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Research Article

OPTIMIZATION OF OSMOTIC DEHYDRATION PROCESS OF BEETROOT (*BETA VULGARIS*) IN SUGAR SOLUTION USING RSM

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ABSTRACT
Beetroot being an alkaline food ranked among the ten most potent antioxidant vegetables. Beetroots (Beta vulgaris) are rich in valuable, active compounds such as carotenoids, glycine betaine, saponin, betacyanines, folate, betanin, polyphenols and flavonoids. Therefore, beetroot ingestion can be considered a factor in cancer prevention. For the optimization of osmotic dehydration by response surface methodology, the experiments were conducted according to Central Composite Rotatable Design (CCRD) with three variables at three levels. The low and high levels of the variables were 30 and 60°C for osmotic solution temperature, 55 and 75°Brix for sucrose solution concentration, 120 and 240 min for duration of dipping in osmotic solution, respectively. The fruit to solution ratio was kept 1:4 (w / w) during all the experiments. The optimum conditions for osmotic solution concentration, temperature and process duration were 75°Bx, 60°C and 240 min, respectively.

Key Words: *Beetroot, Optimization, Sucrose, RSM*

INTRODUCTION
Beetroot (*Beta vulgaris*) and carrot (*Daucus carota L.*) are considered as very important vegetables from nutritional view. Beetroots (Beta vulgaris) are rich in valuable, active compounds such as carotenoids (Dias *et al.*, 2009), glycine betaine, (de Zwart *et al.*, 2003), saponins (Atamanova *et al.*, 2005), betacyanines (Patkai *et al.*, 1997), betanin, polyphenols and flavonoids (Vali *et al.*, 2007). Therefore, beetroot ingestion can be considered a factor in cancer prevention (Kapadia *et al.*, 1996).
The various methods available for preservation of fruits and vegetables are canning, freeze drying, and vacuum drying, hot air drying and osmotic dehydration. Among different methods of food dehydration a well-known process to achieve good-quality product is freeze drying, but it is an expensive method of food preservation relative to the value of that product. Therefore, there is a need for a simple and inexpensive alternate process, which has low capital investment and offers a way to save highly perishable products and make them available for the regions away from production zones. Osmotic dehydration is one of these methods (Shi and Le-Maguer, 2002). Osmotic dehydration of foods such as fruits, vegetables and meat has been extensively studied as it has proved to be an effective process for removing water from tissues, even at low temperatures. Several factors deeply affect the performance of osmotic operations: raw product characteristics (varieties, maturity level, physical and chemical characteristics of the initialproduct); osmotic medium concentration; process temperature; sample geometry; solution agitation; solution/product mass ratio and process duration (Lazarides, 1992; Lenart, 1992).
Osmotic dehydration is a water removal technique, which is applied horticultural products, such as fruits and vegetables, to reduce the water content, while increasing soluble solid content by immersing in aqueous solutions of high osmotic pressure such as sugar and salts (Wanasundara *et al.*, 1996). Osmotic pre-treatment preserves the flavour by dried products, making them acceptable as ready to eat products (Lenart and Grdecka, 1989).

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The aim of this work was to optimize osmotic dehydration process of beetroot as a function of sucrose concentration, osmotic temperature, and time, using response surface methodology with the purpose to achieve maximum possible water loss, solute gain, and sensory score.

MATERIALS AND METHODS

Experimental Design

For the optimization of osmotic dehydration by response surface methodology, the experiments were conducted according to Central Composite Rotatable Design (CCRD) with three variables at five levels. The independent variables were process temperature, solute concentration, and duration of osmotic dehydration process. The low and high levels of the variables were 30 and 60°C for osmotic solution temperature, 55 and 75°Brix for sucrose solution concentration, 120 and 240 min for duration of dipping in osmotic solution, respectively (Ade-Omowaye *et al.*, 2002). The fruit to solution ratio was kept 1:4 (w/w) during all the experiments to minimise problems related to the management of the osmotic solutions like reconcentration, microbial contamination, reutilisation, and discharge of the spent solution (Torreggiani and Bertolo, 2002).

The relationship between levels of different coded and uncoded form of independent variables is given in Table 1. The experiments plan in coded and uncoded form of process variables along with results is as given in Table 2. The experiments were conducted randomly to minimise the effects of unexplained variability in the observed responses because of external factors.

