Optimization of sugars recovery from spoiled date fruits for sustainable bioethanol production from yeasts co-cultures

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Abstract
In the present work, the possibility of exploiting the sugars contained in spoiled date fruits (SD) to produce ethanol at high rates and yields was studied. Box–Behnken statistical experimental design was used for the optimisation of the water extraction of sugars with water with three factors i.e. solids loading, extraction time and extraction temperature, three levels and five replicates at the centre point, and the relative importance of each factor was assessed in terms of the achieved yield of sugars (g recovered sugars/kg SD). Subsequently, fermentation experiments with mono-cultures and co-cultures of newly isolates strains of *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Meyerozyma guilliermondii*, were performed aiming at the maximization of substrates consumption, ethanol yields and titters.

Introduction
The continuously increasing world population and the rapid industrial development have led to the depletion of the global energy reserves and the need to explore alternative energy sources. Biofuels are clean and renewable energy carriers, and among them ethanol produced by fermenting microorganisms is a strong candidate to fill the energy demand gap. Among the most important issues for the industrialization of ethanol production in a sustainable way, is the cost of the raw material and its maximum biotransformation using cost-effective and environmentally friendly methodologies.
The present study investigates the efficiency of bioethanol production via novel yeast strains, using as feedstock the extracted sugars from spoiled date fruits that were discarded as non-edible. The optimization of the extraction process was based on Box–Behnken statistical experimental design with three factors i.e. solids loading, extraction time and extraction temperature, and a relative importance of each independent parameter was assessed in terms of the achieved yield of sugars (g recovered sugars/kg SD).

Materials and Methods
Feedstock
SD were obtained from the local street market of Abha city, Kingdom of Saudi Arabia. The discarded whole fruits containing seeds and skins were collected and packed in PPT bags in batches of 2 kg. Contamination of the SD with insect larvae (4-6 mm, whitish) was observed in some cases but no evident other fungal or bacterial degradation was noticed by bare eye. Upon arrival at the laboratory larvae and fruit seeds were manually removed, and the remaining fruits were washed with warm water and aid dried for 24h. The air dried reach in sugars endocarps/pericarps were then milled in batches with a conventional grinder until formation of a homogeneous pulp. Batches were mixed and divided in 250ml plastic sealed cups which were stored at -21^{o}C.
For the characterization of dates one batch of homogenized pulp prior of freezing was used. The chemical characteristics of the SD pulp were as following: TS (%), 68.37 ± 1.28; Humidity (%),31.63 ± 1.81; VS (%TS), 96.71 ± 0.08; Ash (% TS), 2.25 ± 0.11; pH (10% aqueous solution), 6.12 ± 0.02; Soluble carbohydrates (%),51.32 ± 0.84; Total carbohydrates (%), 66.33 ± 3.67; TKN (NH3-N, %), 0.61 ± 0.04; Proteins (%), 3.75 ± 0.25.

Extraction of sugars
The recovery of soluble sugars from the SD pulp with water was optimized via Box–Behnken design with three selected independent variables (factors), i.e. extraction temperature, extraction time and solids loading. The variables were placed at three equally spaced values (levels), coded as −1, 0, +1 as shown in Table 1, whereas five replicates at the centre point for the estimation of errors were applied to evaluate the main and interaction effects of the factors and to fit a second-order model with quadratic terms. Extractions were performed under constant mechanical agitation at 150rpm, and the extract was recover via centrifugation for 15min at 4.000rpm.

The concentration of soluble sugars was quantified in the extracts and the recovery yield was estimated in each case as g recovered sugars/kg SD.

Table 1. Factors and levels of Box–Behnken design applied for the optimisation of water extraction of sugars from SD

Independent Variable	Code	Code level		
		-1	0	+1
Solids Loading (% w/v)	X1	20	30	40
Temperature (^{o}C)	X2	20	30	40
Time (min)	X3	10	20	30

Fermentation experiments

Fermentation tests were performed using newly isolated yeast strains of the species *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Meyerozyma guilliermondii*, in cocultures and mono-cultures. Cultures were performed in duplicate in batch mode at serum vials with working volume 60 ml. The vials were sealed with rubber stoppers equipped with 0.22 μm filters for CO_2 venting and incubated at 150 g and 30 °C. In all experiments, cells were harvested from pre-culture of the isolates at 10% v/v. For the inoculation, the estimated volume of pre-culture was centrifuged at 4500 g for 15 min and the yeast pellet was re-suspended in a solution containing KH_2PO_4, $MgCl_2$. 6 H_2O and $(NH_4)_2SO_4$ each at final concentrations of 1 g/L culture. The efficiency of alcoholic fermentation was assessed by estimating bioethanol yields in terms of carbohydrate uptake and initial feedstock bioconversion.

Analytical methods

Total solids (TS), volatile solids (VS) and Total Kjeldahl Nitrogen (TKN) were quantified according to Standard Methods (APHA, 1995). Crude protein content was determined by multiplying TKN by a factor of 6.25 (Monlau et al., 2012). Soluble sugars and total carbohydrates were quantified according to DuBois et al. (1956). Reducing sugars were quantified by the DNS (3,5-dinitrosalicylic acid) method and was expressed as glucose equivalents (Miller, 1959). Ethanol was quantified via HPLC-RI (Shodex) with an Aminex HPX–87H column (Biorad) at 60^0C and a Cation H micro-guard cartridge (biorad Laboratories), with H_2SO_4 0.006N mobile phase at a flow rate of 0.6mL/min.

Results

Optimization of the extraction process

The recovery of soluble sugars via warm water is a well-established method applied to different types of biomass and aiming to different end use applications with solids loading in the aqueous suspension, temperature and extraction time are among the most important factors affecting the recovery yields. In this study the differentiation of the yield of recovered sugars from the SD pulp was studied for loading of solids 20 to 40% (on wet mass basis) temperatures, 20 - 40 ^{o}C and extraction time 10 - 30 minutes. Based on the F-value and *p*-value it was shown that the model had a good fit for prediction, whereas the high R^2 indicated that the model was statistically significant and that model equation can indeed be used to adequately describe the water extraction process under a range of operating conditions.

Ethanol production from SD extract

The extraction process was optimized based on the yield of sugars from the initial biomass, indicating the relative importance of the different parameters of the experiment, without taking into account the final concentration of sugars in the extracts, which was, as expected, relatively increased for the higher solids loading, ranging from 55-180 g/L. The effect of the substrate concentration, however, was assessed in the fermentation experiments via both mono-cultures and co-cultures. In general, the fermentation efficiency did not seem to be affected by the solids loading whereas in all cases co-cultures resulted to higher titters and efficiencies.

References

APHA, AWWA, WPCF 1995. Standard Methods for the Examination of Water and Wastewater. M.A. Franson (Ed.) American Public Health Association, Washington, DC.

DuBois, M., Gilles, K., Hamilton, J., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal Chem. 28, 350-356.

Miller, G.L., 1959. Use of dinitrosalicylique acid reagent for determination of reducing sugar. Anal. Chem. 31, 426 – 428.

Monlau, F., Barakat, A., Steyer, J.P., Carrere, H. .2012. Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. Bioresour. Technol 120, 241–247.