ENCAPSULATION OF CAROTENOIDS BIOPRODUCED BY *Sporidiobolus Salmonicolor* CBS 2636 USING THE SPRAY DRYING METHOD

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ABSTRACT

Carotenoids are promising natural pigments in the human diet due to their antioxidant and pro-vitamin A properties. However, they are sensitive to light, heat, and oxygen, which can result in the loss of their beneficial properties through isomerization and oxidation. This study aimed to encapsulate carotenoid extracts bioproduced by the yeast *Sporidiobolus salmonicolor*, using agro-industrial residues (crude glycerol, corn steep liquor, and parboiled rice water), through the spray dryer atomization technique. Gum Arabic (GA), Inulin (IN), and Starch (ST) were used as wall materials. The microparticles were characterized in terms of encapsulation efficiency, achieving approximately 76% efficiency by using 20% (v/v) extract, 80% (v/v) phosphate buffer pH 7, 2% (w/v) Tween 80, 5% wall material (1:1:1 (w/v) GA:IN:ST), and a drying air temperature of 130°C. Encapsulation via spray dryer employing the GA, IN, and ST matrix is promising for preserving carotenoid pigments, with potential applications in the food, pharmaceutical, and cosmetic industries.

Keywords: Spray drying. Carotenoid extracts. Encapsulation efficiency.

1 INTRODUCTION

The coloration of food is vital for consumer perception, leading to the search for natural alternatives. Carotenoids are natural pigments found in various living organisms. However, their application in the industry faces significant challenges due to isomerization and oxidation caused by environmental factors such as light, heat, and oxygen. These conditions can result in the loss of their beneficial properties, making their utilization a challenge for the food industry and other sectors[1,2]. Spray drying encapsulation emerges as a promising technique to protect them[3,4]. Therefore, optimizing the encapsulation of these compounds becomes crucial to ensure their efficacy and stability in different applications[5,6].

This study aimed to optimize the spray drying encapsulation process of carotenoids produced by *Sporidiobolus salmonicolor* CBS 2636, exploring combinations of gum arabic, inulin, and starch as wall materials to ensure the protection and stability of the compounds.

2 MATERIAL & METHODS

The production of carotenoids followed the method described by Colet et al.[7]. The extraction and recovery of total carotenoids were achieved following the method described by Valduga et al.[8,9].

For the formation of microcapsules, a central composite rotational design (CCRD) 2^3 was used, as shown in Table 1, with a replication of the central point, totaling 19 points, for encapsulation. The fixed variables were: the concentrated volume of carotenoid extract (20% v/v), volume of phosphate buffer solution pH 7,0 (80% v/v), and the amount of Tween 80 (2% w/v). The dependent variable was the encapsulation efficiency.

Table 1 Independent variables and levels used in the CCRD 2^3 for carotenoid encapsulation.

Independent Variables	Codes	Levels				
		-1,68	-1	0*	+1	+1,68
Gum Arabic (g/L)	X_1	0	6,8	16,7	26,7	33,5
Inulin (g/L)	X_2	0	6,8	16,7	26,7	33,5
Starch (g/L)	X_3	0	6,8	16,7	26,7	33,5

*Quintuplicate of the central point.

The wall materials (GA, IN, and ST) were weighed according to the experimental design. These components were dissolved in 50 mL of phosphate buffer solution at pH 7,0 in an ultrasonic bath (model: USC-1800A; manufacturer: Unique UltraSonic Cleaner) at 50 ºC, adding 2 g of Tween 80 until completely dissolved. Then, 30 mL of phosphate buffer at pH 7.0 was added, and the mixture was stirred with a mechanical stirrer Turrax (model: RW 20 digital, manufacturer: IKA) at 300 rpm for 5 minutes. Subsequently, 20 mL of carotenoid extract was added, protecting the mixture from light with aluminum foil, and the solution was stirred for another 5 minutes. Drying was performed in a spray dryer (model: SD-05; manufacturer: LabPlant) at an average sample feed rate of 5.83 mL/min and a temperature of 130ºC.

The encapsulation efficiency (%EE) was determined based on the quantification of the surface and total carotenoids of the capsules, following the methodology of Robert et al.[10]. The result was expressed as a percentage of encapsulation efficiency (%EE), with the calculation performed using Equations 1 and 2.

$$SC\% = \frac{ABS_{surface\ carotenoids}}{ABS_{\ total\ carotenoids}}\ x\ 100 \quad (1)$$

$$EE\% = 100 - SC(\%) \quad (2)$$

Where SC (%) is the percentage of carotenoids present on the surface of the capsules, and EE (%) is the percentage of encapsulated carotenoids (encapsulation efficiency).

The results were statistically analyzed using experimental design methodology and analysis of variance (ANOVA), with a significance level of 95% confidence ($p \leq 0,05$), using the software Statistica, version 5.0 (StatSoft, Inc., USA).

3 RESULTS & DISCUSSION

Table 2 presents the matrix of the central composite rotatable design (CCRD) 2^3 with the coded and actual values of the independent variables studied for the encapsulation of carotenoids and the results in terms of encapsulation efficiency (predicted, deviation, and relative deviation).

