SUBCRITICAL WATER BATCH HYDROLYSIS OF SPENT CIDER YEAST: A FIRST STEP TOWARDS RECOVERING HIGH VALUE COMPOUNDS

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Abstract

Spent yeast is a natural by-product generated through the production of fermentable beverages. Traditional markets for spent yeast are declining creating a growing environmental and commercial problem for the European brewing industry, which currently produces an estimated 700,000 tonnes per annum. Therefore the industry is seeking new, sustainable, added-value conversion processes for spent yeast slurry. Given the inherient environmently benign nature of subcritical water we are evaluting the utility and efficacy of the solvent to meditate hydrolysis of spent yeast derived from the cider industry as a first step towards achieving full utilisation of the biomass, i.e. Biorefining. Our current focus is firstly determination and then recovery of vitamin B3 (Nicotinic Acid), a valuable precursor which finds application in a range of health and skin care products. Using a batch reactor, the effect of temperature, pressure and residence time on the extraction efficiency has been evaluated. It was found that temperatures 175^0C and above resulted in a rapid increase in the yield of vitamin B3.

Given the multi factorial nature of the emperical analysis, the response surface method (RSM) was used to develop central composite model of a three factorial experimental design. The yield data was fitted to the model allowing interpolation of data points, these predicted values were validated

Introduction

Within the European food and drink industry over 200 million tonnes of organic waste are produced each year. The brewing and cider making industry produces for example spent grain and yeast, both are wastes. On a European scale spent yeast amounts to 700,000 tonnes per annum. Traditional markets for spent yeast are as dried whole extracts serving the nutraceutical markets and as slurry, with 24% solid content, for animal feed. However all markets for direct application of yeast are in decline and with disposal via land fill not an option there is an economic and environmental imperative to find alternative ways of adding value. It has long been known that yeast is an excellent natural source of major B vitamins and is rich in other biopolymers, nutrients and micronutrients that are of value to the nutraceutical, cosmetic and pharmaceutical industries [1]. For example the cosmetic industries, are interested in sourcing two biopolymers of chitin and β-Glucan which are available in yeast cell [2].

Along with increased interest in sourcing natural intermediates for the nutraceutical, cosmetic and pharmaceutical industries, there are recognised benefits from using environmentally benign solvents when bio-processing. Sub and supercritical carbon dioxide and water are environmental ‘green solvents both have their merits and in part this attributes the polarity which in turn influences solvating capacity. Sub-critical water is achieved at a temperature anywhere between 100 °C and 374 °C its

critical point, while under pressure high enough to maintain its liquid state. Water under sub-critical conditions has been employed for extraction of natural compounds which find application in the food, cosmetic and pharmaceutical industries. [3]. It is also gaining attention as a solvent that enables hydrolysis of naturally occurring polymers for example the conversion of long chain molecules such as cellulose to its valuable sugar monomers [4]. A recent paper by Lamoolphak et al. evaluated the potential of sub-critical water mediated hydrolysis of brewers yeast to extract the protein fraction and further decompose the peptides into amino acids [5]. In the light of this work we report sub-critical water treatment of spent cider yeast and the optimisations of the operating parameters to efficiently extract nicotinic acid from the biomass.

Materials and Methods

Subcritical Water Hydrolysis

150 ml of spent cider yeast slurry supplied by Bulmers with a solid content of 13% (w/v).was subject to batch hydrolysis using a 300 ml stainless steel Unit (Parr Instrument Company ® 5500 mini bench top reactor). The vessel was heated electrically and cooled through the installation of additional cooling loop. The pressure was provided by a nitrogen gas cylinder at pressures indicated in each experiment. After hydrolysis the samples were cooled and split into 50 ml tubes and centrifuged at 17000 g for 10 mins (Beckman) and stored in -20 °C freezer for analysis.

Thin Layer Chromatography;

The crude hydrolysed yeast samples were semi purified using Silica gel G glass TLC plates (Whatman) with water as the mobile phase. 100 µl of each sample was loaded and vitamin B3 detected using a portable UV light source. The band of was scraped from the plate at an Rf value corresponding the vitamin B3 standard. Distilled water was used to elute vitamin B3 from the silica. The supernatant containing the vitamin B3 fraction was further analysed via HPLC.

High Performance Liquid Chromatography;

An Agilent1100 coupled with a UV detector/analyzer (270 nm) fitted with Luna C18 (250 × 4.6 mm) column (Phenomenex) was used with the solvent system described by the column manufacturer for the vitamin analysis. This was a 20 mM phosphate buffered (pH=3.0) of 95:5 ratio of acetonitrile to water. The column temperature was ambient (20 °C) with a flow rate of 1.0 ml/min.

The protein analysis was carried out using a Coomassie Bradford Protein Assay kit (Pierce, IL, USA) using Bovine Serum Albumin (BSA) as the standard. Total organic carbon (TOC) of the hydrolysed suspension, as a measure of soluble organic carbon, was measured with a TOC analyser (Shimadzu).

