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Optimization of Enzymatic Extraction of Sugars from Kabkab Date Fruit

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Abstract: 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. Kabkab, a date cultivar from Kerman province in Iran, which is industrially used for extraction of its sugar, was selected for enzymatic extraction. Comparison of samples, pretreated by either Pectinex® Smash XXL or Cellubrix® L, with untreated date fruits, showed that amount of both extracted sugar and clarity of juices thus produced, were affected by enzymatic pretreatment of fruits. Pretreatment of fruits by each of the two enzymes caused equally about 18% increase in the amount of extracted sugars, while using a precisely determined mixture of two enzymes and a suitable condition, resulted in a further increase of sugar to about 46%, in relation to untreated samples. Furthermore, enzymatic treatment of date fruits positively affects the clarity of juices, with some exceptions.

Key words: Cellulase · Clarification · Date juice · Enzyme treatment · Pectinase · Sugar extraction

INTRODUCTION

Enzyme processing of fruits for more effective extraction and clarification of juices has been advocated by many workers [1-6]. Specific enzymes and enzyme combinations are needed to optimize the extraction of any particular fruit juice concerning yield and quality [7]. 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 increasing 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 [4, 8-10]. 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 [11]. They have been used for separating serum from pulpy fruits like banana, grapes, mango, etc., but the information on the use of these enzymes for juice extraction from date is scanty [12].

Dates (*Phoenix dactylifera* L.) have been an important crop in the desert regions of Middle Eastern countries [13, 14]. At present, approximately 2000 or more different cultivars of date palm are known to exist all over the world, but only a few important ones have been evaluated for their performance and fruit quality [13]. Process industries produce various date products like date-paste, date-syrup, date-dip, date-honey, date-jam, date-vinegar, etc. [15].

In the present study, cellulase and pectinase were used to extract and recover high-quality juice from date pulp. The conditions of the liquefaction treatments (holding time, temperature, concentration of enzymes and pH), significantly affected both the yield and quality of the liquid-form products [7].

In order to optimize the effect of factors, the studies have been carried out statistically, using Taguchi methodology [16]. Therefore, experiments were conducted to evaluate quality characteristics of juices prepared by treating their pulps with pectinolytic and cellulolytic enzymes. The results thus obtained are reported in this communication.

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MATERIALS AND METHODS

Materials: Date fruits of Kabkab variety, at the tamer stage of maturity that are used commonly for juice extraction, were procured from the local market 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: The tamer date fruits of Kabkab variety were pitted manually. Then the date pulp was homogenized with a pulp: water ratio of 1:3 using a food blender (MJ-176 NR, National Co. Ltd., Japan) for 4-5 min until a homogeneous fruit pulp was obtained. The pH of pulp: water mixture was adjusted by 0.1 N hydrochloric acid or 0.1 N sodium hydroxide according to Tables 1 to 3. Pectinex® smash XXL and Cellubrix® L were added alone and in combination, to the pulp. The pulp and enzyme mixtures were incubated at different

Table 1: Factors and their levels used in studying the effect of Pectinex® smash XXL and the results thus obtained

	Factors and their levels[a]			Results[b]	
Treatment No.	pH	Temp. (°C)	Enzyme conc.% (v/v)	Sugar% (w/v)	Clarity (abs.)
1	4.5 (1)	40 (1)	0.015 (1)	14.37±0.49	0.04±0.003
2	5.0 (2)	40 (1)	0.0175 (2)	18.34±0.91	0.047±0.001
3	5.5 (3)	40 (1)	0.02 (3)	17.08±1.36	0.070±0.006
4	4.5 (1)	45 (2)	0.0175 (2)	15.97±0.63	0.042±0.00
5	5.0 (2)	45 (2)	0.02 (3)	16.65±1.01	0.059±0.005
6	5.5 (3)	45 (2)	0.015 (1)	16.45±1.00	0.074±0.00
7	4.5 (1)	50 (3)	0.02 (3)	18.49±0.54	0.041±0.00
8	5.0 (2)	50 (3)	0.015 (1)	14.33±0.58	0.047±0.003
9	5.5 (3)	50 (3)	0.0175 (2)	16.46±1.02	0.082±0.003

a Figures in the parenthesis are coded levels

[b] Average of three replicates± standard deviation

Table 2: Factors and their levels for studying the effect of Cellubrix® L and the results thus obtained