Table 2 Matrix of the central composite rotatable design (CCRD) 2^3 (coded and actual values) and encapsulation efficiency response (predicted, deviation, and relative deviation).

Experiments	GA (g/L)	IN (g/L)	ST (g/L)	%EE	%EE predicted	Deviation*	Relative Deviation ** (%)
1	-1 (6,8)	-1 (6,8)	-1 (6,8)	22,70	-2,53	25,23	111,14
2	1 (26,7)	-1 (6,8)	-1 (6,8)	64,10	61,11	2,99	4,66
3	-1 (6,8)	1 (26,75)	-1 (6,8)	19,65	18,95	0,70	3,57
4	1 (26,7)	1 (26,7)	-1 (6,8)	68,53	62,19	6,34	9,25
5	-1 (6,8)	-1 (6,8)	1 (26,7)	58,09	55,65	2,44	4,20
6	1 (26,7)	-1 (6,8)	1 (26,7)	69,20	61,13	8,07	11,66
7	-1 (6,8)	1 (26,7)	1 (26,7)	65,71	59,93	5,78	8,79
8	1 (26,7)	1 (26,7)	1 (26,7)	28,55	45,01	-16,46	-57,64
9	-1,68 (0)	0 (16,7)	0 (16,7)	4,79	20,92	-16,13	-336,61
10	1,68 (33,5)	0 (16,7)	0 (16,7)	65,54	61,85	3,69	5,63
11	0 (16,7)	-1,68 (0)	0 (16,7)	29,51	48,33	-18,82	-63,78
12	0 (16,7)	1,68 (33,5)	0 (16,7)	59,26	52,84	6,43	10,84
13	0 (16,7)	0 (16,7)	-1,68 (0)	14,88	31,67	-16,79	-112,86
14	0 (16,7)	0 (16,7)	1,68 (33,5)	70,46	66,11	4,35	6,17
15	0 (16,7)	0 (16,7)	0 (16,7)	76,77	75,14	1,63	2,12
16	0 (16,7)	0 (16,7)	0 (16,7)	75,90	75,14	0,76	1,00
17	0 (16,7)	0 (16,7)	0 (16,7)	75,30	75,14	0,16	0,21
18	0 (16,7)	0 (16,7)	0 (16,7)	74,84	75,14	-0,30	-0,40
19	0 (16,7)	0 (16,7)	0 (16,7)	75,12	74,14	-0,02	-0,03

Legend: GA (g/L) = gum arabic; IN (g/L) = inulin; ST (g/L) = starch; %EE = encapsulation efficiency; *Deviation = %EE - %EE predicted. **Relative Deviation = (Deviation/%EE)*100.

The highest EE, ranging from 74,84% to 76,77%, is observed in experiments 15 to 19 (central point) with equal proportions of wall material (16,7 g/L). The results were statistically analyzed ($p<0,05$), and Equation 3 presents the coded second-order model describing the relationship between the EE of carotenoid capsules and the analyzed variables (gum arabic concentration, inulin, and starch) within the studied range. The model was validated through analysis of variance, yielding a correlation coefficient of 0,90 and a calculated F value 1,36 times greater than the tabulated value, allowing for the construction of response surfaces and contour plots (Figure 1).

$$EE = 75,14 + 12,18\ X_1 - 11,96\ X_1^2 + 1,34\ X_2 - 8,7.\ X_2^2 + 10,25\ X_3 - 9,30\ X_3^2 - 5,10\ X_1.X_2 - 14,54\ X_1.X_3 - 4,30\ X_2.X_3 \quad (3)$$

Where: EE = Encapsulation Efficiency (%); X_1 = Gum Arabic (g/L); X_2 = Inulin (g/L); X_3 = Starch (g/L).

It can be observed that the relative deviations were low in the central point assays (15 to 19), where EE is optimized. Thus, the predicted EE results demonstrate that the obtained model is suitable for explaining the carotenoid encapsulation process and can predict efficiency values, even when manipulating the data of the studied variables.

Figure 1 (a, b, c, d, e, and f) presents the contour curves and response surfaces that allow identifying the region of maximum efficiency, located between concentrations of 10 and 26 g/L of GA, IN, and ST, thus demonstrating the optimization of the carotenoid encapsulation process.

The drying process can result in minor losses of carotenoids due to the presence of external factors such as exposure to light and heat. The physical-chemical characteristics of core and wall materials, as well as the properties of the polymeric matrices formed from the wall materials, can influence the interactions and retention of encapsulated compounds. The complexity of determining carotenoid EE should also be considered.

