Utilization of prickly pear waste for baker's yeast production

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Running Title: Production of Baker's Yeast

Highlights

The feasibility of using *OFI* waste as substrate for baker's yeast production was investigated

• *OFI* fruit juice is a suitable substrate for baker's yeast production

• The sugar extraction from *OFI* fruit peels using heat treatment (<150 °C) is recommended as a clean and environmentally benign process. The advantage of this treatment is that acid addition is not needed and no pressure, used.

• *OFI* peel juice represent a potentially inexpensive and renewable carbohydrate feedstock for the fermentation of baker's yeast

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Abstract

The feasibility of baker's yeast production using fruits and peels of *Opuntia ficus indica* (*OFI*) as carbohydrate feedstock was investigated. Two response surface methodologies involving central composite face centered design (CCFD) were successfully applied. The effects of four independent variables on baker's yeast production from *OFI* fruit juice was evaluated using the first CCFD. The best results were obtained with 24 h of inoculum age, 30 °C temperature, 200 rpm of agitation and 10% inoculum size. At the maximum point, the biomass concentration reached 9.29 g/L. A second CCFD was performed to optimize the sugar extraction from *OFI* fruit peels. The potential of these latter as a fermentation substrate was determined. From the experimental results, the *OFI* fruit peel is an appropriate carbon source for the production of baker's yeast. The maximum biomass concentration was 12.51g/L. Different nitrogen supplements were added to promote the yields of baker's yeast. Corn steep liquor was found to be the best alternative nutrient source of casein hydrolysate and yeast extract for baker's yeast production.

Keywords: Baker's yeast, Fermentation, *Opuntia ficus indica*, Response surface methodology, *Saccharomyces cerevisiae*, Thermal pretreatment

List of Abbreviations

OFI: *Opuntia ficus indica*
LBAM: Luria-Bertani agar medium
SSM: Semi-Synthetic Medium
RSM: Response Surface Methodology
CCFD: Central Composite Face Centered Design
HMF: Hydroxymethyl furfural
YE: Yeast extract
AS: Ammonium sulfate
CH: Casein Hydrolysate
CSL: Corn Steep Liquor

1. Introduction

Algeria is a home to many agricultural wastes and surpluses which generally are partially or entirely unutilized, like *Opuntia ficus indica* (*OFI*). The cultivation of Algerian prickly pear cactus is dedicated exclusively to fruit production for fresh consumption, neglecting entirely the cladodes, peel and their byproducts. It is noteworthy that the consumption of fresh fruit causes the production of a huge amount of peel that consequently leads to serious environmental pollution problems and a generalized loss of nutritional value. Fruit peel is an abundant and renewable resource, suitable for animal feed as an important fodder crop during low feed availability periods following drought and dry seasons [1]. Several projects and local programs have supported the diverse application of *OFI* (in food and pharmaceutical areas). Extensive research done on the nutritional and therapeutic properties of *OFI* verified its high potential for human consumption. Presently, efforts are being made to develop the production of *OFI* and its application in various food and non-food products [2-5]. This offers an opportunity to add value to the crop while providing a healthy product that could significantly enhance the well-being of the consumer. The nutritive value of *OFI* is solely dependent on its total carbohydrates, crude protein, crude fat, fibers, ascorbic acid, and minerals [4, 6, 7]. *OFI* fruit is characterized with high percent of sugars (12-18%), mainly glucose and fructose [8, 9, 10]. The composition of the fruit depends mainly on weather conditions, plant age, and development of fruit stage at harvest [11]. On the other hand, peel or skin of cactus pear, which occupies up to 40-48% of the fruit [12, 8], is also rich in sugars and pectic polysaccharides [13-15], with glucose as the main sugar [16]. Anwar et al. [4] reported higher amounts of polysccharides (25%), cellulose (29%) and hemicellulose (8.5%). Also Habibi et al. [14] found that prickly pear peels contained 2.4% lignin and 66% polysaccharides, including 27% cellulose.

Consequently, *OFI* fruit, based on its high nutritive value and easy fermentability, is considered a substantial medium for fermentations [17]. Extensive studies using cactus pear fruit for a panoply metabolites production such as lactic acid [18], enzymes [19, 20]**,** ethanol [21]**,** food colorant [22], single cell oil [23] and single cell protein [24] have been conducted.

Saccharomyces cerevisiae (baker's yeast) is a common constituent in our daily nutrition. Application of agro-industrial wastes in production of baker's yeast is an alternative for refined and costly raw materials. However, many studies are investigated on production of baker's yeast using cost-effective raw materials. Production processes using by-products such as wastes of potato [25, 26], whey and molasses [27-30], cassava and wheat starch

hydrolysate [31, 25, 32], millet flour hydrolysate [32], waste and musts of date [33-36], and fruit wastes [37] have been investigated.

Therefore, this study aimed to investigate the use of *OFI* wastes as the main raw material for the production of baker's yeast from *Saccharomyces cerevisiae*. A central composite face centered design (CCFD) was employed to optimize the baker's yeast production from fruit waste. Experiments were conducted under a variety of operational conditions defined by four independent variables (inoculum age, process temperature, agitation and inoculum size). The role and interaction of each variable and the predicted production of baker's yeast during fermentation were determined. A second CCFD was performed to optimize the sugar extraction from *OFI* peel. The ability of this strain in using *OFI* fruit peel for production of baker's yeast and the effects of different nitrogen sources, were also evaluated.

