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# OPTIMZATION OF PARAMETERS FOR MAXIMUM EXTRACTION OF SUGAR, BY USING PECTINASE AND CELLULASE FROM KABKAB DATE FRUIT USING RESPONSE SURFACE METHODOLOGY

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## ABSTRACT

There are about 400 varieties of dates known in Iran, but only few of them are commercially important. The present study focuses on enzymatic extraction of sugar from Kabkab Date Fruit. Enzymes are integral part of extraction and highly suitable for optimizing processes. Pectinases and cellulases are known enzymes, for facilitating juice extraction from different fruits. These enzymes were used in the present study, for evaluating their effectiveness in sugar extraction process from date fruits. Comparison of combination of both enzymes Pectinex® Smash XXL and Cellubrix® L, with untreated date fruit, at temperature range of 40-62°C showed that, the amount of extracted sugar were effected by enzymatic treatment. This study applied the Response Surface Methodology, to determine optimum extraction condition to produce a high yield of sugar. The highest yield of sugar was obtained at pH 7.5 and temperature 62 °C.

**KEYWORDS**: Pectinase, Cellulase, Sugar Extraction & Enzyme Treatment

## INTRODUCTION

Date is a nutrient fruit which contains digestible sugars like glucose, saccharine and fructose (70%), regime fibers, vitamins such as B1 and B2 and acid folic. Internal part of date is a rich source of iron, calcium, natural antioxidants and other active compounds, so date is a good source of vitamins and macro elements like phosphor, iron, potassium and considerable amount of calcium. [1]. The flesh of a fully ripe date, consist of two-third sugars and one-quarter water, the rest being mainly cellulose, pectin, ash and vitamins. The date is considered as a nutritious fruit as research has indicated the clear contribution of dates to human health when consumed with other food constituents.[2].

The use of cellulases and pectinases has been an integral part of modern fruit processing technology, involving treatment of fruit mashes as they not only facilitate easy pressing and increase in juice recovery, but also ensure the highest possible quality of end products. For example, pectinases can hydrolyze pectin and cause pectin-protein complexes to flocculate, so the resulting juice has a much lower amount of pectin and also a lower viscosity, which is advantageous for the filtration process. These enzymes not only help in softening the plant tissue but also lead to the release of cell contents that may be recovered with high yield [3] The use of pectinase/cellulose enzymes gave the highest recovery of total soluble solids (68%) compared with control without these enzymes (35%).[5]

The enzymatic hydrolysis of pectin substances is influenced by several factors such as temperature, pH, incubation time. Assessing the individual influence of each independent variable is a laborious task, and the Response

Surface Methodology (RSM) is a skillful statistical tool for optimizing the search for the best operational condition. [4]. Experiments were, therefore conducted to evaluate the yield of sugar extracted by treating Date pulp with pectinolytic and cellulolytic enzymes. The result thus obtained, is reported in this communication.

## MATERIALS AND METHODS

### Collection of Fruits

Date fruits of Kabkab variety, at the tamer stage of maturity were purchased from a local shop of Kerman Iran. Pectinex® Smash XXL and Cellubrix® L were obtained from Novozyme (Denmark) representative in Iran, and stored at 4°C. All used chemicals were of analytical grade from known manufacturers.

### Juice Extraction

Date fruits of Kabkab variety, at the tamer stage of maturity were pitted manually Homogenized fruit pulp was obtained by homogenizing pulp: water ratio of 1:3 using a fruit blender (MJ-176 NR, National Co. Ltd., Japan) for 4-5 min. The pH was adjusted by HCL (0.1N) and NAOH (0.1N). Pectinex® smash XXL and Cellubrix® L Pectinex® smash XXL were added in combination. The pulp and enzyme mixture were incubated at a temperature range of 40 °C -62 °C for 1 hour. At the end of incubation, the enzyme was inactivated by heating the mixture at 90 °C for 3 min. Enzymatically treated juices was centrifuged at((model 3-18k, Sigma Co., Germany) 16000 ×g for 15 min and the supernatant was separated.