Preparation of Samples

Fresh, well graded beetroot were collected from local market of sirsa, Haryana on daily basis prior to each set of experiment. They thoroughly washed with water to remove adhering soil and other debris. Then, they were cut into cubes (2cm ×2cm×2cm) using clean knife. No blanching was done prior to osmosis as it is detrimental to the osmotic dehydration process due to loss of semi-permeability of cell membranes (Ponting, 1973). Sugar, the osmotic agent, was purchased from a local market. The osmotic solution is prepared by mixing the sugar with proper amount of pure water.

Osmotic Dehydration

The osmotic dehydration was conducted in stainless steel containers, which is placed in a thermostatically controlled water bath shaker. Beetroot cubes were weighed and then placed into stainless steel containers containing calculated volume of osmotic solution of different concentrations at pre set at desired temperature in hot water bath. The temperature of the osmotic solution was maintained by hot water bath agitating at the rate of 50 oscillations per min. In each of the experiments fresh osmotic syrup was used. All the experiments were done in triplicate and the average value was taken for calculations. Agitation was given during osmosis for reducing the mass transfer resistance at the surface of the fruit and for good mixing and close temperature control in osmotic medium (Chopra, 2001). The beetroot cubes were removed from the container at the specified time and rinsed with fresh water to remove the excess solute adhered to the surface. The osmotically dehydrated beetroot cubes were then spread on an absorbent paper to remove the free water present on the outer surface. Then out of the total osmotically dehydrated beetroot, about 15–20 g sample was put in the preweighed Petri dish for determination of dry matter by oven method .The remaining part of the sample was dried to final moisture content of 10% (wb) in hot air dryer at 60°C air temperature. The dried samples were packed in high density polyethylene (HDPE) (80 micron) bags and kept at ambient temperature for further quality analysis.

Statistical Analysis and Optimization

The second order polynomial equation was fitted to the experimental data of each dependent variable as given below

$$Y = \beta_{k0} + \sum_{i=1} i{=}1\beta_{ki} x_i + \sum \beta_{kii} x_i^2 + \sum\sum \beta k_{ij} x_i x_j$$

Where Y_k = response variable ;Y_1 = water loss (g) per 100 g fresh beetroot ; Y_2 = solute gain (g) per 100 g fresh beetroot; Y_3 = sensory sore; xi represent the coded independent variables (x_1= solution concentration, x_2 = process duration, x_3 = process temperature); where β_{ko} was the value of the fitted response at the centre point of the design , i.e. point (0,0,0), β_{ki} ,β_{kii}, and β_{kij} were the linear, quadratic, and cross product regression coefficients ,respectively.

The analysis of the experimental data was carried out to observe the significant effect of various process variables on the various responses. The β coefficient is that the magnitude of these values helps to compare the relative contribution of each independent variable in the prediction of the dependent variable. Higher the positive value of β of a parameter, higher would be the effect of that parameter and vice versa.

The response surface and contour plots were generated for different interaction of any two independent variables, while holding the value of third variable as constant (at the central value).Such three dimensional surfaces could give accurate geometrical representation and provide useful information about the behavior of the system within the experimental design. The optimization of the osmotic dehydration process was aimed at finding the levels of independent variables viz. osmotic solution concentration, temperature, and process duration, which would give maximum possible water loss, solute gain, and sensory score. When the dehydrated product has to be rehydrated before final use like dehydrated vegetables, the optimization of osmotic dehydration process is always aimed at minimum solute gain. But, in present case, the dehydrated product has to be utilized directly without rehydration. Therefore, the optimization was aimed at the maximum solute gain during osmotic dehydration process. It will also help to make the product shelf stable at ambient conditions Response surface methodology was applied to the experimental data using commercial statistical package, Design– Export version 8.01 (Trail version; Statease Inc., Minneapolis, MN,USA). The same software was used for the generation of response surface plots, superimposition of contour plots, and optimization of process variables.