2.Materials and methods

2.1. Raw material

2.1.1. Extraction of OFI fruit sugar

Fruits of *OFI* were harvested in the month of August from a prickly pear cactus farm outside Setif (AinArnat, Algeria). The wastes from the recovered prickly pear fruits were washed and peeled. The fruits pulps were manually cut into cubes and desiccated for 72 h at 60°C in a drying oven with a cold air current. The ratio of added tap water and dried fruit pulp was 2:1. Heat was applied to the mixture at a temperature of 100 °C for 45 min with constant stirring. The solid residue was separated by filtration. Cellulosic debris was separated from the mixture using a centrifuge at 4000 rpm for 20 min, while the supernatant was used later as the carbon source in the fermentation mediums. Before each experiment, suitable quantity of *OFI* fruit juice was diluted to the desired concentration of reducing sugars.

2.1.2. Extraction of OFI peel sugar

Fruit peels were cut into small pieces and dried in a ventilated oven at 60°C for 72 h. This was followed by grinding of the dried peels for a few minutes in a domestic coffee grinder, sieving and storing at room temperature until further use. For extraction of the sugars from the peel, the samples were subjected to heat treatment with hot air at different temperatures (60, 100, and 140°C) and different substrate loading (5, 10, and 15%) in an Universal Oven UF55 (Memmert, Germany) with an incubation time of 1h.

The peel juice obtained was filtered and centrifuged at 4000 rpm for 20 min. A suitable portion of *OFI* fruit peel juice was diluted to the desired concentration of reducing sugars for use as the carbon source in the fermentation medium. Table 1 presents a summary of the characteristics of the raw *OFI* used.

<<Insert Table 1>>

2.2. Microorganism and growth media

The yeast strain *Saccharomyces cerevisiae* (*S. cerevisiae*) ATCC 4226 used in this study was maintained at 4°C on Luria-Bertani agar medium (LBAM) containing the following components (g/L): peptone, 10 (Sigma); NaCl, 10 (Prolabo); glucose, 20 (Sigma); yeast extract 5 (Biokar) and agar, 15 (Sigma). The culture was periodically sub-cultured to maintain the cultures active and suitable for fermentation.

2.3. Fermentations

Cultures stored in LBAM, were activated in the semi-synthetic medium (SSM) containing (g/L): glucose, 10 (Sigma); yeast extract, 0.5 (Biokar); $(NH4)_2SO_4$, 10 (Sigma); $MgSO_4.7H_2O$, 3 (Merck); KH_2PO_4, 6 (Sigma); NaCl, 0.1 (Prolabo); $CaCl_2.H_2O$, 0.1 (Fluka) [36]. In all the experiments, the inocula were prepared by incubation at 30 ºC; a fermentation medium containing *OFI* juice served as the carbon source. Then, *OFI* juice was supplemented with different nitrogen sources: yeast extract (Biokar), ammonium sulfate (Sigma), urea (Sigma), peptone (Sigma), casein hydrolysate (Sigma) and corn steep liquor (Sigma). The nitrogen sources were tested individually at equivalent 0.217% nitrogen level (nitrogen concentration in SSM). From the results, the initial *OFI* juice sugar concentration was 50 g/L.

The pH of the production medium (*OFI* juice) was adjusted to 4.5 prior to sterilization. The solutions of nitrogen sources were sterilized separately. Fermentations were done in 500 mL Erlenmeyer flasks containing 50 mL medium. All experiments were conducted in triplicate.

2.4. Analytical methods

The optical density of the cell suspension was measured with appropriate dilution at 660 nm, using a spectrophotometer (Spectronic 70). Dry cell weight was determined using samples that were centrifuged, washed with distilled water and dried overnight at 105 °C to constant weight. The values of optical density measured were correlated with the concentrations of cells, in terms of dry weight of cells per litre of suspension (g/L) by using a linear calibration. Determination of reducing sugars content was by the colorimetric method using the UVVis spectrophotometer, (Spectronic Genesis 20) at 540 nm using 3,5-dinitrosalicylic acid (DNS reagent) with glucose as standard [38]. Glucose was measured using an enzymatic kit (Glucose PAP SL, Elitech). Concentration of protein was determined using the Lowry method with bovine serum albumin as the standard [39]. The moisture content of the raw *OFI* was estimated according to the AOAC method [40]**.** Ethanol concentration was determined

by colorimetric assay with a dichromate solution. The absorbance of samples was measured by spectrophotometer (Shimadzu-1601) at 590 nm. This method based on the complete oxidation of ethanol by dichromate in the presence of sulfuric acid with the formation of acetic acid. The calibration curve of ethanol determination was plotted similarly by using known concentration of ethanol as (1% to 5% v/v) [41].

2.5. Kinetic Parameters

The specific growth kinetic (μ), the productivity (δ) and overall cell yield ($Y_{X/S}$) were described as follows:

$$\mu = \frac{dX}{dt} \cdot \frac{1}{X} \quad \text{(Eq. 1)}$$

$$\delta = \frac{X - X_0}{d} \quad \text{(Eq. 2)}$$

$$Y_{X/S} = \frac{X - X_0}{S_0 - S} \quad \text{(Eq. 3)}$$

d : Fermentation time (h)

S : Residual sugar concentration (g/L)

S_0 : Initial sugar concentration (g/L)

X : Cell mass concentration (g/L)

X_0 : Initial cell mass concentration (g/L)

2.6. Experimental design and statistical analysis

2.6.1. Statistical optimization of baker's yeast production from OFI fruit juice

Four variables which influence the baker's yeast production were analyzed and optimized by the Central Composite Face Centered Design (CCFD) in three levels (-1, 0 and +1) as shown in Table (Table 2). Inoculum age (X_1, h), temperature (X_2, °C), agitation (X_3, rpm) and inoculum size (X_4, % v/v) were chosen as the independent variables. Shake flasks were incubated for 24 h. The initial sugar content of the juice was 50 g/L. Cell mass concentration (Y_{cm}) was used as the dependent output variable. For the four factors, a full 2^4 factorial design was used. The total number of experiments was obtained using following formula: $31 = 2^n + 2n + 7$, where n is the number of variables (n=4), this includes 2^4 full factorial CCFD comprising 16 factorial points, 8 axial points, and 7 replicates at the center point.

