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Application of factorial design to the study of the effect of drying conditions on α-tocopherol content in prickly pear seed oil

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Abstract - A full 2^3 factorial design coupled with statistical and graphical analysis of the results, by using analysis of variance (ANOVA) was applied to optimize the process of convective drying on α-tocopherol content in prickly pear seed oil. To facilitate the study and detailed analysis, a statistical model is constructed which is used to predict and optimize the performance of the system. The factors influencing the α-tocopherol content in prickly pear seed oil include temperature, relative humidity and velocity of drying air. A series of experiments is performed on the three process parameters to investigate their effect on α-tocopherol. Experiments were performed at air temperature of 45 and 70°C and relative humidity of 15 and 35% and air velocity of 1 and 2 m/s. HPLC-UV was used to determine the concentration of α-tocopherol in seed oil. The outcome is represented graphically and in the form of empirical model. The analysis of experimental design for process optimization results demonstrates that drying air velocity is the most important factor influencing the concentration of α-tocopherol, whereas the effect of temperature and relative humidity were lower.

Keywords: ANOVA, full factorial design, convective drying, seeds oil, HPLC, tocopherol.

1. Introduction

The potential supply of lipid from fruits and fruit by-products may be enormous and should to be investigated. Palm and coconut oils are good examples of commercially successful oils extracted from fruit. The continued increase in world population and the ever increasing demand for oils necessitates the need to investigate new similar types of plants. For these reasons, new plant sources, especially from underexploited seeds, have been investigated. Millions of pounds of fruit seeds are discarded yearly, resulting in disposal problems, while proper utilisation of these waste products could lead to an important new source of oil (Kamel and Kakuda, 2000). A multi-ingredient fruit, such as cactus or prickly pear (Opuntia ficus-indica), holds promising answers for tailor-made nutraceuticals and functional foods by embracing essential ingredients, such as taurine, amino acids, readily absorbable carbohydrates, minerals, vitamin and soluble fibres (Stintzing et al. 2000, 2001).

The proximate composition of prickly pear cactus (O. ficus-indica) has been investigated (DominguezLopez 1995; El-Kossori et al. 1998; Stintzing et al. 2000, 2001). Seeds constitute about 10–15% of the edible pulp and are usually discarded as waste after extraction of the pulp. Prickly pear seeds were first characterized by Sawaya et al. (1983), who demonstrated that the seeds of *Opuntia ficus indica* are rich in minerals and sulphur amino acids. A reserve protein from the seeds has been isolated and characterized by Uchoa et al. (1998). The prickly pear seed oil composition and its chemical characteristics were investigated by Sawaya and Khan (1982), and then by Salvo, Galati et al. (2002). Sawaya and Khan (1982); Pimienta-Barrios, 1994; and Stintzing et al. 2000 were demonstrated that oil processed from the seeds constitutes 7–15% of whole seed weight and is characterised by a high degree of unsaturation wherein linoleic acid is the major fatty acid (56.1–77%). Coskuner and Tekin (2003) studied the seed composition of prickly pear fruits during the maturation period. Ramadan and Morsel (2003) compared the seed and pulp oil compositions. They detected high levels of tocopherol in the oil that's may contribute to great stability toward oxidation. All tocopherol derivatives were identified in seeds oil with certain differences in the levels of the separated individual tocopherols. γ-tocopherol seems to be the major component in seed oil, the second major component was the α-tocopherol accounting 0.056 g/kg in oil.

Tocopherols stability with the temperature, atmospheric oxygen and light were studied by Boyston et al. (2008). Their results show that temperature is the most important factor especially when it exceeds 100 °C; they found significant interaction between the temperature and oxygen.

Soon et al. (2004) reported that all tested tocopherols isomers exhibited no degradation even after heating for 4 hours at 95ºC when they were exposed to 0% oxygen condition; while over 20% degradation could be observed under 21% oxygen conditions.