	Factors[a] and their levels			Results[b]	
Treatment	pH	Temp. (°C)	Enzyme conc.% (v/v)	Sugar% (w/v)	Clarity (abs.)
1	5.5 (1)	58 (1)	0.01 (1)	14.91±0.82	0.074±0.003
2	6.5 (2)	58 (1)	0.015 (2)	15.94±1.98	0.134±0.041
3	7.5 (3)	58 (1)	0.02 (3)	16.42±0.48	0.191±0.002
4	5.5 (1)	62 (2)	0.015 (2)	16.60±0.17	0.074±0.001
5	6.5 (2)	62 (2)	0.02 (3)	17.02±0.61	0.167±0.012
6	7.5 (3)	62 (2)	0.01 (1)	16.42±0.5	0.174±0.005
7	5.5 (1)	66 (3)	0.02 (3)	15.70±0.4	0.088±0.01
8	6.5 (2)	66 (3)	0.01 (1)	15.35±0.00	0.160±0.008
9	7.5 (3)	66 (3)	0.015 (2)	15.64±0.77	0.167±0.00

[a] Figures in the parenthesis are coded levels

[b] Average of three replicates

Table 3: Sugar content of extracts, as a result of simultaneous application of Pectinex® smash XXL and Cellubrix® L

	Factors[a]		Results[b]
Treatment	pH	Temp. (°C)	sugar% (w/v)
1	5.5 (1)	40 (1)	19.48±0.33
2	6.5 (2)	40 (1)	20.85±0.011
3	7.5 (3)	40 (1)	20.31±0.05
4	5.5 (1)	51(2)	20.84±0.006
5	6.5 (2)	51 (2)	19.90±0.05
6	7.5 (3)	51 (2)	18.67±0.11
7	5.5 (1)	62 (3)	19.91±0.006
8	6.5 (2)	62 (3)	21.08±0.02
9	7.5 (3)	62 (3)	19.91±0.05

[a]Figures in the parenthesis are coded levels

[b] Average of three replicates± standard deviation

temperatures for 1 hour. The incubation temperature was controlled using a water bath. At the end of incubation, the enzyme was inactivated by heating the mixture at 90°C for 3 min. The treated juices were centrifuged (model 3-18k, Sigma Co., Germany) at 16000 ×g for 15 min and the supernatant was separated. A blank sample, to which no enzyme was added, was also run in three replicates to compare the custom way of treatment of date fruits with enzymatic treatments. The costume extraction was done in juice with natural pH by preparing 1:3 ratio of pulp: water. The mixture was heat treated at 90°C for 3 min and centrifuged at 16000 ×g for 15 min and then the supernatant was separated.

Experimental Design: The experiments consisted of three types of enzymatic treatment of date pulp, using: 1) Pectinex® smash XXL, 2) Cellubrix® L and 3) combination of both enzymes. Taguchi method was used to determine the optimum conditions for the enzymatic juice extraction of tamer date fruits of Kabkab variety, as statistical experimental design method. The experimental design and statistical analysis were performed using Qualitek®-4 Software (Nutek Inc., Bloomfield Hills, MI., USA). Three selected independent factors were: pH, temperature and enzyme concentration. Tables 1, 2 and 3 show the selected factors and their levels.

Analysis: The samples were analyzed for pH, seed to pulp ratio, total fibers, total sugars, moisture and pectin content, according to standard AOAC methods [17], before any enzyme treatment. The total sugars and clarity of date extracts, after enzyme treatment, were analyzed and used as responses of the experiment. Sugar content was measured by Lane- Eynon method [17]. Clarity was determined by measuring the absorbance of supernatants at 660 nm using uv-visible spectrophotometer (model T80, PG Instruments Limited, England), where distilled water was used as the reference.

RESULTS AND DISCUSSION

Analysis of Date Fruit: The date fruits from Kabkab variety were analyzed for pH, seed to pulp ratio, total fibers (%), total sugars (%), moisture (%) and pectin contents as Ca-pectate (%). The results were 5.95, 7.6/92.4, 8.39% (w/w), 72.70% (w/w), 16.63% (w/w) and 0.48% (w/w), respectively.

As the first step, the optimum conditions for application of Pectinex® smash XXL on 1:3 ratio of pulp: water mixture of Kabkab variety were investigated. According to the L-9 orthogonal array, which was chosen for studying the effects of 3 factors at 3 levels, the complete design consisted of 9 treatments (each were done in three replicates). The treatments condition, together with the responses, is shown in Table 1. Two responses, which were measured, were total sugars and clarity of the extracts.

The amount of sugar content of blank sample, to which no enzyme was added, was 14.42% (w/v).