Mathematical Calculations

Water loss and solute gain during osmotic dehydration:-

The water loss and solute gain during osmotic dehydration were calculated by the equations given by Ozen *et al.,* (2002); Singh *et al.,* (2007);

Water loss (g) per 100 g of fresh beetroot= $\frac{(Wo-Wt)+(St-So)}{wo} \times 100$

Solute gain (g) per 100 g of fresh beetroot = $\frac{St-So}{WO} \times 100$

Weight reduction=Water loss-solute gain

Where W_o is the initial weight of beetroot (g) , W_t is the weight of beetroot after osmotic dehydration for any time t (min), So is the initial weight of solids (dry matter) in the beetroot (g) and St is the weight of solids (dry matter) of beetroot after osmotic dehydration for time t (min).

Estimation of Dry Matter and Moisture Content

The samples were oven dried at 103 ± 2 º C with lids open until a constant weight loss (AOAC, 1965).

Sensory Evaluation of Osmotic Dehydrated Beetroot

Organoleptic quality of osmotic dehydrated beetroot was determined with the help of a 10-member consumer panel, using a 9-point hedonic scale, following standard procedure. The aspects considered for osmotic dehydrated beetroot were colour, appearance, taste, favour, and overall acceptability. The average scores of all the 10 panelists were computed for different characteristics

RESULTS AND DISCUSSION

Water Loss

The results of second-order response surface model in the form of analysis of variance (anova) are given in Tables 4. The results indicated that the fitted quadratic models accounted for more than 90% of the variation in the experimental data, which were highly significant ($R^2 > 0.90$).The magnitude of P values from Table 4 revealed that all linear and quadratic terms of process variables have significant effect at 5% level of significance ($P < 0.05$) on water loss during osmotic dehydration. Further, interaction of ‘temperature and time’ has significant effect on water loss. The model F-value is 103.39, which implies the model is significant

Table 1: Independent process variables and their levels for osmotic dehydration of beetroot

Independent variables	**Symbol**	**Levels**		
		-1	**0**	**+1**
Sugar concentration (°Brix)	X_1	55	65	75
Solution temperature (°C)	X_2	30	45	60
Immersion time (minutes)	X_3	120	180	240

Table 2: Experimental plan with coded and actual levels of the process variables for the beetroot using Central Composite Rotatable Design

Experiment /sample no	**Sugar Concentration (X1)**		**Solution Temperature (X2)**		**Immersion time (X3)**	
	Actual	**Coded**	**Actual**	**coded**	**Actual**	**Coded**
1	65	0	45	0	180	0
2	75	1	30	-1	240	1
3	65	0	45	0	180	0
4	55	-1	30	-1	240	1
5	55	-1	60	1	240	1
6	55	-1	60	1	120	-1
7	75	1	60	1	120	-1
8	75	1	45	0	180	0
9	65	0	45	0	180	0
10	65	0	45	0	240	1
11	65	0	45	0	180	0
12	75	1	60	1	240	1
13	55	-1	45	0	180	0
14	65	0	45	0	180	0
15	65	0	45	0	120	-1
16	65	0	30	-1	180	0
17	55	-1	30	-1	120	-1
18	75	1	30	-1	120	-1
19	65	0	45	0	180	0
20	65	0	60	1	180	0

Final Equation for water loss in Terms of Actual Factors is:- water los=+26.54464-0.59728×Sugar concentration+0.5258× solution temperature+0.011086 ×immersion time-2.01667E-003×Sugar concentration×solution temperature-5.83333E-005×Sugar concentration×immersion time-6.13889E-004×solution temperature×immersion time+6.28636E-003×Sugar concentration^2-2.56162E-003×solutiontemperature^2+1.76010E-004 ×immersion time^2. Figure 1a shows the increased water loss with increase in solution temperature and osmotic solution concentration. This might be because of the fact that the increase in temperature decreases the viscosity of the osmotic solution and thus reduces the external resistance to mass transfer at product surface to facilitate the outflow of water through cellular membrane (Panades *et al.*, 2006). The increase in water loss with osmotic solution concentration is mainly because of the increase in the osmotic pressure gradient (Azoubel and Murr, 2004). A similar variation in water loss with temperature and time has also been observed in Figure 1b which revealed that increase in water loss with time was more remarkable in high concentration than in low concentration.