Fresh prickly pear seeds are highly perishable and drying is a useful means to increase the shelf life of seeds for further use. The effect of seeds drying conditions on the quality of prickly pear oil is up to now unknown. The major objective of the present work was to optimize the process of convective drying on α-tocopherol content in prickly pear seeds oil. A 2^3 complete factorial design has been used.

2. Materials and methods

2.1. Raw material

Prickly pear seeds used in drying experiments were provided from prickly pear fruit (*Opuntia ficus indica*) grown in Knais, region of Monastir (Tunisia). Mature fruit samples were harvested in August 2012 and taken immediately to the laboratory where seeds were isolated by pressing the whole edible pulp and rinsing the residue with distilled water. The initial moisture content of all samples (varying between 0.9 and 1.5 kg per kg dry matter) were determined by the vacuum oven method at 105°C for 4 h.

2.2. Extraction principle

Several methods can be employed to extract oil from seeds. Cold mechanical pressing of dried seeds is an environmentally friendly method to extract oil where no chemicals are used. This method requires considerably less energy and equipment and the oil extracted is of very high quality.

2.3. α-tocopherol analysis

The standard α-Tocopherol of a purity > 99% was in analytical grade and was purchased from Sigma Aldrich. Methanol, acetonitril, isopropanol (2-propanol) and water, filtered through a 0.45 µm filter, were in HPLC grade and were purchased from Sigma Aldrich.
In our study, saponification of oil samples was not required, which allowed shorter analysis time and greater vitamin stability during analysis (Ramadan and Morsel, 2002).
The α-tocopherol is identified and quantified using an Agilent 1100 HPLC system analytical series, equipped with a quaternary pump and a UV-visible detector (diode array detector). The separation is due to a specific column C18: Hypersil-ODS (125 x 4 mm, 5µm) at room temperature.
The elution solvents used were water (solvent A), methanol (solvent B), acetonitrile (solvent C) and isopropanol (solvent D). The samples were eluted according to the following gradient: 95% A / 5% B in 2 min; 10% B / 30% C / 60% D in 8 min, and finally 25% B/75% C in 12 min. Flow rate was 1 ml/min and sample injection volume was 20 µl. Identification of compound was achieved by comparing their retention time values with those of standards.
Stock solution (1000 mg/l) was obtained dissolving 100 mg of α-tocopherol in 100 ml methanol/isopropanol/acetonitrile mixture (1:3:1,v/v/v). The standard solutions were obtained progressively diluting stock solution in methanol/isopropanol/acetonitrile mixture (1:3:1,v/v/v) to yield the concentrations of 12.5, 25, 50,100 et 200 µg/ml. The standard solutions were injected on the HPLC column. Concentrations were subjected to regression analyses to calculate the calibration equation and correlation coefficient.
Sample solutions were prepared by dissolving the oil in the eluent solvents. 20µl of each aliquot was injected on the HPLC column. Three replicates for each analysis are performed.

2.4. Experimental Design

The experimental design methodology (EDM) has been successfully applied to optimizing conditions in food research (Ibanoglu S and Ainsworth 2004) but very few studies have focused on optimization of drying conditions on oils quality. Common practice for optimization operating conditions of such a process consists of varying one parameter and keeping the others at a constant level. The major disadvantage of this single variable optimization is disregard of the interactive effects between the studied variables. Consequently, the net effect of various parameters on the final response is not exhibited. In order to overcome this problem, optimization studies have been carried out using experimental design. This technique is an integration of experimental strategies, mathematical methods and statistical inference which allows simultaneous variation of several factors to find the optimal level giving the most interesting response. EDM reduces the number of experimental trials needed to evaluate multiple parameters and their interactions.

2.5. Study parameters

The optimization of the variables affecting the quality of oil was carried out following a 2^3 complete factorial design. The response measured was the amount of tocopherol in the oil extracted from the seeds of prickly pear. The studied factors were temperature, relative humidity and air velocity for convective drying.

