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Evolution of bentonite and gelatin effects on clarification of variety of date fruit Kaluteh juice with response surface methodology

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Abstract

Date is one of important products that play important role in economy and nutritional health in some tropical regions of the world. Liquid sugar of date is produced by date syrup after extraction of phases, purification, and elimination of pectin compounds, protein, fiber, and color. This liquid has some characterizes such as 73% sugar with Brix 68-74% and it consists of mainly by glucose and fructose with similar ratio to honey hive and corn syrup with high fructose (HFCS) and It's color varies from brown to bright yellow. In this study, bleaching and clarification of *keloteh* variety date syrup to produce liquid sugar were investigated by response surface methodology (RSM) and face central composite design (FCCD). Three main factors including temperature (30-70°C), pH (4-6), and time (40-120 minute) to determine the optimal condition of maximum activity of these two components (bentonite and gelatin) by constant adding were investigated. Also there are some factors such as color, ash, and absorption was determined. The optimal value of added bentonite and gelatin is determined 1-3 g/L and 0.02-0.08 g/L, respectively, which the desired amount is 3 and 0.05g/L, respectively. In low temperature and pH, the increased action of bentonite and gelatin is observed during passing the time and also high amount of impurities were removed. The lowest amount of these responses for color at 420 nm is (4302 ICUMSA), ash (0.421%) and absorption at 660 nm is 0.059. It is revealed that this technique can diminish the values of syrup color (68.25%), ash (8.7%), and absorption up to 89.46%, by comparing the values in the optimal point of clarified syrup by bentonite and gelatin with traditional method.

Introduction

Date is a nutrient fruit which contains digestible sugars like glucose, saccharine and fructose (70%), regime fibers, vitamins such as B_1 and B_2 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 (Ismail *et al.*, 2001; Zaid, 2002; Al-Farsi *et al.*, 2006; Kulkarni *et al.*, 2008). Date fruit has a delicious and sweet taste also it is very nutritious and rich of calorie (The amount of calorie which produced per gram date is approximately 2500-3000 calorie), vitamins, and minerals (Saleem *et al.*, 2005; AL-Farsi *et al.*, 2005). Date nectar or date honey is a date syrup extract. After removing the colloidal materials and bulk of dyestuff from the extract; the resulting extract is a sweet and golden juice with a flavor similar to tough caramel like honey (Ashraf Jahani, 2002; Elleuch *et al.*, 2008). Liquid sugar date can be produced from date juice after the extraction, purification and also remove the pectin compounds, protein, fiber and dyes (Al- Farsi, 2003).

As it mentioned in some references date liquid sugar is produced from grade 2 or 3 fruits, it has high value added which competes with similar by-products including honey apiary (Anonymous, 1972; Abo-baker *et al.*, 1988). Date juice is used directly or as a combination in formulation of some foods like some products in bakery, beverages, confectionaries or sesame paste and date juice (Barreveld , 1993; Roukas *et al.*, 1997; Razavi *et al.*, 2007; El-Sharnouby *et al.*, 2009). In processing juices, wine and date juice the most important stage is exclusion of opacity factors, dyes like pectin, protein, resins, polysaccharides, tannins and other polyphenols (Chatterjee *et al.*, 2004; Berardini *et al.*, 2005; El-Sharnouby *et al.*, 2009; Onsekizoglu *et al.*, 2010).

Generally, transparentizing methods can be done through centrifuge, enzyme methods or by using translucid substances like gelatin, bentonite, aluminum hydroxide, silica cell and poly vinyl

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pirolidon (Youssif *et al*., 1990; Chatterjee *et al*., 2004; Berardini *et al*., 2005; Moure *et al*., 2006; El-Sharnouby *et al*., 2009; Onsekizoglu *et al*., 2010; Abbès *et al*., 2011). Oszemianski and Vojudli (2007) studied variations of antioxidant activity, phenolic compounds, and the color of *Abdard* and *Shampion* varieties apple juice during classical clarification procedure and with using transparent materials such as bentonite, gelatin, silica sol, and chitosan.

The absorbing feature of bentonite is an important factor in purification of food oils including soy oil, date oil, canola and beverages like beer, wine and mineral water as well as products like sugar and honey (Didi *et al*., 2009). By study on transparentizing red wine by gelatin, it is clear now that minimum activity of gelatin is in relation with pure density. Statistical analysis highly confirms changes in opacity, total polyphenol, color intensity and brown polymers which relates to transparentization by gelatin (Versari *et al*., 1998).