Experimental design and statistical analysis

Response surface modelling was used to evaluate the effect of three independent variables, namely temperature (°C), residence time (min) and pressure (bar) on the yield of vitamin B3 (%), protein concentration (mg/ml) and total organic carbon (ppm). The independent variables are listed in Table 1. Experimental points were chosen according to a face-centred Central Composite Design (CCD).

Table 1. Independent variables used in the RSM design

Symbols	Variable	Coded levels		
		-1	0	1
X1	Temperature	125	175	225
X2	Time	30	45	60
X3	Pressure	30	45	60

For statistical analysis, data were evaluated and fitted to a quadratic polynomial selected model. The determined model was evaluated using results from analysis of variance (ANOVA). This model also was used to plot the graphs and surfaces.

Results

The model

The responses were modelled with a second order polynomial in terms of independent variables:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{11} X_1^{\,2} + b_{12} X_1 X_2 + b_{22} X_2^{\,2} + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{33} X_3^{\,2}$$

The coefficients then derived by regression and are listed in the Table 2.

Table 2. Estimated coefficients of response model

	Vitamin Yield (%)	Protein (mg/ml)	TOC(ppm)
X_1	0.13	-238	59
X_2	0.21	14	-23.5
X_3	0.014	-107	-69.5
X_1 X_1	0.23	80.6	-409
X_1 X_2	8.16 e-3	-165.7	-214.7
X_1 X_3	9.19 e-3	-64.2	-255.2
X_2 X_2	4.17 e-4	-47.8	-75.3
X_2 X_3	3.571e-3	-23.7	3.25
X_3 X_3	1.67 e-5	73.65	-16.3
R^2	0.99	0.98	0.92
Intercept	0.13	319.4	2527

Effect of Temperature on vitamin concentration

Data from the model and experiments show that the extraction of vitamin B3 increases with temperature. The RSM model also suggests that this parameter is the most significant parameter compared to the other two parameters (pressure and time). The vitamin concentration is plotted versus temperature and pressure for a residence time of 45 min and is shown in Figure. 1.

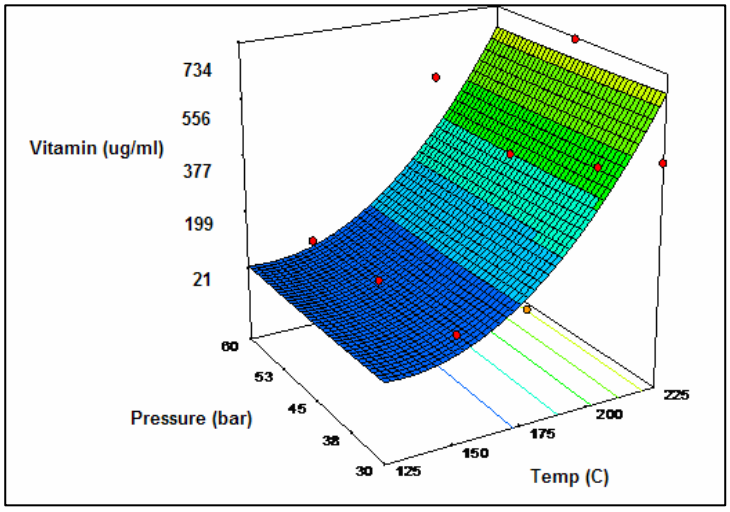

Fig. 1. The effect of temperature and pressure at time = 45 min

Figure.1 shows that yield increases sharply at around 175 C and goes to its maximum at 225 °C. The graph also illustrates that the temperature effect is not modulated by pressure in the range of temperatures studied. This behaviour was also observed at different residence time.

Effect of residence time on vitamin concentration

Figure. 2 illustrates the effect of residence time versus temperature on the solubility of vitamin B3 concentration at a constant pressure of 45 bar. The model predicts that the effect of residence time is not significant at different temperatures and this was confirmed by experimental data.

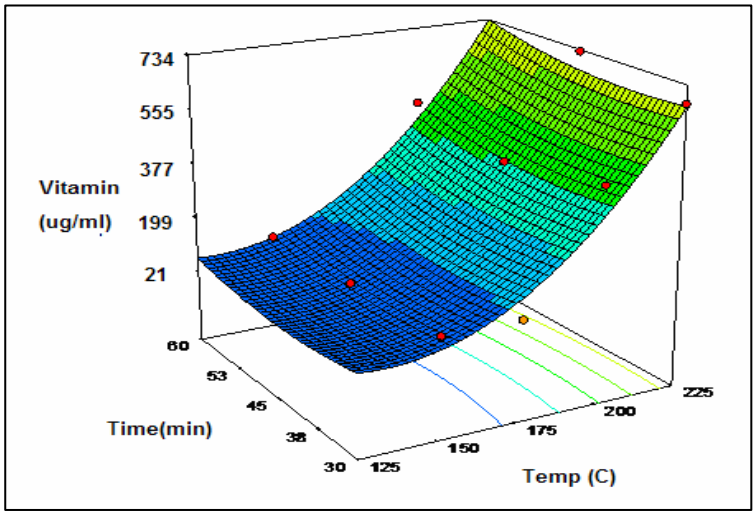

Figure 2. The effect of residence time and temperature on the yield at constant pressure o 45 bar

3.4- Soluble Protein

The maximum amount of soluble protein was detected at 125 °C and 30 minutes and decreases at higher temperature and residence time this is shown in Fig. 3, The effect of pressure and residence time is negligible comparing to the temperature.