Table 3: Central Composite Rotatable Design with experimental values of response variables

Sugar concentration (^{0}Brix)	Solution temperature (^{o}C)	Time (minutes)	Water loss (g per 100 g of fresh beetroot)	Solute gain (g per 100 g of fresh beetroot)	Weight reduction (g per 100 g of fresh beetroot)	Sensory score
65	45	180	28.47	6.08	22.39	7.3
75	30	240	33.18	6.49	26.69	9.7
65	45	180	28.76	6.07	22.70	7.5
55	30	240	30.57	7.02	23.55	7.9
55	60	240	31.41	7.15	24.26	8
55	60	120	27.58	5.68	21.90	7.1
75	60	120	29.12	5.21	23.91	6.8
75	45	180	30.68	5.85	24.83	8.2
65	45	180	29.15	5.96	23.19	8
65	45	240	32.08	7.04	25.04	8.4
65	45	180	28.65	6.08	22.57	7.5
75	60	240	33.56	7.12	26.44	9.5
55	45	180	28.61	6.32	22.29	7.4
65	45	180	28.76	6.08	22.68	7.2
65	45	120	27.22	5.28	21.94	6.2
65	30	180	27.84	5.77	22.07	6.8
55	30	120	23.78	5.09	18.69	5.8
75	30	120	27.28	4.58	22.70	5.7
65	45	180	29.17	5.93	23.24	7.5
65	60	180	29.04	6.64	22.40	7.5

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Table 4: ANOVA Table showing the variables as a linear, quadratic and interaction terms on water loss and coefficients for the prediction models

Source	Sum of squares	df	Mean square	F value	*P*-value prob›f
Model	94.11	9	10.46	103.39	< 0.0001
A-Sugar conc.	14.09	1	14.09	139.32	< 0.0001
B-solution temp.	6.50	1	6.50	64.23	< 0.0001
C-immersion time	66.67	1	66.67	659.19	< 0.0001
AB	0.73	1	0.73	7.24	0.0227
AC	9.800E-003	1	9.800E-003	0.097	0.7620*
BC	2.44	1	2.44	24.15	0.0006
A^2	1.09	1	1.09	10.75	0.0083
B^2	0.91	1	0.91	9.03	0.0132
C^2	1.10	1	1.10	10.92	0.0080
Residual	1.01	10	0.10		
Lack of Fit	0.62	5	0.12	1.59	0.3108*
Pure Error	0.39	5	0.078		
Cor Total	95.12	19			
R^2			0.9894		
Adj R^2			0.9798		

**Non-significant at 5% level*

Table 5: Showing the variables as a linear, quadratic and interaction terms on solute gain and coefficients for the prediction models

Source	Sum of squares	df	Mean square	F value	*P*-value prob›f
Model	9.04	9	1.00	180.30	< 0.0001
A-Sugar conc.	0.37	1	0.37	65.66	< 0.0001
B-solution temp.	0.69	1	0.69	123.35	< 0.0001
C-immersion time	7.87	1	7.87	1412.54	< 0.0001
AB	0.052	1	0.052	9.27	0.0124
AC	0.012	1	0.012	2.20	0.1691*
BC	0.039	1	0.039	7.01	0.0244
A^2	2.051E-004	1	2.051E-004	0.037	0.8517*
B^2	7.475E-003	1	7.475E-003	1.34	0.2737*
C^2	0.012	1	0.012	2.24	0.1655*
Residual	0.056	10	5.574E-003		
Lack of Fit	0.032	5	6.390E-003	1.34	0.3771*
Pure Error	0.024	5	4.758E-003		
Cor Total	9.10	19			
R^2			0.9464		
Adj R^2			0.9884		

**Non-significant at 5% level*

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Table 6: Showing the variables as a linear, quadratic and interaction terms on weight reduction and coefficients for the prediction models

Source	Sum of squares	df	Mean square	F value	***P*-value prob›f**
Model	57.14	9	6.35	62.92	< 0.0001
A-Sugar conc.	19.00	1	19.00	188.27	< 0.0001
B-solution temp.	2.96	1	2.96	29.31	0.0003
C-immersion time	28.72	1	28.72	284.63	< 0.0001
AB	1.17	1	1.17	11.62	0.0067
AC	0.044	1	0.044	0.44	0.5242*
BC	1.86	1	1.86	18.47	0.0016
A^2	1.12	1	1.12	11.07	0.0077
B^2	0.76	1	0.76	7.49	0.0210*
C^2	0.88	1	0.88	8.74	0.0144
Residual	1.01	10	0.10		
Lack of Fit	0.42	5	0.083	0.71	0.6444*
Pure Error	0.59	5	0.12		
Cor Total	58.15	19			
R^2			0.9806		
Adj R^2			0.9631		