In determination of opacity of extracted juice of banana it showed a linear relation with proteins and polyphenols (Wang *et al*., 2004). The application of gelatin and bentonite for absorption of phenol materials from apple skin and core as well as mango juice and skin and also using these two combinations before ultrafiltration in clarification of apple juice has been described in many investigations (Haddad *et al*., 2003; Berardini *et al*., 2005; Onsekizoglu *et al*., 2010). As the brown color of pear juice lightens by combination of bentonite and gelatin, we did use 3 to 1 proportion which then gained juice is refined and freeze. This is best condition for maintaining residues and impurities. Using -4°C and then temperature 75-78°C improves dye and effective clarification (Haddad *et al*., 2003). The aim of this study is to determine the optimal condition for clarification and bleaching of date syrup using different concentrations of bentonite, gelatin and various levels of pH, temperature and time by using response surface methodology (RSM) and face central composite design (FCCD) in order to obtain a suitable process method to generate liquid sugar from the date.

Materials and Methods

Primary substances

This study was performed on *Keloteh* variety of date. The dates were bought wholesale from the market in Mashhad, Iran, and were maintained in the storage condition at Temperature above 0ºC. The used gelatin (Erbigel) was gelatin type A with the Bloom number of 100 and utilized in the form of 5% solution in the water. On the other hand, bentonite was sodium-calcium bentonite (Na-Calite, Germany).

The extraction of date syrup

The trials were performed into two phases. In the first stage, a significant amount of dates were out of the mentioned storage condition, and then placed to reach to the laboratory temperature (room temperature about 25ºC). The reason for this was as the date tissue become thick during primary storage and the homogenization process was not done well during blending. To increase the contact surface between water and date and exacerbate the diffusion rate, the dates were divided into small pieces and mixed with 500 g of distilled water with different ratios (first, the distilled water is reached to the experiment temperature into the water bath, type w 350 B). The mixture of water and date was homogenized by manual blender with the low speed for 2 minutes to more extraction of the date sugar syrup. Then, the mixed significant pH was regulated by citric acid 5 N and sodium hydroxide 1N. The beaker containing a significant sample was transferred into the water bath with different temperature (30-70°C) after different times (40-120 minute). In a significant temperature, the sample was cold up to the ambient temperature by cold water. The soluble was cooled down by the smooth fabric filters; then the cold solution and mentioned parameters were measured (Al-Hooti *et al*., 2002; Abbès *et al*., 2011). The sample was analyzed based on humidity, ash, total sugar, and protein (AOAC 2000).

Clarification and bleaching of date syrup

100 mg of date syrup was poured in a 250 mL Erlenmeyer flask. Also, gelatin and bentonite were utilized for clarification. Before using, bentonite must be soaked in water for some times. Furthermore, the 20% solution of bentonite was prepared 8-12 hours before experiment (Xifang *et al*., 2007). Then, 3 g/L of bentonite was added to the Erlenmeyer flask in the form of 20% solution. The mixed pH must be decreased slightly due to acidic properties of bentonite. The obtained mixture was regulated by citric acid and sodium hydroxide in a desired pH. The date syrup and bentonite were mixed by the magnetic blender in a low speed in the environment temperature (25±1°C) for 10 minutes as long as the reactions were completed. Then, 0.05 g/L of gelatin solution was added. After 10 minutes blending; the mixture of syrup, bentonite, and gelatin was transferred into the water bath with different times and temperatures. The mixture was cold up to the room temperature by water. The final mixture was gone through the non-deposit phase of Watman filter paper (No 42) which

used to measure significant parameters.

The measurement of required parameters of date syrup

The properties of the date juice were determined according to method of International Commission for Uniform Methods of Sugar Analysis (ICUMSA) (2000). The absorption which introduces the rate of date syrup clarity was measured in the wavelength of 660 nm and the colorimetry was performed too by spectrophotometer (Model Cambridge England) in 420 nm. After reading, the amount of color was calculated by using the following equation (Eq.1) the ash was measured by conductometry (Model 380BA, UK).

Equation (1): $$F_{IE} = 10^5 \frac{A}{L \times W_{TS} \times \rho}$$

Where F_{IE} is color according unit ICUMSA (cm^2/kg), A is absorbance, W_{ts} is solid (or dry substance) content, L is the path length of light or cell thickness in cm, ρ is density (g/cm^3) of juice.

Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (pH, x_1; temperature, x_2 and time, x_3) on the color (ICU_{420}), turbidity (660 nm) and ash content (%). Face Central Composite Design was employed for designing the experimental data. Experimental data was modeled by using the Design Expert software version 6.01 (Statease Inc., Minneapolis, USA) and three-dimensional representations of the response surface generated by the model. Numerical optimization techniques of mentioned software were used to determine the optimal point. The response functions (Y) were color, turbidity and ash content. These values were related to the coded variables (xi, i = 1, 2, and 3) by a second-order polynomial using Equation below.

Equation (2):

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \varepsilon$$

The coefficients of polynomial model were represented by b_0 (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects).

The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked accounting to R^2 and adjusted R^2 (Kumari *et al.*, 2008; Keshani *et al*., 2010).

Result and Discussion

Model fitting

The coefficients of determination (R^2), adjusted R^2 and lack of fit are used to verify the model. The model is statistically appropriate when lack of fit is not significant and has the highest value of R^2 and adjusted R^2 (Koocheki *et al*., 2009). Based on the results obtained from the statistical analysis (Table 1), the second-order polynomial response surface model (Eq. 2) was fitted to each of the response variables (Y).

The chemical composition of date

This date was analyzed for some properties like humidity, ash, protein, and total sugar. The results have been listed in Table 2.

The study of impact of independent variables on responses

Color

The influence of independent variables on the color of date syrup has been shown in the shapes of response surface (Figure 1). The effect of time and temperature (temperature is constant and equal to 50°C) and pH (pH = 5) on the color's changes of date syrup have been observed in this figure. With regard to the independent variables coefficient in the equation (2), temperature has a main effect on the color's changes of date syrup. The significance of linear effects ($P < 0.001$) and the square ($P < 0.05$) of these changes are confirmed this impact. No significant changes have occurred in the color of syrup with the temperature's change from 30°C to 50°C; but with increasing temperature up to 70°C, the color of syrup has sharply increased. Koyuncu *et al*. (2007) reported that with increasing temperature, the absorption of compounds that cause turbidity and color in the apple juice is raised by bentonite.

Since that time was not significant effect on the color of syrup ($P > 0.05$), so it can be concluded that the color of syrup does not have high relation with time passing. With the levels' change of this variable, the rate of color variations is very low. It is observed that the relation between the color and pH is linear based on the shape of surface. This process is confirmed by the significance of linear effect of model ($P < 0.001$). The results showed that with the decreased pH from 6 to 4, the absorbance's rate of factors causing color in the date syrup was increased by bentonite and gelatin. As a result, the color of syrup was almost decreased linearly. In the investigations

Table 1. Statistical analysis results of fited model on the response data

Source	Standard deviation	R^2	Adjusted-R^2	P-Value for lack of fit
Color				
Linear	585.6	0.85	0.82	0.04
Quadratic	463.9	0.94	0.89	0.1^{ns}
Turbidity				
Linear	0.026	0.75	0.70	0.058^{ns}
Quadratic	0.022	0.89	0.81	0.37^{ns}
Ash				
Linear	0.013	0.50	0.41	0.0059
Quadratic	0.0066	0.92	0.86	0.11^{ns}

Table 2. The composition of *keloteh* date

Humidity (%)	Ash (%)	Total sugar (%)	Protein (N* 6.25%)
23.76	1.84	74.76	2.311

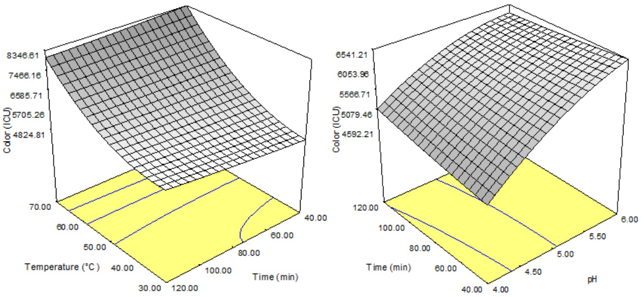

Figure 1. The graph of response surface of color (a) the influence of time and pH (T = 50°C) (b) the impact of temperature and time (pH = 5) Ash

which performed by Shen *et al.* (2009) and Li *et al.* (2010) about the absorption of anionic dyes by bentonite, they concluded that the elimination of these colors is increased by two factors included present of bentonite and decreasing pH. Based on the results and shapes of response surface, it was observed that the best result gained for decreased color by gelatin and bentonite in the low pH and temperature. With regard to the regression coefficients, the response function (equation of model) was obtained as an equation (3) to estimate the color of syrup.