2.6. Study domain

Factors levels were chosen by considering literature data about drying seeds. Table 1 lists the independent parameters, their symbols and their real and coded levels (-1, 0 and +1).

Table 1: Parameter levels and coded values used in the experimental design

		Code	Unite	Level -1	Level 0	Level +1
Factors	Temperature	X_1	°C	45	57.5	70
	Humidity relative	X_2	%	15	25	35
	Velocity of air	X_3	m/s	1	1.5	2
Response	Tocopherol	Y	mg/kg			

2.7. Experimental matrix

A factorial matrix of three factors has been defined. For such a matrix, 12 experiments were required: 8 for the complete factorial matrix (2^3) and four carried out at the centre. The duplication of centre points, coded 0, is used to determine the experimental error. In our case, four central replicates were employed. The experiments were run in random order to avoid systematic errors due to extraneous factors (Goupy J 2001).

2.8. Mathematical model

In a complete factorial design, a linear mathematical model could be established that shows the influences of each parameter and the interactions between parameters on the measured response (Y). Therefore, the symbolic mathematical model is expressed by the following equation 1:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1X_2 + b_{13} X_1X_3 + b_{23} X_2X_3 + b_{123} X_1X_2X_3 + \varepsilon$$

Where b_0 represents the average theoretical value of the response. Coefficients b_1, b_2 and b_3 represent the factor effects of X_1, X_2 and X_3 respectively. The coefficients b_{12}, b_{13}, b_{23} and b_{123} represent the interaction effects of X_1-X_2, X_1-X_3, X_2-X_3 and X_1-X_2-X_3 respectively, and ε is an error term.

2.9. Statistical analysis

The analysis of results was performed with statistical and graphical analysis software NemrodW (Mathieu et al. 2000). This software was used for regression analysis of the obtained data and to estimate the coefficient of regression equation. ANOVA (analysis of variance), which is the statistical testing of the model in the form of a linear term and an interaction term, was also used to test the significance of each term in equation 1 and the fitness of the obtained regression model (Huiping L et al. 2007).

3. Results and discussion

The α-tocopherol in prickly pear seeds oil was quantitatively determined by a HPLC-UV method. The calibration curve was used for the calculation and good linearity was achieved in the range 12.5-200 µg/ml (r^2 = 0.99). The observed response values with different combinations of the three variables used in our experimental design are listed in Table 2.

Table 2. Design matrix corresponding to the 2^3 factorial design.

	Experiments	MOY	X1	X2	X3	Y
Complete factorial matrix	1	1	-1	-1	-1	0.970
	2	1	1	-1	-1	0.830
	3	1	-1	1	-1	0.482
	4	1	1	1	-1	0.491
	5	1	-1	-1	1	0.249
	6	1	1	-1	1	0.463
	7	1	-1	1	1	0.725
	8	1	1	1	1	0.311
Centre points	9		0	0	0	0.569
	10		0	0	0	0. 586
	11		0	0	0	0.521
	12		0	0	0	0.519

α-tocopherol was found in variable concentrations (0.249 – 0.970 mg/kg). The amount of α-tocopherol in prickly pear seed oil was similar to data reported by Vanesa et al. 2001 for Chia seed oil (0.4 – 9.9 mg/kg), but lower than those reported by Ramadan and Morsel (2003) for prickly pear seed (56 mg/kg) oils obtained by solvent extraction. This is explained by the α-tocopherol extrability. In fact, tocopherol contents were significantly higher ($p<0.05$) in oils obtained by solvent extraction than by pressing (Tuberoso et al. 2007).