**The Table Shows the Effect of pH and Temperature on the Yield of Sugar in Percentage**

| Run | Factor 1 A:pH | Factor 2 B:Temperature degree celsius | Response 1 sugar percentage |
|---|---|---|---|
| 1 | 6.5 | 40 | 20.85 |
| 2 | 6.5 | 45.5 | 20.87 |
| 3 | 7 | 45.5 | 18.2 |
| 4 | 6 | 56.5 | 21.01 |
| 5 | 6.5 | 56.5 | 19.9 |
| 6 | 7.5 | 62 | 18.67 |
| 7 | 5.5 | 62 | 20.03 |
| 8 | 7 | 51 | 18.61 |
| 9 | 7.5 | 40 | 19.01 |
| 10 | 6.5 | 51 | 19.93 |
| 11 | 5.5 | 51 | 20.07 |
| 12 | 7.5 | 51 | 18.6 |
| 13 | 6 | 51 | 21.06 |
| 14 | 5.5 | 40 | 19.11 |
| 15 | 7 | 56.5 | 18.67 |
| 16 | 6 | 45.5 | 20.9 |
| 17 | 6.5 | 62 | 21.08 |

### Experimental Design and Analysis

The ranges of experimental parameters were selected based on preliminary trials. The factors considered are :pH and temperature. The response is percentage of sugar yield. The experiment consisted of treatment of Date pulp by Pectinex® smash XXL and Cellubrix® L Pectinex® smash XXL combination at 40-62°C. Response Surface (User-Defined) Methodology was used to determine the optimum temperature and pH conditions for enzymatic extraction of tamer date fruits of Kabkab variety, as statistical experiment design method. The experimental design and statistical anaylsis were performed, using Design –Expert version10.

## RESULTS AND DISCUSSIONS

### Statistical Analysis

The equation below describe the effect of pH and temperature on the yield of sugar

$$Y=20.15-3.17A-0.22B-0.28AB-1.24A^2+0.43B^2+0.11A^2B+0.36AB^2+2.44A^3+0.28B^3$$

Y-yield of sugar in percentage

A-pH

B-Temperature

The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. Response surfaces and contour plots were generated with the help of commercial statistical package, Design-Expert version 10. The variation of sugar yield with pH and temperature are graphically represented in the 3-D surface plot and contour plots (fig a and b)

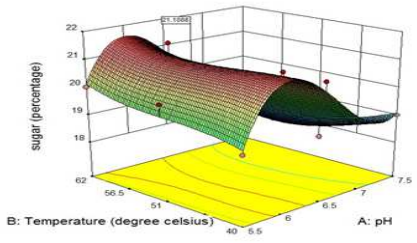


**Figure (a): 3-D Response Surface plot**

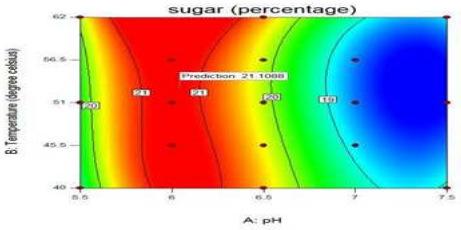


**Figure (b): Contour Plots**

### Optimization

Fig d shows the optimum conditions of the extraction process to yield maximum sugar. Sugar yield in percentage is shown in the figure by numerical optimization. The contour indicated that the predicted yield of sugar is 21.08% at pH7.5 and temperature 62°C.

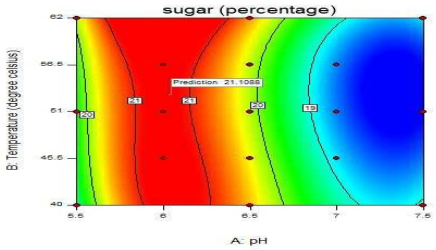


**Figure c: Contour Plot Sugar Extraction**

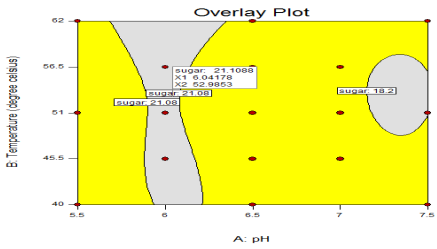


**Figure d: Optimum Conditions for Sugar Extraction**

In graphical optimization overlay plot (fig d) was obtained by applying superimposing surface methodology to contour plots of the response variables to select optimum combination of pH and temperature for the maximum extraction of sugar. The optimum was obtained was 21.08% at 62°C and pH 7.5.

## CONCLUSIONS

Treatment of date juice by each of the Pectinex® smash XXL or Cellubrix® L , caused about 18% increase in the amount of extracted sugar (*Samira Bahramian, MehrdadAzin, 2011). Simulatenous application of both increased the*

*amount of extracted sugar*. Among the different extraction process in date syrup production, the use of pectinase and cellulase enzymes cause the optimum soluble solid extraction (68%), compared to blank sample (35%) without enzymatic processing. At optimum pH7.5 and temperature 62°C maximum amount of yield sugar was obtained

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