<<Insert Table 2>>

The design was generated with Minitab 16 software (Minitab Inc, State College, PA-www.minitab.com). For model validation, an optimal value for cell mass concentration was determined by a second order polynomial model presented in equation (Eq. 4):

$$Y_{cm} = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 \quad \text{(Eq. 4)}$$

Where Y_{cm} (cm: cell mass) is the predicted response for cell mass concentration, β_0 is the model constant, β_1, β_2, β_3 and β_4 are linear coefficients, β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} are interaction coefficients, β_{11}, β_{22}, β_{33} *and* β_{44} are squared coefficients. The coefficient of determination R^2 was used to express the quality of fit of the polynomial model equation.

2.6.2. Statistical optimization of sugar extraction from OFI fruit peel

The objective of the second CCFD was to optimize the sugar extraction from *OFI* fruit peel. Thus, a CCFD is made up of 2^k factorial points (k means factors=2), 2k axial points and five replicated at center point, resulting in a total of 13 experiments.

Temperature (X_i, °C) and substrate loading (X_{ii}, % w/v) served as the independent variables, and they have the following three levels: -1 (low), 0 (center), and +1 (high) as shown in Table 3.

<<Insert Table 3>>

The empirical second order polynomial equation (Eq. 5) is used to prove the relationship between the factors (X_1 and X_2) and the investigated response (Y_s).

$$Y_s = A + BX_i + CX_{ii} + DX_iX_{ii} + EX_i^2 + FX_{ii}^2 \quad \text{(Eq. 5)}$$

Where Y_s (s: sugar) is the response equation (sugar), A is the model constant, B and C are linear coefficients, D is the interaction coefficient, E and F are squared coefficients. Minitab 16 software was used to calculate the predicted responses, analyze the experimental data, and plot the surface plots.

3. Results and discussion

3.1. Baker's yeast production from *OFI* fruit juice

3.1.1.Optimization of Culture Conditions

Based on CCFD, response surface methodology (RSM) was used for optimization of fermentation process design factors. Table 4 presents the statistical combinations of actual values of variables along with the predicted and experimental responses. Maximum of cell mass production (8.52 g/L) was reached under these conditions: 26 h , 30 °C, 100 rpm and 10% inoculum size, respectively.

The second-order regression equation provided levels of cell mass production as a function of inoculum age, temperature, agitation and inoculum size which can be presented in terms of coded factors according to the following equation (Eq. 6):

$$Y_{cm} = 7.83 + 0.43X_1 - 2.88X_2 + 0.11X_3 + 0.55X_4 + 0.073X_1X_2 - 0.03X_1X_3 + 0.02X_1X_4 - 0.01X_2X_3 - 0.2X_2X_4 + 0.11X_3X_4 - 0.28X_1^2 - 2.91X_2^2 - 0.34X_3^2 + 0.11X_4^2 \quad \text{(Eq. 6)}$$

Fitting of the response function to the experimental data was done using regression analysis. The coefficient of determination (R^2) which was found to be close to 1 (0.97) proves the ability of the model to successfully predict the response surface of cell mass production. The ANOVA for cell mass production is presented in Table 5. A Model *F*-value of 257.31 (P = 0.00) implies model significance. The larger the magnitude of the *F*-value and smaller the *P*-value, the more significant is the corresponding coefficient. This implies the high significance of the linear (X_2) and square (X_2^2) effects of temperature as evident from their respective *p*-values (P = 0.00). The square effect of agitation (X_3^2), the linear coefficient (X_4) and interactive effects of X_2 and X_4 (X_2X_4) were significant for cell mass production with $P \leqslant 0.05$.

<<Insert Table 4>>

<<Insert Table 5>>

The interactive effects of variables on cell mass production were studied by plotting 3D surface curves against two independent variables with the other variable being kept at its central (0) level. The results of the curves are presented in Figure 1a-f.

As shown in Figure 1a, as the inoculum age and temperature increase, the cell mass increases until it reaches an optimal region (at temperature range from 30 to 33°C and inoculum age range from 24 to 26 h). However, increase in temperature beyond the optimum level resulted in decrease in the cell mass concentration. It is clear that growth temperature is an important factor in *S. cerevisiae* production process. Similar results were obtained by Beiroti and Hosseini [42], who studied baker's yeast production from date juice. The highest concentration of biomass was observed at the following conditions: temperature of 30 °C, inoculum age of 24 h, agitation of 200 rpm and inoculum size of 10%. Alemzadeh et al. [43] and Yalcin et al. [44] reported 30 °C as the optimum temperature for a maximum biomass production. Similar results were confirmed by Arroyo-Lopez et al. [45] who found that the temperature greatly influenced the metabolic rate of yeast compared to other variables like pH and glucose levels. From another study conducted by Vanoni et al. [46] on the effects of

temperature on the growth and nuclear and budding cycle in populations of the yeast *S. cerevisiae* in batch culture, the results showed that at 30 °C the maximal rate of exponential growth is achieved. According to Zakhartsev et al. [47], yeast metabolism when exposed to temperatures that are above optimal (above 31°C) varies in order to dissipate more heat. According to Tai et al. [48], the molecular mechanisms necessary for this heat dissipation include increased diffusion rates and increased fluidity of the cell membrane due to changes in phospholipids.

The effects of the inoculum age and agitation on the cell mass production are shown in Figure 1b. Cell mass increased with the increase of inoculum age and agitation. The maximum cell concentration was at inoculum age range from 22 to 25 h and agitation range from 180 to 220 rpm.