**Non-significant at 5% level*

Table 7: Showing the variables as a linear, quadratic and interaction terms on sensory score and coefficients for the prediction models

Source	Sum of squares	df	Mean square	F value	***P*-value prob›f**
Model	19.45	9	2.16	47.74	< 0.0001
A-Sugar conc.	*1.37*	1	1.37	30.25	0.0003
B-solution temp.	*0.90*	1	0.90	19.88	0.0012
C-immersion time	*14.16*	1	14.16	312.87	< 0.0001
AB	*0.031*	1	0.031	0.69	0.4254*
AC	*1.71*	1	1.71	37.81	0.0001
BC	*0.78*	1	0.78	17.26	0.0020
A^2	*0.47*	1	0.47	10.40	0.0091
B^2	*0.15*	1	0.15	3.39	0.0952
C^2	*0.021*	1	0.021	0.45	0.5161*
Residual	0.45	10	0.045		
Lack of Fit	*0.073*	5	0.015	0.19	0.9533*
Pure Error	*0.38*	5	0.076		
Cor Total	19.90	19			
R^2			0.9773		
Adj R^2			0.9568		

**Non-significant at 5% level*

(a) (b)

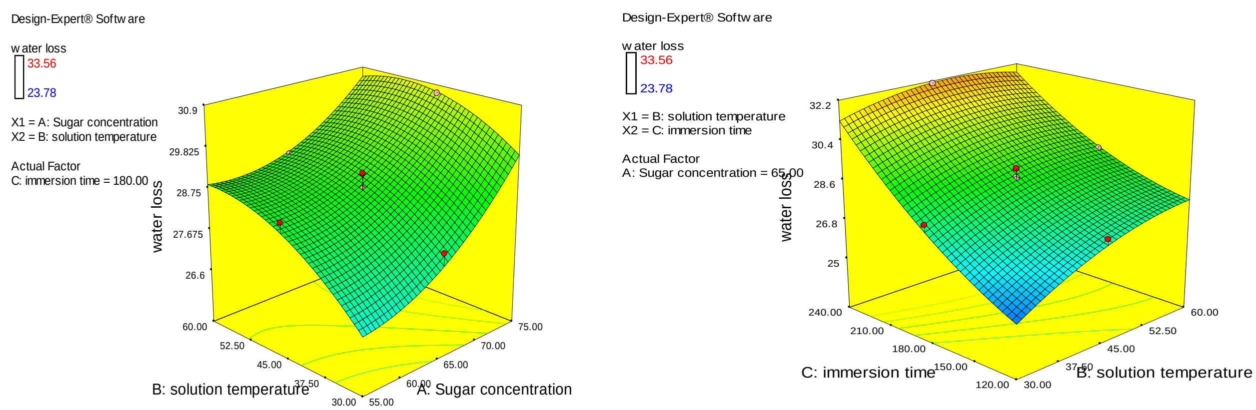

Figure 1: Influence of process variables on water loss (a) sugar concentration and temperature for 180min of process duration (b) temperature and time at 65 ^{o}BX

(a) (b)