Equation (3):

$$Color = -3504.08 + 3147.06x_1 - 155.9x_2 + 35.98x_3 - 266.18x_1^2 + 1.88x_2^2 + 0.018x_3^2 + 13.42x_1x_2 - 4.55x_1x_3 - 0.28x_2x_3$$

Ash

Ash is one of important factor in this study. The influence of independent variables on the ash of extracted syrup from the keloteh variety date has been shown in Figure (2) as three-dimensional shapes of response surface. The results of variance analysis of ash data showed that pH has the direct influence on the amount of ash of syrup. Furthermore, with decreasing pH, the ash content of syrup has a decreased. The only significant impact for temperature was its quadratic effects ($P < 0.001$). The results showed that the activity of bentonite and gelatin are decreased with increasing temperature from 30°C to 70°C due to better function of bentonite and gelatin

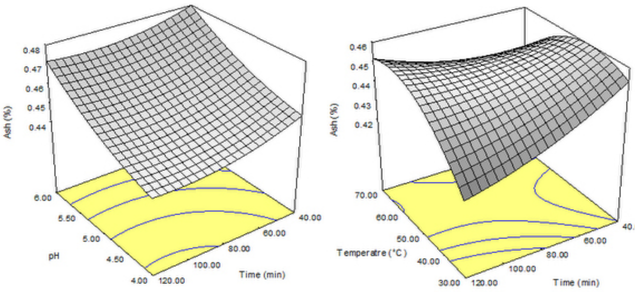

Figure 2. The graph of response surface of ash (a) the influence of temperature and time (pH = 5) (b) the effect of pH and time (T = 50°C)

in low temperatures. As it can be observed, the amount of ash has decreased at higher temperature. In comparison with previous investigation Garg *et al.* (2004) declared that temperature has positive impact on the absorption of elements by bentonite. Therefore, the absorption rate of cobalt from the aqueous solution and the absorption of compounds causing turbidity of apple syrup were increased with high temperature.

The results of variance analysis of ash data suggested that its linear effect has been only signified in model so that the absorbance of elements is increased by bentonite with increasing reaction time from 40 to 120 minutes. Furthermore, the amount of ash of syrup is declined. In equation (4), the regression coefficients show that increased time has positive effect on reducing the ash of syrup. These results are explanatory of Mekhemer et al. investigations (Mekhemer *et al.*, 2008). Based on the obtained results, it was found that the lowest amount of ash is accessible in low pH and temperature and long time. With regard to the calculated regression coefficients, the response function (equation of model) was obtained as an equation (4) to estimate the ash of syrup. Also with optimization of used condition more improvements can be obtained. It can be recommended to use long time and low pH and temperature for decreasing amount of ash in final product.

Equation (4):

$$Ash = 0.524 - 0.037x_1 + 1.89x_2 - 1.64x_3 + 4.13x_1^2 - 3.96x_2^2 + 2.89x_3^2 + 1.75x_1x_2 + 3.12x_1x_3 + 1.78x_2x_3$$

Absorbance

The influence of independent variables on the absorbance of extracted syrup from the *keloteh* variety date has been shown in Figure (3) as three-dimensional shapes of response surface. The results of variance analysis showed that temperature was simply effective on the absorbance of date syrup

Table 3. Predicted optimum condition for bleaching and clarification process of date juice

Factor	Low	High	Optimum
Temperature (°C)	30	70	38
Time (min)	40	120	82
pH	4	6	4.28

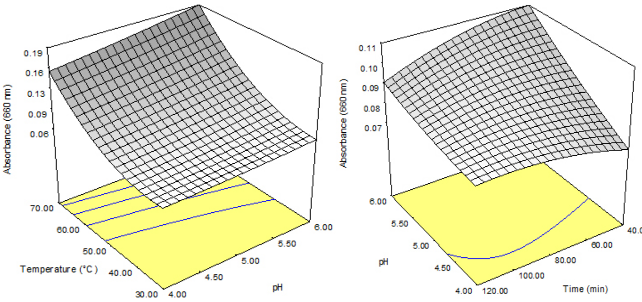

Figure 3. The graph of response surface of absorption (a) the influence of pH and time (T = 50°C) (b) the effect of temperature and pH (time = 80 minute)

