OPTIMIZATION OF SIMULTANEOUS SOLID STATE HYDROLISIS AND ALCOHOLIC FERMENTATION OF CHESTNUT

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Introduction. Galicia (NW of Spain) is one of the most productive chestnut areas in Europe but the main part of crop is underutilized (1). In basis of their starchy nature, overproduction of fruits can be used to obtain an alcoholic beverage from distillation of fermented chestnut. Simultaneous solid-state hydrolysis and fermentation can be used for this purpose because allow harmonize jointly, the maximization of the conversion of starch to ethanol and enhance flavour production by yeast (1).
The aim of this work is to optimize synergistic joint action of a mixture of a-amylase and glucoamylase to hydrolyze starch and simultaneously the operational conditions of solid-state fermentation to improve the ethanol yield.

Methods. Peeled, chopped and sterilized chestnut was used as substrate by six different ethanol producers strains of *Saccharomyces cerevisiae*. By means complete second order factorial experimental designs effect of particle diameter (D) and content of humidity (L) were studied as independent variables for both process solid-state hydrolysis alone and simultaneously to the alcoholic fermentation. Dependent variables evaluated at different times to optimize the process were percentages of starch solubilization (%S) and hydrolysis (%H) and ethanol production.

Results. Table 1 shows that was no possible to reach the total solubilization and hydrolysis of chestnut in solid state process and the effect of the independent variables in the response was scarce comparing with independent term. Contrary the reported in soluble liquid hydrolysis of chestnut starch (1), solubilization and hydrolysis are correlated indicating a dependence relationship between them. L variable had strongest effect than D in hydrolysis and the optimum is close to the center of experimental domain. Smallest particles diameter provided the best responses, but under 0,75 mm agglomeration and diffusional restriction could occurs.
Table 2 shows the ethanol production after 48 hours of incubation of six *Saccharomyces cerevisiae* strains in cultures performed in the same experimental domain than the former hydrolysis. Very similar amounts of ethanol were obtained in all cases and the effect of the variables on the production was scarce comparing with the independent term. Contrary to the hydrolysis process alone, L variable had more effect than D one revealing that to produce ethanol the suitable amount of liquid in the system to satisfy the metabolic needs of yeast is more determinant that the substrate availability from the enzymatic process.

The value of D and L variables as well the levels of the ethanol productions reached and the were comparable to the reported for other starchy materials (2)

Table 1. Parameters of the quadratic models obtained for two hydrolysis time. NS: Non significant coefficient. It: independent term of the model r^2adj: Adjusted regression coefficient. Res_{max}: Maximun response expected from models. D_{opt} and L_{opt} are de values of diameter of particle and amount of humidity to obtain maximum response.

	%S (12 h)	%S (24 h)	%H (12 h)	%H (24 h)
it	78,6	83,0	60,9	65,1
D	-5,4	-2,6	-5,4	-2,6
L	NS	1,0	NS	1,0
DL	NS	NS	NS	NS
D^2	NS	NS	NS	NS
L^2	-1,5	-1,1	-1,5	-0,9
r^2adj	0,920	0,802	0,920	0,820
$Res_{máx}$	85,5	86,6	67,8	68,7
D_{opt}	-1,267	-1,267	-1,267	-1,267
L_{opt}	0,000	0,468	0,000	0,523
% it	91,9	95,9	89,8	94,8

Table 2. Parameters of the quadratic models obtained for the ethanol production (in mg/g) in a 48 hours simultaneous hydrolysis and solid-state fermentation. RST: residual total sugars. Other keys as in table 1

cepa	S1.01	S1.02	S1.03	S1.04	S1.06	S1.07
ti	140,8	143,0	137,5	134,7	143,8	149,9
D	NS	-2,9	NS	5,3	NS	NS
L	10,5	11,0	8,7	NS	7,8	5,0
DL	NS	NS	NS	6,6	NS	NS
D^2	NS	NS	NS	NS	NS	NS
L^2	-8,3	-6,6	-7,1	-4,9	-11,1	-8,1
r^2adj	0,900	0,921	0,852	0,543	0,749	0,777
$Res_{máx}$	144,2	151,2	140,1	145,0	145,1	150,7
D_{opt}		-1,267		1,267		
L_{opt}	0,636	0,832	0,608	0,857	0,351	0,310
% it	97,6	94,6	98,1	92,9	99,1	99,5
RTS	77,0	80,8	74,9	77,4	77,5	80,5

Conclusions. High ethanol productions can be obtained in simultaneous solid-state hydrolysis and fermentation process in optimal conditions of D and L variables.

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