ADSORPTION OF CELLULASE ON CEREAL BRANS: A SIMPLE FUNCTIONAL MODEL FROM RESPONSE SURFACE METHODOLOGY*

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ABSTRACT

A functional model based on Langmuirian adsorption as a limiting mechanism was proposed to explain the effect of cellulase during the enzymatic pretreatment of bran, conducted prior to extraction of proteins, by wet alkaline process from wheat and buckwheat bran materials. The proposed model provides a good fit (r = 0.99) for the data generated thru predictive model taken from the response surface methodology, permitting calculation of a affinity constant (b) and capacity constant (k), for wheat bran (b = 0.255 g/IU and k = 17.42%) and buckwheat bran (b = 0.066 g/IU and k = 78.74%).

INTRODUCTION

There is considerable interest in utilizing cereal brans for food purposes, for example in production of proteins concentrates for food enrichment. The protein concentrate may be obtained by submitting the bran to a wet alkaline extraction process (WOERMANN & SATTERLEE[8]), to wich an enzymatic pre-treatment based on carbohydrases may be added to enhance they yield of protein, without recourse to extremes of pH during the extraction (TOYAMA[6]).

Recently, GROSSMANN[2] and WASZCZYNSKYJ[7] have employed the response surface methodology (RSM) in selecting the conditions for enzymatic pre-treatment (using cellulase, hemicellulase and pectinase) of buckwheat and wheat bran respectively.

Considering that RSM provides only an empirical and predictive mathematical model, within the experimental region, the objective of this particular study was to explore the possibility of developing a functional model (related to physico-chemical phenomena) to explain the action of cellulase on brans cited, during the pre-treatment step. The data for our study comes from the proper RSM analysis, without recourse to additional experimentation, or in other words, extending the utility of response surface methodology.

MATERIALS AND METHODS

Enzymatic pre-treatment

The pre-treatment of wheat bran and buckwheat bran conducted by WASZCZYNSKYJ[7] and GROSSMANN[2] respectively, in our laboratory, consisted of placing 10 g of bran material (particle size 0.297 - 0.420 mm) in 50 - 30 cm^3 acetate buffer (pH 3.7 - 5.7) containing enzyme, followed by constant agitation (120 - 160 rpm) for at least 3 hours at 37°C. Subsequent to the pre-treatment, extraction of nitrogen (N) was made based on WOERMANN & SATTERLEE[8] procedures. The results (response variable) were expressed as % of total N (Kjeldahl), referring to the N content of the cereal brans, after correcting for N content of enzyme included in the pre-treatment.

Mathematical model

GROSSMANN[2] and WASZCZYNSKYJ[7] have employed the response surface methodology (RSM), derive from rotatable central composite design (COCHRAN & COX[1]), with a total of 32 experiments. The results were adjusted to a quadratic polynomial, followed by analysis of variance (ANOVA) and regression analysis. Finally, gradient technique (nonlinear programming) was used to select optimum conditions for extraction of total N from the bran.

With the aid of predictive model obtained by the cited authors, we have obtained data for the following situation (optimum conditions), to explore a functional model for explaining the action of cellulase on bran: cellulase (Sigma, *T. viride*) 5 - 35 IU/10 g of bran, incubation pH (constant) = 5.7 and incubation time (constant) = 3 hours.

The polynomials obtained were:

$$Y_w = 20.336 + 0.4193X - 0.00582X^2 \qquad (1)$$

for wheat bran, and

$$Y_b = 40.491 + 0.5173X - 0.00283X^2 \qquad (2)$$

for buckwheat bran, wherein,

Y's = % of total N solubilized in each case

X = amount of cellulase (IU/10g of bran)

The data thus obtained (Tables 1 and 2) were tested using a physical model, which may be described as an hyberbolic function similar to type I Langmuir adsorption isotherm, where

$$y = k\theta = \frac{k.b.x}{1 + b.x} \qquad (3)$$

* Trabalho financiado pela Coordenação de Pesquisa e Pós-Graduação, Universidade Estadual de Londrina, Pr. Brasil e apresentado no 40o. Congresso Anual do Institute of Food Technologists, Nova Orleans, EUA, de 8 a 11 de junho de 1980.

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or, following a linearization, we have

$$\frac{1}{y} = \frac{1}{k\theta} = \frac{1}{k} + \frac{1}{kbx} \qquad (4)$$

Whrein, y = % increase in total N solubilized, as compared to control (i.e., without enzymatic pre-treatment), θ = fraction of total surface of the substrate (bran) covered by the adsorbed cellulase, x = concentration of cellulase employed in the pre-treatment (IU/g of subtrate), k = capacity constant (%) and b = affinity constant $(IU/g)^{-1}$. However, the application of Eq (3) or Eq (4) does not mean strict adherence to the basic postulate of the Langmuir adsorpition, i.e., cellulase forming a monolecular layer on bran, and that adsorption is independent of cellulase already adsorbed (HAYWARD & TRAPNELL[3]).