In order to analyze the results and determine the optimum condition for sugar content and clarity of date juice extracts, Qualitek-4® Software was used in standard method.

In Fig. 1, the main effects of the factors are shown. By performing the statistical analysis, the optimum levels of factors was determined to be: level 1 for temperature (40°C), level 3 for pH (5.5) and level 3 for enzyme concentration (0.02% v/v). At this condition, the amount of sugar in juice should be 17.7% (w/v), which had a small difference (4.3%) with the 18.49% (w/v) sugar that was obtained practically in the treatment No. 7. Since this difference was insignificant, the verification test was not necessary to be performed.

Fig. 2. shows the main effect of factors regarding the clarity of the juice. Statistical analysis showed that the optimum condition for decreasing the turbidity of the juice is obtainable at level 1 of all of the factors, i.e. at 40 °C, pH 4.5 and 0.015% (v/v) of the enzyme. In such a condition, the absorbance of the juice may be as low as 0.036, which was not significantly different from the value of 0.04, which was practically obtained during the experiment (treatment No. 1) and again, the verification test was not necessary to be done. The absorbance value of blank sample was 0.131.

The same design as described for Pectinex® smash XXL, was chosen for evaluation of Cellubrix® L effect on Kabkab date variety by Taguchi method. Effect of each independent variable (temperature, pH and enzyme concentration) was sought for, at three levels. According to Taguchi L-9 orthogonal array, treatments (each in 3 replicates) were performed and total sugars and clarity were measured as the responses (Table 2).

In Fig. 3, the effect of various levels of independent variables on Cellubrix® L activity on sugar content of Kabkab juice are shown. As it is obvious, temperature at level 2 (62°C), the pH at level 3 (7.5) and enzyme concentration at level 3 (0.02%) were the best condition for application of Cellubrix® L in this process. At this optimum condition, the percent of juice sugar, recommended by Taguchi method, was 17.76% (w/v).

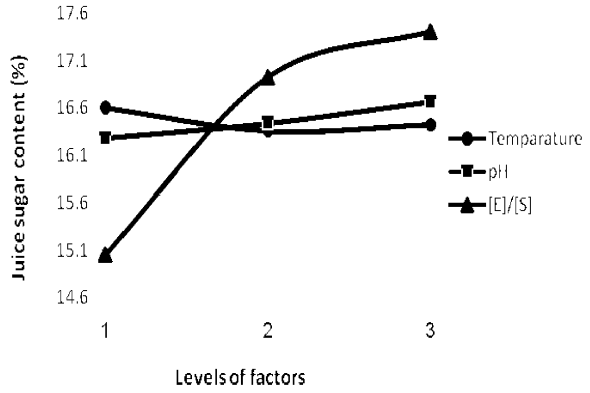

Fig. 1: The main effect of factors on sugar content of juice after treatment by Pectinex® smash XXL.

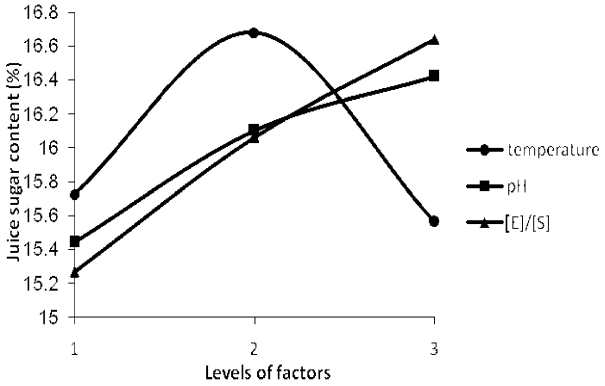

Fig. 3: The main effect of factors on sugar content of juice after treatment by Cellubrix® L.

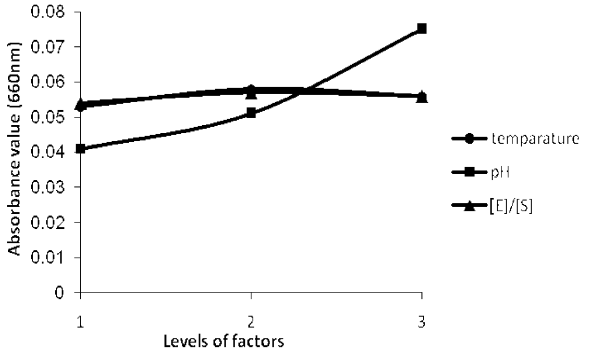

Fig. 2: The main effect of factors on juice clarity after treatment by Pectinex® smash XXL.