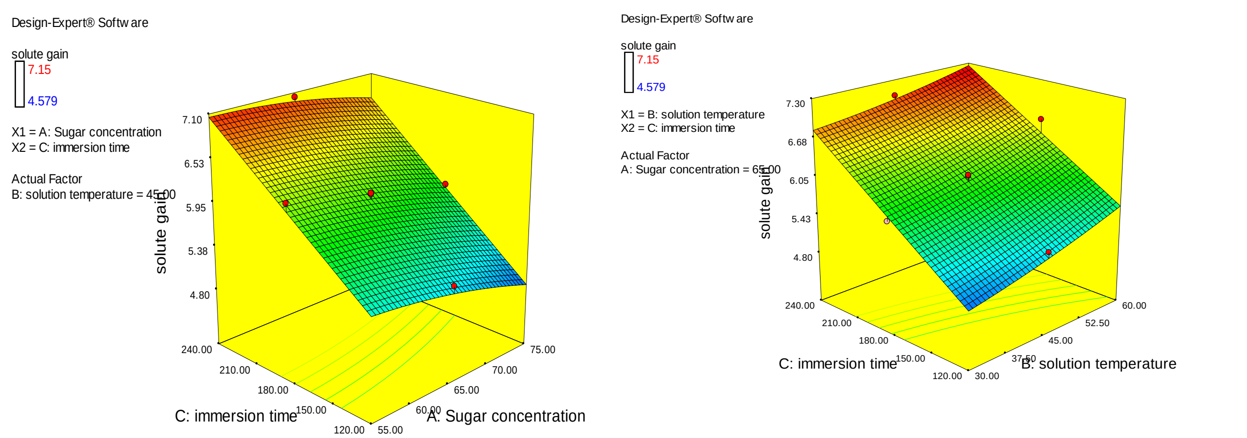

Figure 2: Influence of process variables on solute gain (a) sugar concentration and time at 45 ^{o}C of osmotic solution temperature (b) temperature and time at 65 ^{o} Bx of osmotic solution concentration

(a) (b)

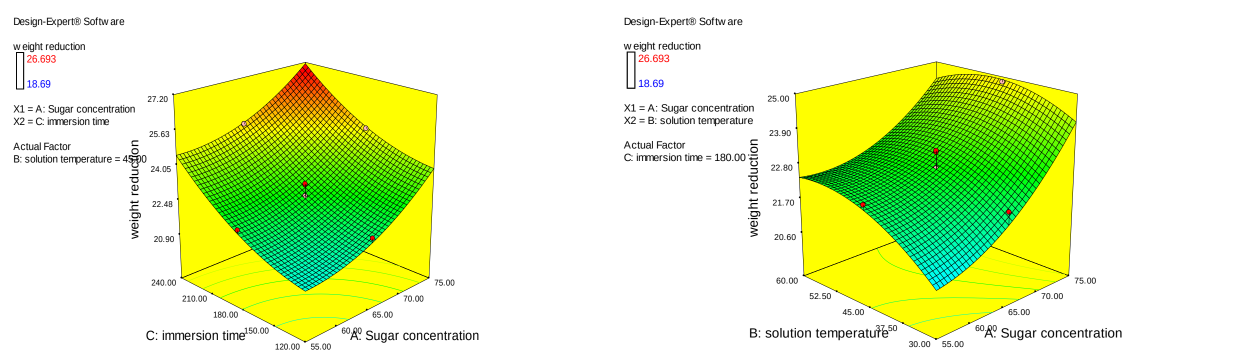

Figure 3: Influence of process variables on weight reduction (a) sugar concentration and time at 45 ^{o}C of osmotic solution temperature (b) sugar concentration and solution temp for 180 min of process duration

(a) (b)

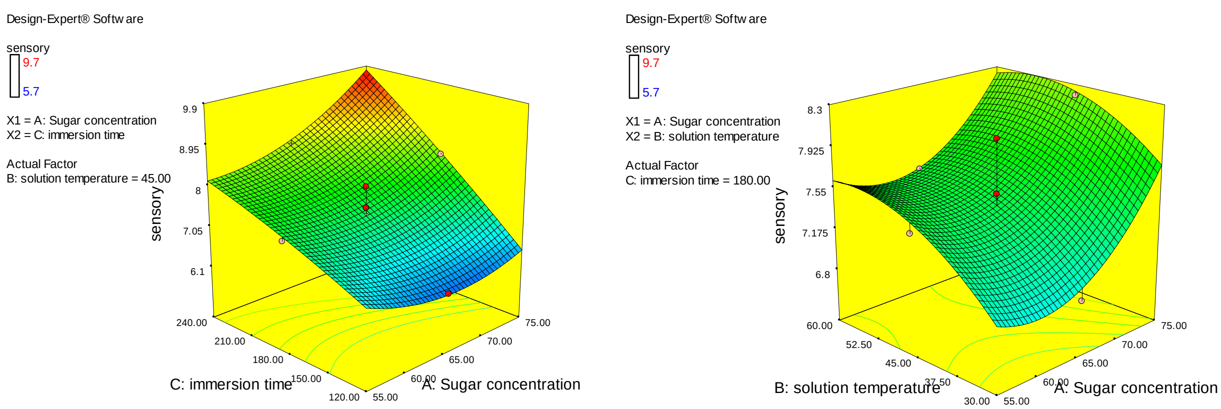

Figure 4: Influence of process variables on weight reduction (a) sugar concentration and time at 45 ^{o}C of osmotic solution temperature (b) sugar concentration and solution temp for 180 min of process duration