The effects of the inoculum age and size on the cell mass production are shown in Figure 1c. It should be noted that an increase in the inoculum age and size ended in high yields of cell mass production. The maximum cell concentration was at inoculum age range from 24 to 26 h and inoculum size range from 9 to 10%.

In Figure 1d, the 3D response surface plot was developed for the cell mass concentration with varying temperature and agitation. The maximum cell concentration was at temperature range from 30 to 32°C and agitation range from 150 to 250 rpm.

The effects of different temperature and inoculum size on cell mass production are given in Figure 1e. The interaction between the temperature and the inoculum size was significant (P=0.005). The response curve demonstrate that higher cell mass concentration are obtained at low temperature and high inoculum size.

The 3D response surface plot in Figure 1f shows the cell mass concentration as a function of agitation and inoculum size. Higher cell mass concentrations were obtained with higher inoculum size (ranging from 9.5 to 10%) and agitation (ranging from 200 to 250 rpm).

<<Insert Figure1>>

The optimum conditions necessary for the maximum cell mass production includes inoculum age, 24 h; temperature, 30 °C; agitation, 200 rpm and inoculum size, 10%. Experimental model validation was tested by conducting a batch experiment under optimal operating conditions (Table 4). From the results of validation experiments from three replications, the experimentally determined production values are closely related to the statistically predicted values, confirming the authenticity of the model.

3.2. Optimization of sugar extraction from *OFI* fruit peel

Heat treatment was carried out for 1 hour under different temperatures and different substrate loading according to the design earlier described. Central composite design of response surface methodology (RSM) was used to determine the levels of the factors (temperature and substrate loading) and the effect of their interaction on sugar extraction. From the second-order regression equation, levels of sugar concentration are presented as a function of temperature and substrate loading, which can be presented in terms of coded factors according to the following equation:

$$Y_s = 39.67 + 24.69X_i + 6.87X_{ii} + 2.30X_i^2 - 0.81X_{ii}^2 + 3.99X_iX_{ii} \quad \text{(Eq. 7)}$$

ANOVA was conducted to determine the significant effects of process variables and the results are presented in Table 6. From the *P*-values of each model term, it can be concluded that, the linear coefficients (X_i and X_{ii}) and interactive coefficient (X_iX_{ii}) are the most significant coefficient ($P = 0.00$). The large *F*-value indicates that majority of the variance in the response could be explained by the equation of the regression model. Accordingly, high *F*-value (500.79), very low *p*-value (P=0.000) and insignificant result from the Lack of Fit model ($P = 0.493$) obtained suggest that the experimental result of the model is highly significant.

<<Insert Table 6>>

Plot of 3D surface curve was used to study the interaction effects of variables on sugar extraction. Figure 2 presents the effects of the temperature and substrate loading on the sugar concentration. An increase in the substrate loading with temperature resulted in an increase in the sugar concentration. The maximum sugar concentration was at substrate loading range from 14 to 15% and temperature range from 135 to 140 °C.

The upward trend observed may be attributed to the pretreatment temperature. Increasing the temperature implies a corresponding increase in the number of hydrogen ions present in the solution. Veluchamy and Kalamdad [49] reported that the hot air oven pretreatment significantly affected lignocellulose content of pulp and paper mill sludge. They showed that the organic and inorganic compounds were efficiently solubilized at 80 °C for 90 min in hydrothermal pretreatment. Thus, hemicellulose is broken down mainly into xylose and glucose. The benefit of hot water pretreatment is the acidic characteristic of water and its

dissociation into hydronium ions at elevated temperatures which speeds up the hydrolysis of lignocellulosic biomass [50]. This pretreatment shows great potential for degrading lignocellulosic material thus making it easily accessible to enzymes by disrupting the inter-polymeric association between lignin, hemicellulose, and cellulose [51]; and this leads to minimal production of potentially inhibitory products [52]. Chen et al. [53] in their work on the investigation of the degradation of carbohydrates and lignine of the aspen wood during hot water extraction (HWE), show that the degradation of xylose did not occur until 150 °C. Kilpeläinen et al. [54] who worked on extraction of birch sawdust using pressurized hot water, reported only trace amounts of furfurals in the extracts after heat treatment at 150 to 160 °C. The amount of hydroxymethyl furfural (HMF) was under 6 μg/L for all extraction temperatures between 150-190 °C. However, no furfural or HMF was detected in hot water pretreatment of boreal aspen woodchips at 160 °C and 210 min [55]

<<Insert Figure 2>>

The validity of the model was tested using sugar extract experiments under optimal operation conditions (temperature 140°C and substrate loading 15%). Three repeated experiments were conducted. The sugar concentration obtained from experiments (76.47 g/L) was very similar to the response predicted (76.71 g/L) by the regression model, which proved the validity of the model.

3.3. Cell mass production from *OFI* fruit peel juice

The potential of *OFI* fruit peel juice as a fermentation substrate was determined after a heat temperature extraction step. The capacity of *S. cerevisiae* for cell mass production was tested in a medium containing *OFI* fruit peel juice as the carbon source using the optimal conditions obtained with the production of cell mass from *OFI* fruit juice (inoculum age, 24 h; temperature, 30 °C; agitation, 200 rpm and inoculum size, 10%). To investigate the influence of initial sugar concentration on cell mass production, *S. cerevisiae* was cultivated for 24 h with *OFI* fruit peel juice at various sugar concentrations (10 to 70 g/L).

Table 7 shows the cell mass concentration in the four different levels of sugar evaluated.