among independent variables. Thus, just linear effect ($P < 0.001$) and square ($P < 0.05$) of temperature were signified in model. The influence trend of independent variables on the absorbance of syrup is similar to their impact on the color of syrup. The reason may be due to present of the colloidal and suspended materials which result in development of visual color in syrup. Thus, they are also associated with the color of syrup. In addition, these materials lead to turbidity and increased absorption. Therefore, any changes that may cause elimination or reduction of the colloidal materials in the syrup, lead to decrease the color in addition to increase the clarification of syrup. In a study performed by Ray *et al.* (2007) about the clarification of *Musamby* orange juice with classical procedures, was distinguished that filter aid materials like gelatin had positive charge and could be effective in the reduction of pectin and descent of turbidity by physical reactions and surface absorption of negatively charged pectin substances.

With increasing temperature from 30°C to about 50°C, the absorption rate is not much changed. But the absorption rate of syrup and consequently the turbidity of it are sharply increased with continuing increased temperature up to 70°C. Also similar result was observed for color changes with temperature so that the color of syrup was raised with increasing temperature (Figure 1). With increasing time from 40 to 120 minutes and decreasing pH from 6 to 4, the absorption rate of syrup and consequently the turbidity of it are declined. Also, it was observed similar trend for color. The reason for better function of bentonite in low pH is that bentonite has more negative load in acidic condition in the separation of compounds which cause color and turbidity. In addition, the power of clarification is raised parallel to increased load concentration (Anonymous, 1982). Therefore, the lowest amount of absorption and consequently the most clarification of syrup are obtained in low pH and temperature and also in a longer time in this study based on the results. With regard to the calculated regression coefficients, the response function (equation of model) was obtained as an equation (5) to estimate the absorption rate of syrup.

Equation (5):

$$Abs = 0.072 - 7.35x_1 - 5.4x_2 + 1.8x_3 + 1.54x_1^2 + 7.51x_2^2 - 2.16x_3^2 + 3.5x_1x_2 - 1.68x_1x_3 - 1.34x_2x_3$$

The optimization of date syrup clarification

The optimal operating condition was sought for bleaching and clarification of date syrup using constant values of bentonite (3 g/L) and gelatin (0.05 g/L) in different pH, time and temperature on the parameters like color, ash, and the absorption of syrup by numerical optimization technique for software (Design Expert). For this purpose, the aims of optimization are detected and then response surfaces and independent variables would be regulated. Each of three responses considered in minimum values. In the optimal condition, the lowest value for color, ash, and absorption was 4302 ICU_{420}, 0.421%, and 0.059, respectively. In the optimal condition of bleaching and clarification of syrup, the values of independent variables for pH, temperature, and time were 4.28, 38°C, and 82 minutes, respectively (Table 3). The value of parameters such as color, ash, and absorption was 13552 ICUMSA, 0.46%, and 0.560 for the raw syrup, respectively. With comparison of resulting values in the optimal point of syrup that clarified by bentonite and gelatin than the initial juice, it was revealed that this bleaching and clarification technique could reduce the value of color, ash, and absorption up to 68.25%, 8.7%, and 89.46%, respectively.

Conclusion

Though a wide area is cultivated for date palms in Iran, only 11 – 12 percent of this amount is used in food processing and packaging industries and due to the high level of waste and low-quality dates, much of the produced dates is consume as animal feed. Therefore, improvement of the process for the production of clarified date juice and liquid sugar date is one of the new methods in conversion industries to convert waste dates to products with higher value in order to provide domestic needs and export some of these products to reduce imports of similar products and also obtaining of exchange. In this study, the keloteh variety date was selected. The effect of different factors on the bleaching and clarification was studied and optimized.

With the study of impact of factors such as pH, temperature, and time in the bleaching and clarification which performed by bentonite and gelatin, it was also revealed that temperature and pH are the main factors that control the activity of bentonite and gelatin. But, time has the lower impact than these two variables. The results showed that bentonite and gelatin known as transparent materials and purifiers have more activity and more decrease the impurities of syrup in low temperature and pH over the time. Thus, the lowest amount of these responses in the optimal point for the color in 420 nm, ash and absorption in 660 nm was 4302 ICUMSA, 0.421%, and 0.059, respectively. A significant reduction was observed in the rate of color and turbidity in comparison with the primary sample, While, the ash of syrup did not have much change. This shows that the ash of syrup is not increased by bentonite which is used for clarification in addition toelimination of impurities and clearness of syrup.

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