3.1. Effect of factors and their interactions on α-tocopherol

The study of the effect of different factors on the response was performed using the analysis design procedure of the NemrodW software. The main effects of the three variables studied and the interaction effect involving these factors are shown in Table 3. The value of the constant was found to be 0.560, which does not depend on any factor or factor interaction. A positive sign of the coefficient represents a synergistic effect, while a negative sign indicates an antagonist effect. The analysis of Table 3 shows that all the effects except interaction between temperature and air velocity are highly significant on the α-tocopherol inside the range of the experimental studies. Figure 1 shows the corresponding Pareto chart, used for identification of the most important factors. The length of the rod is proportional to the importance of the effect. Rods are directed toward the right when the effect is positive, toward the left when it is negative. The charts indicate that the effect of temperature x air velocity is statistically insignificant. The interaction between relative humidity and air velocity has the highest standardized effect on α tocopherol followed by the effect of air velocity. Hence, the term X_1X_3 should not be considered for the empirical relation.

Table 3. Estimated regression coefficients for the complete factorial design

	Effect	t_{exp}	Standard error	Signif, %
b_0	0,560	60,06	0,09	***
b_1	-0,041	-3,63	0,011	*
b_2	-0,063	-5,51	0,011	**
b_3	-0,128	-11,23	0,011	***
b_{12}	-0,060	-5,25	0,011	**
b_{13}	-0,009	-0,76	0,011	
b_{23}	0,144	12,61	0,011	***
b_{123}	-0,097	-8,51	0,011	**

t_{exp} Value of the coefficient of regression for the error, measures it how big the effect is regarding the mistake standard or residue

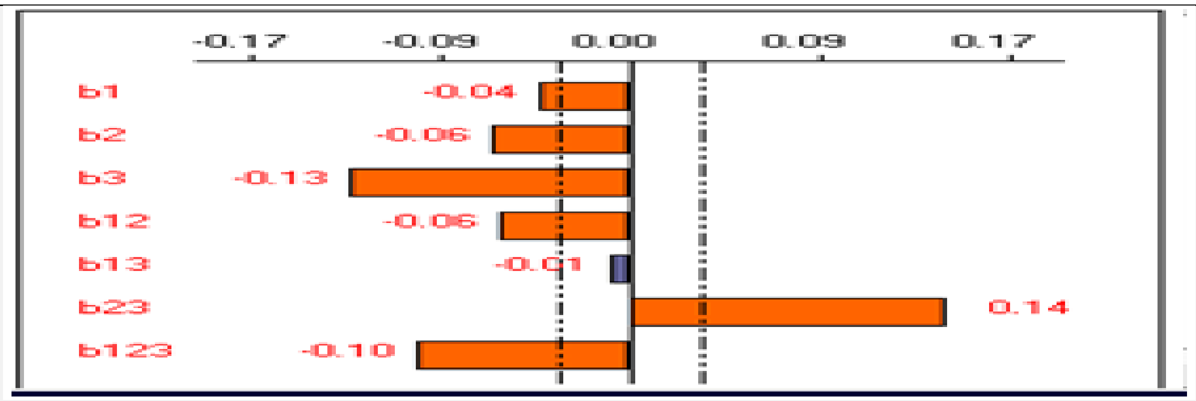

Figure 1 .Pareto chart representation of the parameter effects on the response

3.1.1. Effects study

For the results exploiting the mean content of α-tocopherol for each experiment at the same level must be calculated. For example, the mean of level -1 of temperature is $y_{T_{-1}} = (0.970 + 0.482 + 0.249 + 0.725)/4 = 0.6065$, these means are reported in table 4. Table 4 shows the air velocity effect is 1.6 times more at 1 m/s (0.6932) than at 2m/s (0.437). The same table shows the effect of relative humidity demonstrating that its increase influences the concentration of α-tocopherol. As a matter of fact, α-tocopherol decreases between level -1 (15%) and level +1 (35%). The loss of α-tocopherol is 1.25 times more when the experiment is performed at 35%.

