weight after being triturated. This sample was stored in a vacuum desiccator prior to characterization.[2]

Table 4. Design of the RSM

experiment	sucrose concentration (%)	temperature (°C)	incubation time (h)
1	22.5	25	24
2	22.5	25	8
3	22.5	31	16
4	22.5	37	8
5	10.0	25	16
6	35.0	31	24
7	10.0	31	24
8	22.5	31	16
9	22.5	31	16
10	22.5	37	24
11	22.5	31	16
12	35.0	37	16
13	35.0	31	8
14	10.0	37	16
15	10.0	31	8
16	35.0	25	16
17	22.5	31	16

4.2. Factorial Design to Optimize Dextran Production. Once all the experiments had been completed (Table 4), RSM was carried out to determine the best productivity conditions; with these parameters, the corresponding kinetic test was made, and finally, optimization was achieved using the Design expert 7.0 software.[33]

4.3. Physicochemical Properties Evaluated on Dextran Produced by *L. mesenteroides* SF3. *4.3.1. Moisture Content.* The moisture content (H) was estimated via water loss using a drying oven (133 000, Boekel Scientific). The H was calculated via eq 1[34,35]

$$H = \left[\left(\frac{M_i - M_f}{M_i}\right)*100\right] \quad (1)$$

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where M_i and M_f are the masses of samples before and after drying, respectively. Both measurements are expressed in grams (g).

4.3.2. Water Activity. The water activity was measured using a water activity meter (3-PRE Series, AquaLab). Triplicate samples were analyzed, and their mean was calculated.

4.3.3. Hygroscopicity. Hygroscopicity was determined following the method proposed by Tonon et al. A sample of dextran (approximately 1 g) was placed at 25 °C in a container with a NaCl-saturated solution. After 7 days, the sample was weighed and its hygroscopicity was expressed as mass (in g) of adsorbed moisture per 100 g of dry solids (g/100 g).[36]

4.3.4. Solubility. The solubility (S) was determined following the methodology described by Anderson.[37] Basically, the dextran (1 g) was suspended in distilled water (10 mL) for 1 min and then kept at rest for 15 min. Subsequently, the samples were re-agitated and then centrifuged (SOB-J40-16, SOLBAT) at 1000 rpm for 15 min. The solids were re-diluted (40 mL) and centrifuged under similar conditions. The solubility was expressed as a percentage as per eq 2

$$S = \left[\left(\frac{M_d - M_s}{M_d}\right)*100\right] \quad (2)$$

where M_d is the dextran mass and M_s is the solids mass. Both magnitudes are measured in grams (g).

4.3.5. Absorption Capacity. The absorption capacity (in water and oil) was determined by the weight uptake. The sample (1 g) was mixed in 10 mL of water or oil and then centrifuged at 1000 rpm for 15 min; the absorption capacity was expressed as a percentage.[37,38]

4.3.6. Emulsion Activity. Oil-in-water emulsions were prepared by mixing dextran solution (1 g in 25 mL water) with 25 mL of commercial corn oil using a homogenizer Ultra Turrax model (Ika T25 Basic Staufen, Germany) at 13 500 rpm for 3 min and later centrifuged at 1000 rpm for 15 min; the emulsifying capacity was calculated as per eq 3

$$\text{emulsifying capacity} = \left[\left(\frac{H_e}{H_w}\right)*100\right] \quad (3)$$

where H_e is the height of the emulsified layer and H_w is the height of the whole layer in the centrifugal tube.[39]

4.3.7. Density. The apparent and packing densities were determined by placing a specific amount of dextran powder in a graduated cylinder. The measurement of the volume before and after compacting the sample was recorded.[40]

4.4. Determination of the Molecular Weight of Dextran. The molecular weight of dextran was calculated using a PerkinElmer Series 200 HPLC system, TSKgel G5000PWxl column (30.0 cm × 7.8 mm) and Series 200 RI detector. Dextran (1 mg/mL) was loaded on the column and diluted using water with a constant flow rate (0.3 mL/min). Different standards such as Blue dextran (2 000 000 Da; Sigma, USA) and Industrial dextrans (1 400 000; 788 000; 410 000; 112 000; 365 000 Da; Sigma, USA) were used for the estimation of the molecular weight of dextran produced by *L. mesenteroides* SF3.[12]

4.5. Statistical Analysis. The data of the response and optimization surface model were obtained using the Design expert 7.0 software. The data were obtained in triplicate and expressed as the means ± standard deviation. One-way analysis of variance was performed. Tukey's multiple range tests were used to compare the means. Differences among the means of $p < 0.05$ were considered significant.

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Notes

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