<<Insert Table 7>>

The result obtained show that cell mass production increased with increasing initial *OFI fruit* peel juice sugar concentration up to 50 g/L. The use *OFI* fruit peel juice with sugar concentration greater than 50 g/L increased the production of cell mass less significantly. *OFI* fruit peel juice is able to support the growth of *S. cerevisiae*, it can serve as a low-cost substrate for the production of baker's yeast.

Figure 3 presents the relationship between cell mass production and sugar consumption vs time on *OFI* fruit juice and *OFI* fruit peel juice. It's important to emphasize that *OFI* juice was not supplemented with nutrients to be used as fermentation medium. In both culture media the sugar use was additionally amid the exponential stage. The behaviour of the *S. cerevisiae* on *OFI* fruit juice is different from this on *OFI* fruit peel juice. In the latter, the strain consumed practically all the sugar present in the medium after 24 h fermentation (3g/L residual sugar concentration). Similar behavior was observed during the fermentation of spent coffee grounds hydrolysate by different yeast strains. Notably, it has been shown that *S. cerevisiae* (RL-11) consumed faster the sugars than the other strains, with almost total depletion after 24 h fermentation (residual sugar concentration 5 g/L). Indeed, the Kinetics of sugars consumption for the three yeasts is related to the variety of sugars present in this medium [56].

In the *OFI* fruit juice, the residual sugar concentration was 2.5 fold higher than in the *OFI* peel juice, it could be due to a low concentration of important nutrients (e.g. nitrogen source, mineral salts...etc) in the medium, which is in accordance with observations by Layokun et al. [57], who have worked on cashew apple juice that it contained a mixture of fermentable sugars (glucose, fructose and sucrose) as a substrate for the single cell protein production using *S. cerevisiae* NCYC 1250. These authors show that the consumption of sugars in the unsupplemented medium is lower compared to the supplemented medium with nitrogen source and mineral salts.

With *OFI* fruit juice as carbon source, the cell large scale manufacturing achieved a most extreme concentration of 9.29 g/L toward the finish of the exponential stage with the greatest explicit specific growth rate, yield coefficient and production values of 0.17 h^{-1}, 0.18 g/g and 0.35 g/L/h, respectively. After 24 h the cell mass production increased less significantly, the most extreme concentration of 10.44 g/L was obtained at the end of fermentation. The growth of *S. cerevisiae* on *OFI* fruit peel juice exhibited a diauxic pattern, with two growth stages. In the first growth stage, the cell mass production reached a most extreme concentration of 12.51 g/L at the end of the exponential phase (24 h) with the most extreme specific growth rate, yield coefficient and productivity values of 0.22 h^{-1} (μ_1), 0.25 g/g and 0.48 g/L/h,

respectively, which was associated with ethanol accumulation in the culture during the fermentation. In the second growth stage (24 h to 50 h), after an intermediate lag phase between 20 to 25 h, the cells growth was continuous although the residual sugar content was low and reached a maximum concentration of 20.72 g/L after 50 h of fermentation with the specific growth rate of 0.07 h^{-1} (μ_2). The increase of cell mass was attributable to the re-assimilation of produced ethanol in the first stage that relies largely on glycolysis for energy production. In the presence of sugars, together with other fundamental supplements, for example, amino acids and minerals, *S. cerevisiae* will conduct fermentative digestion to ethanol and carbon dioxide as the cells endeavor to make energy and recover the coenzyme NAD+ under anaerobic conditions [58]. It is during this phase that the majority of the ethanol is excreted, and *S. cerevisiae* cells undergo even progressively distressing conditions [59, 60] and modulate their metabolic activities in order to adapt to these environmental changes [61]. The yeast cells specially consume glucose when both glucose and ethanol were accessible, until the point that all the glucose was consumed totally [62, 63]. Without a doubt, the difference in the main development stage to the second development is related to a switch-over in enzymatic responses, and the production of new enzymes [64].

OFI fruit peel juice as carbon source showed high concentration of produced cell mass, comparing with *OFI* fruit juice. This may be due to higher nitrogen content in the peel which is necessary for the development of the organism and to the presence of glucose and certain minerals, i.e. calcium, potassium, magnesium and manganese [9, 15, 16, 65]. In addition to that, the presence of microelements such as zinc, copper and iron, although in trace quantities, are basic activators and modulators of various biological activities which are significant to yeast performance and survival [66].

It is clear that the fermentation process, the composition of the medium, the strain used and the nature of the carbon and nitrogen sources influence the cell mass production.

<<Insert Figure 3>>

3.4. Effect of nitrogen source on cell mass production

Nitrogen is a fundamental supplement amid fermentation since it impacts both yeast development and metabolism. It is important for the production of amino acids, enzyme co-factors, a few carbohydrates and different substances. Also, yeast cell development, and by-product formation are influenced by changes in the amount and source of nitrogen in the culture media [58, 67-69]. As certain yeast species are nutritionally exacting and require a few amino acids and nutrients for development, it is critical to pick the correct nitrogen and

carbon sources. Different organic nitrogen sources were added to the production medium to evaluate their suitability to support baker's yeast production. The effect of these sources on the cell mass production for 50 h cultivation is given in Table 8. The results demonstrate that the type of nitrogen source has a strong influence on cell growth. As shown, casein hydrolysate and yeast extract were the best nitrogen sources to support cell growth reaching about 23.92 g/L and 23.84 g/L cell mass, respectively (about 2.3-fold higher cell mass concentration compared to the control culture: *OFI* fruit juice without nitrogen source) with yield of 0.5 g/g. Most of the previously published studies mentioned only lower maximum cell mass concentrations. During their experiments performed in flasks with palm date sugar, Khan et al. [33] reached a concentration of 11.70 g/L, and in this latter case with a much lower productivity (0.12 g/L/h) compared to our result (0.46 g/L/h). Alemzadeh and Vosoughi [43], obtained with date sugar (20 g/L), a maximum concentration of cell mass of 6.6 g/L with yield of 0.33 g/g, Beiroti and Hosseini [42] obtained a maximum concentration of 7 g/L with yield of 0.34 g/g. Yalcin and Ozbas [44] also reported a low cell mass concentration (3.5 g/L) from glucose. In their work, they investigated the effects of pH and temperature on growth and glycerol production kinetics of two indigenous wine yeast strains *S. cerevisiae*.