Solute Gain

Table 5 indicates that all linear terms of process variables have significant effect ($P < 0.05$) on solute gain. Further, quadratic effect of temperature and time and interaction of 'temperature and time' have significant effect on solute gain during osmotic dehydration ($P < 0.05$). The model F-value 180.30 implies the model is significant. As shown in Figure 2a, the solute gain increased with increase in osmotic solution concentration is mainly because of high concentration difference between the beetroot and osmotic solution (Falade and Igbeka, 2007). Figure 2brevealed that solute gain enhanced with osmotic solution temperature might be because of decrease in viscosity of the osmotic solution resulting in high diffusion rates of solute (Singh *et al.*, 2007).

Final Equation for solute gain in Terms of Actual Factors is:-

solute gain =+5.32444-0.043753×Sugar concentration+0.017480×solution temperature +7.30718E-003× immersion time+5.35833E-004×Sugar concentration×solution temperature +6.52083E-005× Sugar concentration × immersion time -7.76389E-005× solution temperature ×immersion time-8.63636E-005×Sugarconcentration^2-2.31717E-004×solution temperature^2+1.87121E-005× immersion time^2

The increase in water loss and solute gain with time, temperature, and concentration may also be because of agitation given during osmotic dehydration process which reduces the mass transfer resistance between the surface of beetroot and osmotic solution (Panagiotou *et al.*, 1999).

Weight Reduction

The magnitude of P-value from Table 6 indicates that all the linear and interaction terms have significant effect on the wight reduction of the osmotic dehydrated beetroot ($p < 0.05$) at 5% level of significance.

Final Equation for weight reduction in Terms of Actual Factors is:-

weight reduction=+21.22020-0.55352×Sugar concentration+0.50838×solution temperature+3.77919E-003× immersion time-2.55250E-003×Sugar concentration × solution temperature-1.23542E-004×Sugar concentration ×immersion time-5.36250E-004× solution temperature ×immersion time+6.37273E-003× Sugar concentration^2-2.32990E-003×solution temperature^2+1.57298E-004×immersion time^2

Sensory Score

The magnitude of P-value from Table 7 indicates that all the linear and interaction terms have significant effect on the sensory score of the osmotic dehydrated beetroot ($p < 0.05$) at 5% level of significance. The

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quadratic term of solution concentration and time has a non-significant effect on the sensory score, i.e. quality of osmotic dehydrated product, at the 5% level of significance ($p > 0.05$).

Final Equation for sensory score in Terms of Actual Factors is:-

sensory score=+20.13864-0.62073× Sugar concentration+0.20413× solution temperature -6.00947E-003× immersion time-4.16667E-004 Sugar concentration ×solution temperature +7.70833E-004 ×Sugar concentration × immersion time-3.47222E-004× solution temperature ×immersion time+4.13636E-003× Sugar concentration^2-1.05051E-003× solution temperature^2-2.39899E-005 × immersion time^2

Figure 3 (a) shows the effect of sugar concentration and temperature on sensory score and the effect of temperature and time on sensory score shown in figure 3(b). This implies that the overall quality of the osmotic dehydrated beetroot product depends upon processing temperature, time and osmotic solution concentration.

Conclusion

Response surface methodology was effective in optimizing process parameters for the osmotic dehydration of beetroot in osmotic aqueous solutions of sucrose having concentrations in the range 55–75^{0}Bx, temperature 30–60 ^{0}C, and process duration 120–240 min. The regression equations obtained in this study can be used for optimum conditions for desired responses within the range of conditions applied in this study. Graphical techniques, in connection with response surface methodology (RSM), aided in locating optimum operating conditions, which were experimentally verified and proven to be adequately reproducible. Optimum solution by numerical optimization obtained was 75^{0}Bx osmotic solution concentration, 60^{0}C osmotic solution temperature, and 240 min of process duration to get maximum possible water loss, solute gain, and sensory score.

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