Table 4: Means of effect and interactions

Effects	Level	Mean	Interactions	Level	Level	Mean
Temperature			Temperature/relative humidity			
	-1	0.6065		-1	-1	0.6095
	+1	0.5237		-1	+1	0.6035
Air velocity				+1	-1	0.6465
	-1	0.6932		+1	+1	0.401
	+1	0.437	Temperature/air velocity			
Relative humidity				-1	-1	0.726
	-1	0.628		-1	+1	0.487
	+1	0.5022		+1	-1	0.6605
				+1	+1	0.387
			Relative humidity/air velocity			
				-1	-1	0.9
				-1	+1	0.356
				+1	-1	0.4865
				+1	+1	0.518

Temperature is a factor of lesser importance. Increasing temperature from 45 to 70°C leads to increase the loss of α-tocopherol by 1.15 times. That's mean that α-tocopherol is stable at heat treatments. Stability of α-tocopherol is explained by the role of phenolic compounds that protect the α-tocopherol from oxidation during the heating. In fact, according to Tlili et al (2009), the amount of phenolic compounds in prickly pear seeds was found to be 268mg/100g of seed.

Our results show that α-tocopherol is more sensitive to oxygen (provided by air drying) than the temperature. These results are consistent with those found by Soon et al. (2004) whose reported that α-tocopherol has not been degraded when has been exposed to 0% oxygen even after heating for 4 hours at 95°C, whereas more than 20% degradation was observed in conditions of 21% oxygen.

3.1.2. Interaction study

Fig 2 depicts a plot of average output for each level of the factor with the level of the second factor held constant. These plots called interaction plots are used to interpret significant interactions between the process parameters. Interaction is present when the response at a factor level depends upon the levels of other factors. Since they can magnify or diminish the main effects of the parameters, evaluating interactions is extremely important.

In the Interaction plot for α tocopherol, the lines in temperature versus air velocity plot are approximately parallel, indicating a lack of interaction between the two factors. It suggests that mutual interaction between temperature and air velocity has negligible effect on α tocopherol. In the second plot, there exists antagonistic interaction between relative humidity and air velocity as the lines of the graph cross each other. Similarly, the third plot depicts synergic interaction between temperature and relative humidity. The greater the departure of the lines from the parallel state, the higher the degree of interaction.

The study of the interactions between the different factors revealed that the more meaningful interaction was the first-order interaction air velocity-relative humidity. This interaction had a positive relationship with the tocopherol content

The highest amount of α-tocopherol comes from seeds dried at 45°C, 15% and 1 m/s, however it can be deduced from the result obtained that there is a negative effect of the temperature, relative humidity and air velocity.