Other works performed in a fermenter mentioned similar yields. Aransiola et al. [31] reported yields of 0.472, 0.462 and 0.470 g/g in the study of baker's yeast production under batch conditions in a bioreactor using hydrolysates obtained from acid, acid-enzyme and enzyme-enzyme hydrolysis of raw cassava starch, respectively. Solomon et al. [70] reported yield of 0.48 g/g, in the study of single cell protein production on blackstrap molasses and Lotz et al. [25] estimated the biomass yield to be 0.53 g/g, when *S.cerevisiae* was cultivated on glucose (24.7 g/L) with addition of potato protein liquor (10%), and 0.46 g/g, when *S. cerevisiae* was cultivated on glucose (21.1 g/L) with addition of potato protein liquor (5%). Layokun et al. [57] estimated the biomass yield to be 0.5 g/g when this microorganism was cultivated on cashew apple juice for the production of single cell protein.

Yeast cells perceive the nature and accessibility of nitrogen compounds and effectively modify their transcriptional, metabolic, and bio-engineered capacities to coordinate that discernment.[62].

On the other hand, cell mass production was higher (23.84 g/L) with *OFI* fruit juice supplemented with yeast extract than *OFI* peel juice supplemented with the same nitrogen source (19.5 g/L). This may be due to the inhibitory action of high total protein content in peel juice medium with initial nitrogen concentration in addition to the protein of yeast

extract. Thus, *OFI* peel juice alone may be sufficient to provide nitrogen source. Hence, the addition of nitrogen source was not essential.

Casein hydrolysate and yeast extract showed more cell mass production from *OFI* fruit juice, followed by CSL and peptone compared to urea and ammonium sulfate. Similar growth behaviour was observed by Da Cruz et al. [71] in fermentation using maltose as carbon source at 2%. In this study, higher biomass accumulation (9.5 g/L) using *S. cerevisiae* was observed in the media with peptone and casein hydrolysate compared to the media with ammonium sulfate (2.5 g/L). Concentrated sweet sorghum juice was used by Yue and al. [72] in a study of the impact of various nitrogen sources ($CO(NH_2)_2$ or $(NH4)_2SO_4$) on the fermentation and development of yeast cells in very high-gravity fermentations. These authors found that *S. cerevisiae* better assimilates organic nitrogen than inorganic. Thomas and Ingledew [73] used wheat mashes in a study of the effect of amino acids on the fermentation and growth of yeast cells. From their results, mixtures of amino acids stimulated growth and decreased the fermentation time.

The higher biomass concentrations with organic nitrogen sources could possibly also be attributed to improved nitrogen utilization for anabolic processes due to the presence of amino acids. Hence, yeast cells couple their synthetic capacity and development rate to the quality and measure of accessible metabolizable nitrogen [62]. It has been reported that biomass yield was higher with the mixture of amino acids than it was with either glutamic acid and ammonium as the nitrogen source [74]. According to Makinen et al. [75], increased concentration of amino acids in the wort increased the fermentation rate and accelerated the growth of the yeast under both aerobic and anaerobic conditions. Moreover, yeast extract is a rich source of trace elements and vitamins which are important for cell development [76].

These results suggest that the nitrogen source supplementation enhances cell mass production compared to the results obtained without supplementation. Although, the sources of nitrogen such as casein hydrolysate and yeast extract have been reported to support microbial process, the economic viability of these sources for baker's yeast production on an industrial scale are in doubt due to their cost. In conclusion for the fermentation utilizing *OFI* fruit juice, among the diverse nitrogen sources, CSL could be considered as a cheap potential source of nitrogen as an option in contrast to the expensive nitrogen sources.

<<Insert Table 8>>

Conclusion

The present study features a strategy for reusing, reprocessing and possible usage of *OFI* waste for valuable uses as opposed to their release to the earth which may cause adverse environmental effects. The feasibility of producing baker's yeast from *OFI* waste as a source of carbon using *Saccharomyces cerevisiae* was investigated. The baker's yeast production from fruit was carried out using response surface methodology (RSM) based on central composite face centered design (CCFD). This latter proved to be reliable and powerful tool for modeling, optimizing and studying the interactive effects of four process variables (inoculum age, temperature, agitation, and inoculum size) of baker's yeast production from *OFI* fruit. The results also demonstrate the suitability of *OFI* fruit peel as an economically feasible alternate substrate for use in baker's yeast production. Different nitrogen sources were used for direct fermentation of *OFI* juice to cell mass production. Corn steep liquor was found to be the best alternative nutrient source of casein hydrolysate and yeast extract for baker's yeast production. These results clearly indicate the high potential of *OFI* juice for baker's yeast production by *S. cerevisiae* for subsequent industrial applications.

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Conflict of interest: The authors declare that there is no conflict of interest.

