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Development and optimization of a new culture media using extruded bean as nitrogen source

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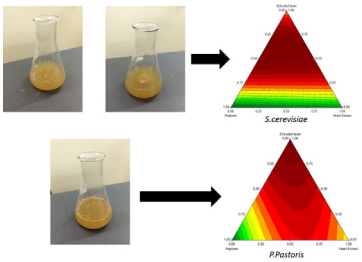
GRAPHICAL ABSTRACT



ABSTRACT

The composition of a culture medium is one of the most important parameters to be analyzed in biotechnological processes with industrial purposes, because around 30–40% of the production costs were estimated to be accounted for the cost of the growth medium [1]. Since medium optimization using a one-factor-at-a-time approach is time-consuming, expensive, and often leads to misinterpretation of results, statistical experimental design has been applied to medium optimization for growth and metabolite production [2–5]. In this scenario, the use of mixture design to develop a culture medium containing a cheaper nitrogen source seems to be more appropriate and simple. In this sense, the focus of this work is to present a detailed description of the steps involved in the development of a optimized culture medium containing extruded bean as nitrogen source.

- In a previous work we tested a development of new culture media based on the composition of YPD medium, aiming to reduce bioprocess costs as well as to improve the biomass production and heterologous expression.
- The developed medium was tested for growth of *Saccharomyces cerevisiae* and *Pichia pastoris* (GS 115).
- The use of culture media containing extruded bean as sole nitrogen source showed better biomass production and protein expression than those observed in the standard YPD medium.

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Method details

Briefly, the development of the new culture media containing extruded bean as substitute of traditional nitrogen source (peptone and yeast extract) involved the following steps:

(1) Production of the extruded bean flour.
(2) Use of statistical approach to optimize the composition of nitrogen source.
(3) Evaluation of the yeast growth profile in the new media compared to a commercial YPD media.
(4) Estimation of the potential use of the new culture media for heterologous expression.

Production of extruded bean flour

Hard-to-cook common beans (*Phaseolus vulgaris*, cv carioca) were ground in a hammer mill and sifted in a screen of 0.425 mm. Then, the moisture of the bean flour was adjusted to 25% and the flour was kept out at refrigerator (4 °C) overnight aiming to reach a wetting balance. Before extrusion, the bean flour was kept out at room temperature for 2 h and the moisture content was confirmed gravimetrically [6]. The extrusion process was carried out using a Cerealtec International single screw extruder (CT-L15), with four zones of heating, compression ratio screw of 3:1, 5 mm die and screw speed of 150 rpm. The temperatures used in each heating zone of the extruder were 80 °C, 140 °C, 150 °C and 130 °C [7,8]. The extrudates were dried at room temperature for 12 h, milled and sifted in a screen of 0.425 mm. This extruded bean flour was used as nitrogen source in the culture media.

Mathematical model determination for optimization of culture media

The initial composition of culture media was based on the commercial medium YPD, widely used for yeasts growth. This medium is composed by 1% yeast extract, 2% peptone and 2% dextrose. So, in order to evaluate the effectiveness of substituting peptone and yeast extract for extruded bean, the composition of the culture media was optimized using a 3-factor simplex-lattice design. This mixture design was used to study the relationship between the proportion of the different nitrogen sources and their respective responses in the optical density of *Saccharomyces cerevisiae* and *Pichia pastoris* (GS 115). The design was implemented using Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA) and the proportion of each nitrogen source varied from 0 to 2% (Table 1).

Table 1
Mixture design and assigned concentrations of each compound at different levels of the mixture design.

Experiment	Peptone (X1)	Yeast extract (X2)	Extruded bean (X3)
1	2%	0	0
2	0	2%	0
3	0	0	2
4	1%	1%	0
5	1%	0	1%
6	0	1%	1%