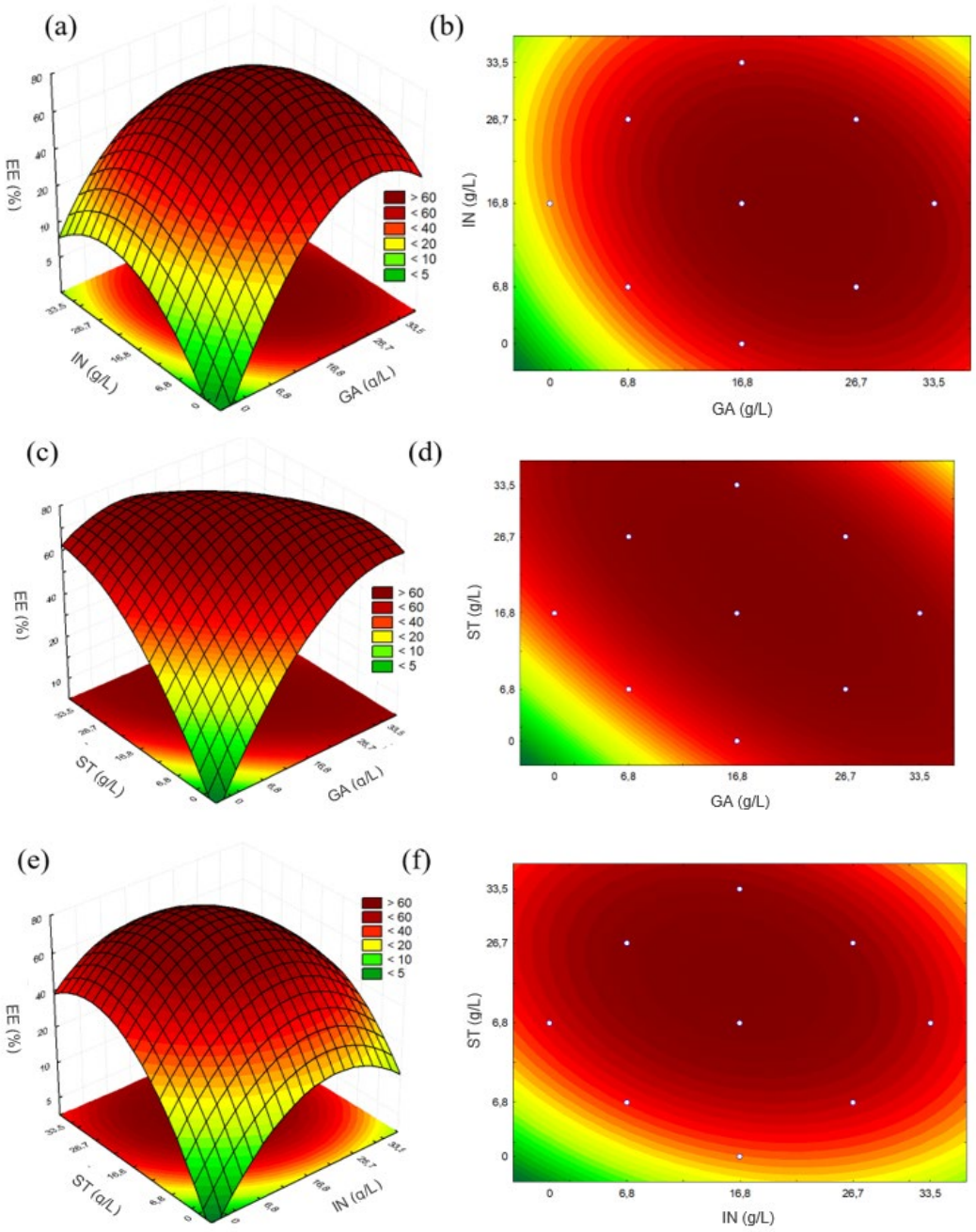

Figure 1 Response surface and contour plot for encapsulation efficiency as a function of Gum Arabic and Inulin (a) and (b); Gum Arabic and Starch (c) and (d); and Inulin and Starch (e) and (f), respectively.

In a previous study[11], carotenogenic extracts produced by the yeast *Sporidiobolus salmonicolor* were encapsulated using a GA:MD ratio of 1:1 (25 g/L), resulting in a maximum EE of 60%. Comparing these results with those obtained in this study, where a maximum EE of 76,77% was achieved using GA:IN:ST at a ratio of 1:1:1 (16,7 g/L) as wall materials, a significant improvement in this parameter can be observed. This increase in efficiency can be attributed to the specific properties of these agents, such as GA's ability to form encapsulating films and trap carotenoids, the complementary contribution of IN and ST to the formation of the polymeric matrix, as well as the interaction between these components.

Other research highlights the positive influence of GA and other substances on the encapsulation efficiency of compounds such as norbixin and lutein[12,13]. These studies emphasize the relevance of proper formulation of wall materials to maximize encapsulation effectiveness.

4 CONCLUSION

Based on the obtained results, it was possible to encapsulate the carotenogenic extract produced by the yeast *Sporidiobolus salmonicolor* using the spray drying technique. The encapsulation process involved 20% (v/v) of the extract, 80% (v/v) phosphate buffer at pH 7, 2% (m/v) Tween 80, 50,1 g/L of wall material (1:1:1 (m/v) GA:IN:ST), and a drying air temperature of 130°C, resulting in an encapsulation efficiency of approximately 76%. Therefore, spray drying with the composite matrix of GA, IN, and ST holds promise for preserving carotenogenic pigments and their application in the food, pharmaceutical, and cosmetic industries.

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