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Figure Legends

Figure 1. Surface plots (a - f) for interactive terms in cell mass production from *OFI* fruit

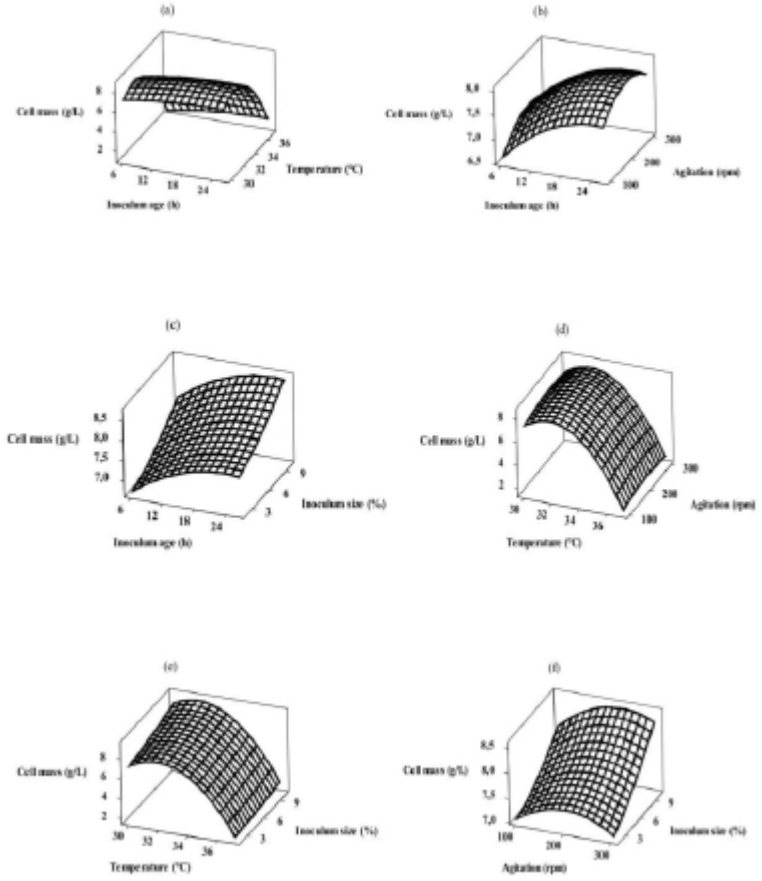

Figure 2. Surface plot for interactive terms in *OFI* peel sugar concentration

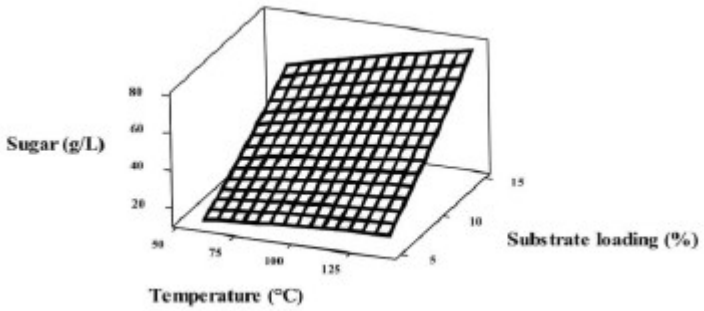

Figure 3. Cell mass production and sugar consumption by *S. cerevisiae* at optimized conditions. Symbols: ▲, Cell mass production on *OFI* fruit juice; ■, Residual sugar concentration of *OFI* fruit juice; ●, Cell mass production on *OFI* fruit peel juice; ▼, Residual sugar concentration of *OFI* peel juice; ✦; Ethanol concentration of *OFI* peel juice

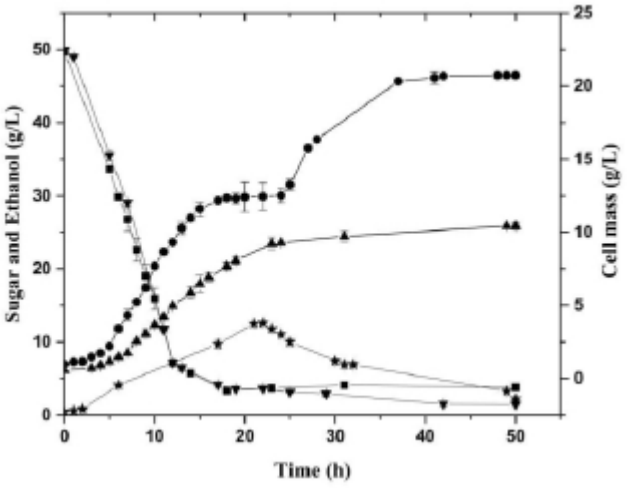

Table 1. Characteristics of raw materials of *OFI* fruit pulp and peel used as carbon sources

Characteristics	*OFI* fruit	
	OFI pulp	*OFI* peel
Moisture content	84.57±0.07	82.91±1.23
pH	5.36±0.11	5.08 ±0.042
Fraction occupation	43.76 ±1.32	56.22 ±0.07
Reducing sugars[a]	50.30 ±1.71	52.24±1.33
Glucose[b]	26.54 ±1.72	20.10 ±1.08
Protein[b]	1.106 ±0.015	10.45±0.18

[a] Sugar concentration (g/L) of *OFI* juice used in fermentation experiments at optimum conditions
[b] (% w/w, dry matter)

Table 2. Levels of independent variables in the experimental design for baker's yeast production from *OFI* fruit.

Variables	Symbol	Actual levels of coded variables		
		-1 (low)	0 (middle)	+1 (high)
Inoculum age (h)	X_1	6	16	26
Temperature (° C)	X_2	30	33.5	37
Agitation (rpm)	X_3	100	200	300
Inoculum size (%)	X_4	2	6	10

Table 3. Levels of independent variables in the experimental design for sugar extraction from *OFI* fruit peel.