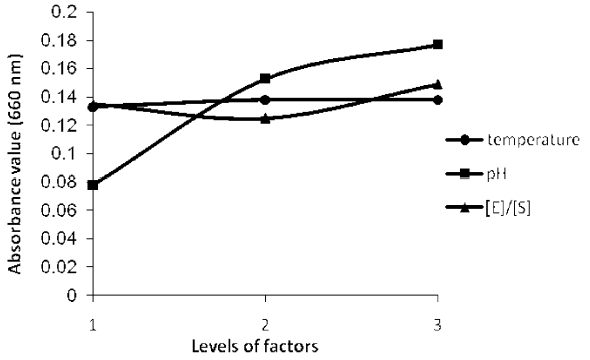

Fig. 4: The main effect of factors on juice clarity after treatment by Cellubrix® L.

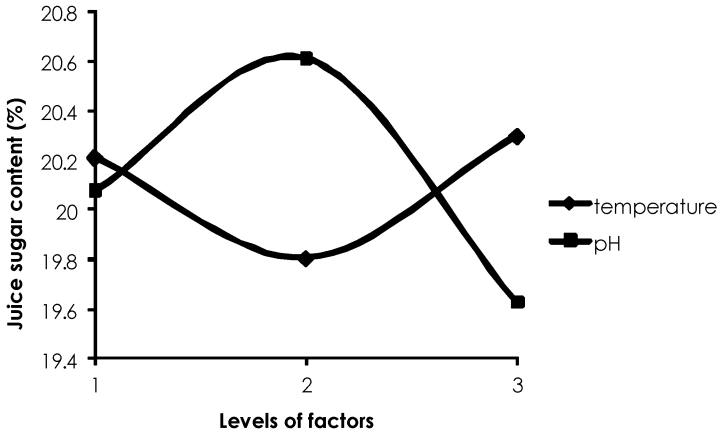

Fig. 5: The main effect of factors on simultaneous application of Pectinex® smash XXL and Cellubrix® L on increasing sugar content of date juice

According to verification test, the amount of sugar at this condition should be 17.01% (w/v), which, its difference with recommended one was not significant.

On investigation of the effect of cellulolytic enzyme on juice clarity, the best condition for this purpose was setting the variables at level 1 for temperature (58°C), level 1 for pH (5.5) and level 2 for enzyme concentration (0.015%). The absorbance value at this condition was 0.062.

Therefore, according to the results thus obtained, regarding the amount of extracted sugar, enzyme concentration has had the most significant influence.

Table 4: Comparisons of different treatments on juice sugar content of different date varieties

Reponses	Blank	Pectinex® smash XXL	Cellubrix® L	Both enzymes
Sugar content of juice at optimum condition (%)	14.42[c]	17.08[b]	17.01[b]	21.08[a]
Clarity of juice as absorbance value	0.131	0.036[j]	0.062[j]	-

[a, b, c] Figures with the same letter has no significant difference, á=0.05 (three replicates).

[j] Only the results are shown here.

When the clarity was chosen as the criterion, the pH has had the most significant influence, among other factors, when each of the enzymes was applied on the date fruit.

Simultaneous Effect of Pectinex® Smash XXL and Cellubrix® on Juice Extraction: After determining the effect of each singular enzyme on sugar content and clarity of juices of Kabkab variety, the simultaneous effect of these enzymes was investigated. For this reason, by considering the best condition for activity of each of the enzymes, a new set of treatments were designed. In Table 3 the design of 9 combinations of three independent variables including 3 replicates is shown. The concentration of both enzymes in these set of treatments was 0.02% (v/v).

The juice sugar content at the best condition was 21.08% (w/v). As it is seen in Fig. 5, temperature at level 3 (62°C) and pH at level 2 (6.5) were optimal for the maximum sugar yield. The absorbance value of juice at this treatment condition was 0.175 which was higher than the untreated one (0.131). The clarity of the juice was not included in this latter experiment.

CONCLUSION

According to Tukey's (HSD) mean analysis (alpha 0.05), treatment of Kabkab juice by both enzymes simultaneously had significant effect on increasing sugar content (21.08%) compared to blank sample (14.42%), where treatment by single enzymes, were not significantly different (Table 4).