RESULTS & DISCUSSION

Tables 1 and 2 demonstrate the values obtained using Eq. (1) and (2). These values were refitted by the method of least squares, based on Eq. (4). As a result, the Langmuirian adsorption equation for cellulase on wheat bran was found to be:

$$\frac{1}{y} = 0.0574 + 0.225\left(\frac{1}{x}\right), \qquad 0 < y \leqslant 7.5\% \qquad (5)$$

with,
r^2 (coefficient of determination) = 0.9952
$\hat{\sigma}$ (standard error) = 0.0103
and, the similar equation for cellulase adsorption on buckwheat bran was:

$$\frac{1}{y} = 0.0127 + 0.192\left(\frac{1}{x}\right), \qquad 0 < y \leqslant 14.6\% \qquad (6)$$

with,
r^2 = 0.9998
$\hat{\sigma}$ = 0.0016

In both cases, these results indicate a good adjustment of the data to the linearized Langmuirian equation, especially with regard to buckwheat bran.

Therefore, we could suggest that in our experiments, the action of cellulase is related to the surface phenomena, with the mechanism of adsorption as the control. The constant agitation and relatively small particle size of the substrate could have reduced substantially the resistences to mass transfer, minimizing its importance in the process.

The surface phenomena normally is divided in three steps: adsorption of reagents, (bio) chemical reaction, and desorption of products.

In the case study presented, there are evidences that adsorption is a limiting step as indicated by a good fit of the data to a functional model similar to Langmuir adsorption isotherm. This could be confirmed (Tables 1 and 2) by the fact that an increase in cellulase concentration during pre-treatment resulted in an increase of % total N solubilized (LEVENSPIEL[4]). In fact, PEITERSEN et alii[5] have shown that the adsorption of cellulase over cellulosic fibers is the first step in the enzymatic hydrolysis of cellulose.

The parameters of Langmuirian adsorption equation for cellulase on the cereal brans tested could be obtained comparing each of the Eqs. (5) and (6) with Eq. (4), respectively. The calculated capacity constant (k) and affinity constant (b) for wheat bran were k = 17.42%; b = 0.255 $(IU/g)^{-1}$ and those for buckwheat bran were k = 78.74%; b = 0.066 $(IU/g)^{-1}$.

The results presented indicate that the buckwheat bran has relatively higher capacity for adsorption of cellulase, and therefore more responsive to pre-treatment with this enzyme. However, its lower affinity leads to increased demand for cellulase. But, these conclusions should be treated with care, since the data used to arrive at Langmuirian adsorption isotherm were obtained from predictive equations.

Future research in the are may include the study of the following variables: raw material (nature, particle size and surface area) enzyme (type and concentration), temperature and pH.

Table 1 — Data used for testing the Langmuir isotherm equation related to adsorption of cellulase on wheat bran

Amount of cellulase on 10 g of bran X (IU)	Total N solubilized Y (%)	Increase total N solubilized y = Y – 20.336 (%)	Concetration of cellulase x (IU/g bran)
5	22.287	1.951	0.5
10	23.947	3.611	1.0
15	25.316	4.980	1.5
20	26.394	6.058	2.0
25	27.181	6.845	2.5
30	27.677	7.341	3.0
35	27.882	7.546	3.5

Table 2 — Data used for testing the Langmuir isotherm equation related to adsorption of cellulase on buckwheat bran

Amount of cellulase on 10g of bran X (IU)	Total N solubilized Y (%)	Increase total N solubilized y = Y – 40.491 (%)	Concentration of cellulase x (IU/g bran)
5	43.006	2.515	0.5
10	45.381	4.890	1.0
15	47.613	7.122	1.5
20	49.705	9.214	2.0
25	51.654	11.163	2.5
30	53.463	12.972	3.0
35	55.129	14.638	3.5

RESUMO

Modelo funcional baseado na adsorção de Langmuir como mecanismo limitante proposto para explicar o efeito da celulase durante o pré-tratamento enzimático de farelos, visando à extração de proteínas, através do método alcalino-úmido. O referido modelo ajusta-se muito bem (r = 0,99) aos dados gerados com base em modelo preditivo obtido da metodologia da superfície de resposta. Pode-se calcular a constante de afinidade (b) e a constante de capacidade (k) para o farelo de trigo e farelo de trigo mourisco (sarraceno), usando uma equação análoga à isoterma de adsorção de Langmuir. Os resultados indicaram que o farelo de trigo mourisco apresenta uma capacidade mais alta para adsorver celulase e, conseqüentemente, pode-se esperar uma resposta maior ao pré-tratamento com esta enzima.

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