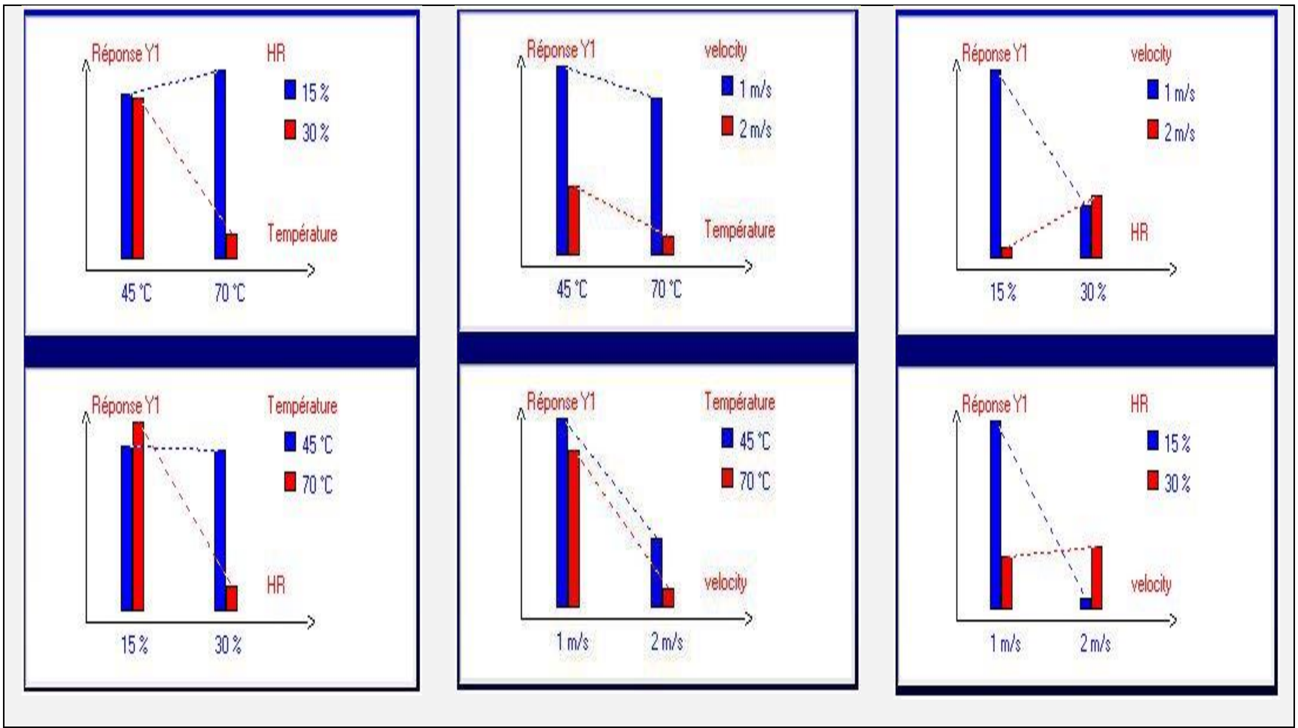

Figure 2: Interaction plots of α-tocopherol

3.1.3. Fitted model

The interaction X_1X_3 has been stated to be statistically insignificant i.e. the product of temperature and air velocity has negligible effect on the tcopherol and a reduced model was created wherein the factors X_1X_3 and $X_1X_2X_3$ are ignored. The removal of 3-way interaction factor $X_1X_2X_3$ is essential due to the hierarchical nature of the model. The final outcome as given by NemrodW software after incorporating these changes is given below. The empirical relation in terms of coded factors generated by a complete factorial design 2^3 for the responses in this study is:

$$[Y] = \mathbf{0.560 - 0.041}\,\boldsymbol{X_1} - \mathbf{0.063}\,\boldsymbol{X_2} - \mathbf{0.128}\,\boldsymbol{X_3} - \mathbf{0.060}\,\boldsymbol{X_1X_2} + \mathbf{0.144}\,\boldsymbol{X_2X_3} + \boldsymbol{\varepsilon}$$

The experimental error (e) was calculated based on the standard deviation of the central point with four runs and was determined as 0.032.

The quality of fitting the first-order polynomial was expressed by the coefficient of regression, R^2. The R^2 values provide a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. As R^2 approaches unity, the better the empirical model fits the actual data. The smaller values of R^2 represent less relevance of the dependent variables in the model used in explaining the variation behaviour.

Furthermore, the regression coefficient is estimated to be acceptable (R^2 = 0.991). The value gives good agreement between the experimental and predicted values of the fitted model. It implies that 99.1% of the variability in the response could be explained by the model.

4. Conclusion

In this study, we evaluated the effect of the convective drying factors on the α-tocopherol content in the prickly pear oil by using as strategy the methodology of experimental design that aims to obtain maximum results for a smaller number of experiments. All data are used simultaneously to calculate every effect, which leads to a greater precision of the obtained results as well as the mathematical modelling of the experience.

The results clearly showed that the experimental design is a suitable method to optimize convective drying. A 2^3 complete factorial design was applied to establish a first-order model. After analysis of the effects, this model permitted to show that air velocity (oxygen) has the greatest effect on degradation. Interaction between relative humidity and air velocity is the most significant.

Further studies are necessary to determine the effect of the convective drying factors on oil yield and chemical composition of prickly pear seeds oil.

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