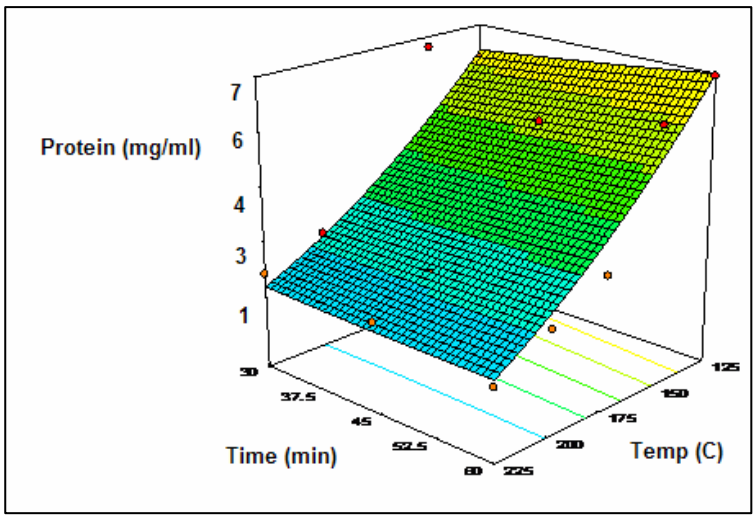

Fig 3. The effect of pressure and temperature on soluble protein fraction at 45 minutes

3.5-Total Organic Carbon (TOC)

The soluble organic carbon is an indication of the amount of organic compounds hydrolysed and solubilised in the aqueous medium under the process parameters. Again, the Effect of temperature on TOC was the most significant parameter and this is shown in Fig. 4. The TOC amount increases with increasing the temperature and reaches its maximum at around 175 °C before it starts to decrease. This could be explained by possible charring that happens at higher temperatures which results in the formation of gaseous products.

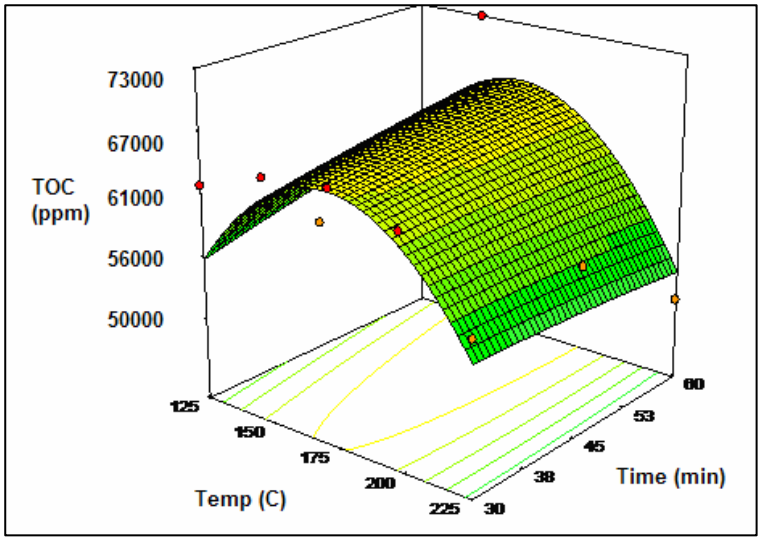

Fig. 4 – The effect of residence time and temperature on the TOC of extract

4- Conclusion

The possibility of using sub-critical water to hydrolyse yeast cells to release vitamin B3 was investigated. Three different operating parameters were studied with respect to their influence on the extraction and included in response surface methodology. Temperature was the most significant parameter for the hydrolysis of yeast cells. Pressure appeared to have little effect on vitamin release. Residence time did not show a positive effect on the yield and it is believed to have negative consequences because of vitamin sublimation at higher temperatures. The presented data also suggest that the soluble protein content falls in the range of working temperatures (125- 225 °C) used in this study.

The procedure which was used in this study includes the use of Thin Layer Chromatography (TLC) as a method to extract and separate vitamin B3 fraction from other compounds in the available crude mixture. This part of the study could be further investigated to evaluate the ability of silica based chromatography to purify vitamin B3 fraction from hydrolyzed biomass. The same procedure could be applied for other hydrophilic vitamins, especially when a crude complex extract of biomass is going to be studied.

5- Future Work

This work is a part of a bigger study which its philosophy is based on the design of a platform which uses environmentally friendly techniques to extract valuable products from biomass wastes. Using straight forward extraction techniques based on supercritical and subcritical fluid technology and chromatography separation concept to isolate vitamins and other products are also under investigation which includes several fractionation stages. In the next steps, the hydrolysis reaction with similar operational conditions will be investigated using a

continuous plug flow tubular reactor and silica column chromatography separation will be developed.

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