Treatment of date juice by each of the Pectinex® smash XXL or Cellubrix® L, caused about 18% increase in the amount of extracted sugar, while simultaneous application of both enzymes, caused about 46%increase in this factor (Table 4). Al-Hooti *et al.* (2002) also showed that among the different extraction process in date syrup production, the use of pectinase and cellulase enzymes cause the optimum soluble solid extraction (68%) when compared to blank sample (35%) without enzymatic processing [13]. According to Zare *et al.* (2006), the process variables for best combination of response function are enzymes (pectinase and cellulase in 2:1 ratio) concentration 0.017%, temperature 62°C, pH 7.5 in 30 min. incubation time. At this condition the amount of total sugars, reducing sugars and total soluble solid increase 3.33%, 3.22% and 3.6%, respectively [18].

While Pectinex® smash XXL was significantly effective in increasing the clarity of the juice, which is an important factor in manufacturing the date juice, Cellubrix® L made the final juice more turbid. So, according to the purpose of production of the date juice, one may choose to utilize each or both of the enzymes.

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REFERENCES

1. Benjamin, N.D. and M.F. Abbas, 1985. Clarification of date juice by chemical and enzymatic processes. J. Agriculture and Water Resource, 4: 155-168.
2. Cliff, M., M.C. Dever and R. Gayton, 1991. Juice extraction process and apple cultiver influences on juice properties. J. Food Sci., 56: 1614-1617.
3. Landbo, A.K., K. Kaack and A.S. Meyer, 2007. Statically designed two step response surface optimization of enzymatic prepress treatment to increase juice yeild and lower turbidity of elderberry juice. Innovative Food Science and Emerging Technologies, 8: 135-142.
4. Liew Abdullah, A.G., N.M. Sulaiman, M.K. Aroua and M.J. Megat Mohd Noor, 2007. Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. J. Food Engineering, 81: 65-71.

5. Sreekantiah, K.R., S.A. Jaleel and T.N. Ramachandra Rao, 1971. Utilization of fungal enzymes in the liquefaction of soft fruits and extraction and clarification of fruit juices. J. Food Science and Technol., 8: 201-203.
6. Xu, F., Z. Wang, S. Xu and D.W. Sun, 2001. Cryostability of frozen concentrated orange juices produced by enzymatic process. J. Food Engineering, 50: 217-222.
7. Shalmon, P., A.R. Gur-Arie and A.H. Levi, 1999. Extraction of plume juice after liquefaction with commercial enzymes. Italian J. food Sci., 11: 29-38.
8. Lee, W.C., S. Yusof, N.S.A. Hamid and B.S. Baharin, 2006. Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). J. Food Engineering, 73: 55-63.
9. Mutlu, M., K. Sarioglu, N. Demir, M.T. Ercan and J. Acar, 1999. The use of commercial pectinase in fruit juice industry. Part é: viscosimetric determination of enzyme activity. J. Food Engineering, 41: 147-150.
10. Rai, P., G.C. Majumdar, S.D. Gupta and S. De, 2007. Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. J. Food Engineering, 78: 561-568.
11. Sreenath, H.K., K.R. Sudarshanakrishna and K. Santhanam, 1994. Improvement of juice recovery from pineapple pulp/residue using cellulases and pectinases. J. Fermentation and Bioengineering, 78: 486-488.
12. Joshi, V.K., S.K. Chauhan and B.B. Lal, 1991. Extraction of juices from peaches, plums and apricots by pectinolytic treatment. J. Food Science and Technol., 28: 64-65.
13. Al-Hooti, S.N., J.S. Sidhu, J.M. Al-Saqer and A. Al-Othman, 2002. Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. Food Chemistry, 79: 215-220.
14. Sablani, S.S., A.K. Shrestha and B.R. Bhandari, 2008. A new method of producing date powder granules: Physicochemical characteristics of powder. J. Food Engineering, 87: 416-421.
15. Ahmed, J. and H.S. Ramaswamy, 2006. Physico-chemical properties of commercial date pastes (*Phoenix dactylifera*). J. Food Engineering, 76: 348-352.
16. Sin, H.N., S. Yusof, N. Sheikh Abdul Hamid and R. Abd Rahman, 2006. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J. Food Engineering, 73: 313-319.
17. AOAC, 2002. Official methods of analysis (18th ed), method numbers: 945.27, 985.29, 923.09, 925.04 and 952.04. Association of Official Analytical Chemists, Washington, DC.
18. Zare, F., M. Azin, H. Nikopoor and M.T. Mazloomy, 2006. Investigation the effect of pectinase and cellulase on date syrup extraction. Food Science and Nutrition J., 1: 15-21.