Variables	Symbol	Actual levels of coded variables		
		-1 (low)	0 (middle)	+1 (high)
Temperature (°C)	X_1	60	100	140
Substrate loading % (w/v)	X_2	5	10	15

Table 4. Experimental and predicted values of yeast cell mass concentration recorded in the experimental set up of RSM

Run	Inoculum age X_1 (h)	Temperature X_2 (° C)	Agitation X_3 (rpm)	Inoculum size X_4 (%)	Cell mass (g/L)	
					Experimental	Predicted
1	16	30.0	200	6	7.68	7.80
2	26	37.0	100	10	2.04	2.24
3	6	30.0	300	10	8.08	7.94
4	6	37.0	100	2	0.81	0.68
5	16	33.5	200	6	7.80	7.83
6	16	33.5	200	6	7.92	7.83
7	26	30.0	100	2	6.84	6.88
8	16	33.5	200	6	7.84	7.83
9	6	30.0	100	10	7.40	7.39
10	16	33.5	200	6	8.08	7.83
11	6	37.0	300	2	0.72	0.71
12	16	33.5	200	2	7.28	7.39
13	26	30.0	100	10	8.52	8.25
14	26	37.0	100	2	1.86	1.71
15	16	37.0	200	6	1.93	2.03
16	6	30.0	100	2	6.12	6.14
17	16	33.5	200	6	8.32	7.83
18	16	33.5	200	6	7.72	7.83
19	26	37.0	300	10	2.89	2.59
20	6	37.0	100	10	1.23	1.10
21	16	33.5	200	6	7.88	7.83
22	26	30.0	300	2	7.01	6.86
23	6	37.0	300	10	1.41	1.57
24	26	33.5	200	6	7.96	7.99
25	16	33.5	300	6	7.76	7.60
26	26	37.0	300	2	1.40	1.61
27	26	30.0	300	10	8.32	8.66
28	16	33.5	200	10	8.40	8.51
29	6	30.0	300	2	6.24	6.24
30	16	33.5	100	6	7.00	7.38
31	6	33.5	200	6	6.92	7.11
32[a]	24	30	200	10	9.29	8.8

[a]: Optimum conditions of cell mass production

Table 5. ANOVA with estimated regression coefficients for cell mass production from *OFI* fruit

Source	Degree of freedom	Sum of squares	Mean Squares	*F*-Value	*P*-Value
Model	14	242.42	17.31	257.31	0.000
X_1	1	3.47	0.04	0.65	0.431
X_2	1	149.76	19.94	296.34	0.000**
X_3	1	0.22	0.19	2.87	0.109
X_4	1	5.56	0.38	306.06	0.029*
X_1^2	1	50.62	0.20	3.06	0.099
X_2^2	1	31.46	22.07	328.04	0.000**
X_3^2	1	0.26	0.30	4.50	0.050*
X_4^2	1	0.03	0.03	0.54	0.473
X_1X_2	1	0.08	0.08	1.27	0.276
X_1X_3	1	0.01	0.01	0.16	0.616
X_1X_4	1	0.01	0.01	0.17	0.684
X_2X_3	1	0.00	0.00	0.08	0.783
X_2X_4	1	0.69	0.69	10.30	0.005**
X_3X_4	1	0.19	0.19	2.91	0.107
Residual Error	16	1.07	0.06		
Lack of fit	10	0.83	0.08	2.03	0.2
Pure Error	6	0.24	0.04		
Total	30	243.50			

** Very significant
* Significant at 5% level

Table 6. Estimated regression coefficients for sugar extraction from *OFI* fruit peel.

Source	Degree of freedom	Sum of squares	Mean Squares	*F*-Value	*P*-Value
Model	5	4018.99	803.80	500.79	0.000
X_i	1	3657.58	3657.58	2278.77	0.000**
X_{ii}	1	283.18	283.18	176.43	0.000**
X_i^2	1	12.82	14.61	9.11	0.019*
X_{ii}^2	1	1.81	1.81	1.13	0.323
X_iX_{ii}	1	63.60	63.60	39.62	0.000**
Residual Error	7	11.24	1.61		
Lack of fit	3	4.71	1.57	0.96	0.493
Pure Error	4	6.53	1.63		
Total	12	4030.22			

** Very significant
* Significant at 5% level

Table 7. Kinetic parameters of growth of *S. cerevisiae* using different sugar concentrations of *OFI* fruit peel sugar

Sugar concentration (g/L)	Cell mass[a] (g/L)	Yield (g/g)	Productivity (g/L/h)
10	3.4±0.14	0.32	0.13
30	8.15±0.07	0.27	0.32
50	12.51±0.08	0.25	0.48
70	14±0.14	0.19	0.54

[a] Values are expressed as Mean ± Standard Deviation

Table 8. Kinetic parameters of growth of *S. cerevisiae* on *OFI* juice supplemented with different nitrogen sources

OFI juice	Cell mass[a] (g/L)	Yield (g/g)	Productivity (g/L/h)
OFI fruit juice	10.44±0.08	0.21	0.20
OFI fruit juice + YE[b]	23.84±0.16	0.50	0.46
OFI fruit juice + Peptone[b]	18.88±0.16	0.62	0.36
OFI fruit juice + CH[b]	23.92±0.14	0.50	0.46
OFI fruit juice + CSL[b]	22.72±0.13	0.48	0.40
OFI fruit juice + AS[b]	10.72±0.07	0.22	0.44
OFI fruit juice + Urea[b]	17.28±0.16	0.40	0.33
OFI peel juice	20.72±0.08	0.40	0.40
OFI peel juice + YE[b]	19.5±0.07	0.42	0.38
OFI peel juice + AS[b]	13.68±0.10	0.30	0.26

[a] Values are expressed as Mean ± Standard Deviation
[b] The amount of nitrogen was set at equivalent 0.217% nitrogen level
YE: Yeast Extract
AS: Ammonium Sulfate
CH: Casein Hydrolysate
CSL: Corn Steep Liquor