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Optimization of Lycopene Extraction from Tomato Waste with the Integration of Ultrasonic - Enzymatic Processes by Response Surface Methodology

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ABSTRACT

Lycopene is a powerful antioxidant and a valuable potential combined that today it is tried to increase achieving it by using tomato and its products. In this study, the effect of the sample weight of 0.5, 1 and 1.5 grams at different ultrasound waves times of 0, 30 and 60 min and the cellulase enzyme concentration of 20, 40 and 60 unit on extraction of lycopene from tomato waste was investigated and process optimization was performed using response surface methodology. The design of statistical analysis showed that the amount of sample, the time of using of ultrasound waves and enzyme concentration has a significant effect on the amount of lycopene extraction. Optimal amount of sample, ultrasonic time and enzyme concentrations were obtained 1.5 g, 30 minutes and 40 units by statistical analysis using response surface methodology (RSM). Based on the results, using integration processes of ultrasonic and enzymatic is suitable for the extraction of lycopene because the lycopene can be extracted without the use of organic solvents which have negative effects on health and it is not economical. The extraction of lycopene only with ultrasonic waves causes isomerization and destruction of lycopene, but if it is used as a pre-treatment of enzymatic extraction process leads an increase in extraction of lycopene.

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INTRODUCTION

Epidemiological evidence indicates that consumption of fresh and processed tomato products causes a reduction of the risk of cancer [1] and a lower incidence of coronary heart disease[2]. These beneficial effects are primarily attributed to the antioxidant activity of tomato products increased oxidation. Increasing of oxidation damage in the pathogenesis of these diseases has been proposed [3, 4]. Tomatoes contain a wide variety of antioxidants, including vitamin E, ascorbic acid, carotenoids, flavonoids and phenolic [5, 6]. Among them the carotenoids lycopene attracted much attention, as an important role in preventing diseases in recent years, is responsible for the red color of grown tomatoes [7, 8].

Chemically, lycopene is a great molecule that has a structural formula of $C_{40}H_{56}$, a molecular weight of 536 Dalton and red color and is responsible for red color of many fruits such as water melon, pepper, grapefruit, etc. [9, 10] and its structure shown in Figure 1, has 13 double bonds contains 11 conjugated bonds and 2 unconjugated bonds. Existence of lycopene as a powerful antioxidant has been associated with the idea of having other important biological properties, including induction of apoptosis, inhibition of cell proliferation and increasing the synaptic interface[11]. Lycopene is susceptible to oxidative degradation [12, 13], thus, extraction, storage, transport and analysis of lycopene should be conducted under controlled environmental conditions [14]. The presence of a long chain of conjugated carbon - carbon double causes lycopene affected by chemical changes when exposed to light and heat [6, 15]. Most common forms of lycopene are all-trans form of, 5- cis, 9 - cis, and 15 - cis[1]. Lycopene is commercially available but is very expensive, which causes searching for a good way to replace the production technology. For example, residual waste tomatoes obtained from processing is one of the promising fields and is a rich source of lycopene [6]. The wastes are about 3-5 percent of total weight that have been proposed according to estimates by the World Council of tomato [16]. Tomato waste is a rich source of carotenoids because it gradually accumulates in the outer layer during the ripening of tomatoes [17].

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Conventional extraction method is time consuming and low efficiency while supercritical fluid extraction has been performed using inorganic solvents as an advantage, but it requires expensive equipment and its energy consumption is very high.

Now we know that ultrasound and enzymatic methods can improve the speed and extraction time and improves the extraction of bioactive compounds [18-21]: Ultrasound can be used as an appropriate process for the extraction of lycopene, which causes disruption of the cell wall. One of advantages of ultrasound in food processing is to increase the extraction rate that will result in reduced extraction time and enhanced throughput [14, 22, 23]. Enzymatic method causes extraction by breaking down the cell wall, and using enzymatic pretreatment improves the extraction [17, 24].

To the best of our knowledge, no previous studies have been conducted on the ultrasonic - enzymatic processes for the extraction of lycopene from tomato waste, using hydrolytic enzymes such as cellulase and the ultrasonic extraction. This study was conducted to optimize the process parameters (amount of sample, ultrasonic time and enzyme concentrations) of extraction of lycopene from tomato waste with RSM, and to find the optimum operating conditions that maximize the lycopene extraction.

MATERIALS AND METHODS

All required materials were cellulase enzymes (Sigma, United States) and solvents including acetone (Iran), ethanol (Fariman, Iran), hexane (Scharlow, Spain) and sodium acetate with purity of 98, 96, 98, 99% and acetic acid (Merck).

In this study, extraction method of lycopene from tomato waste using ultrasound and then using the spectrophotometer at the wavelength of 503 nm (λ_{max}) was measured [2, 18, 24] and the effect of different parameters such as power and time were investigated. All-trans lycopene content was calculated by spectrophotometer according to standard methods in milligrams using the specific extinction coefficient [2, 20]:

$$Lycopene(mg) = A \times dil \times ml \times 10 \,/\, E_{1cm}^{1\%}$$

Where A: absorbance of the solution a 1 cm cuvette, dil: dilution factor, ml: final volume of the sample, $E^{1\%}_{1cm}$: special extinction coefficient for lycopene in hexane 3450.

Lycopene evaluation:

Lycopene concentration in solvent extraction spectrometry at room temperature was determined using quarter absorption spectrum with the length of 1 cm and binary ray UV-Vis spectrophotometer the (UNICO 2001SUNV, USA). The absorption spectrum was similar to the standard of lycopene and it was splayed at a wavelength of 503 nm.

Tomato waste powder and a mixture of sodium acetate and acetic acid with pH=4.70 were selected for the extraction of lycopene from tomato waste.

Flask was attached to a condenser and was immersed an ultrasonic water bath (UP400S, Hielscher, Germany; Ultrasonic frequency 24KHZ, total power consumption 400w). The flask was taken after extraction and the mixture was separated by centrifugation and filter paper.

Lycopene extraction was performed in 50 or 100 ml of a mixture of sodium acetate with pH = 4.70 using batch method. Ultrasound waves was used as an auxiliary energy and extraction accelerators in this method. These waves causes breaking the cell wall by created incorporation. After drying tomatoes' husk, 1 g of the sample is added to the flask whose walls were covered with foil to prevent light, then the sodium acetate buffer, pH = 4.60 is added to it due to using of enzymes treatment in next steps[2] and is placed under ultrasound at 35 º C. In enzymatic extraction step, different amounts of cellulase enzyme (20, 40, 60 U) is added to the solution and it is placed inside Shaker incubator at 37 ° C for 4 hours and rate of 200 (rpm). Finally, solution is filtered, its liquid phase is removed, and the amount of lycopene is measured[2].

Experimental design:

As shown in Table 1, a CCD in the form of the 2^3 full factorial design was used, in which three independent variables were converted to dimensionless ones (*A*, *B*, *C*,), with the coded values at 3 levels: −1, 0, +1. The arrangement of CCD as shown in Table 1 was in such a way that allows the development of the appropriate empirical equations (second order polynomial multiple regression equations) [25, 26]:

The predicted response (y) was therefore correlated to the set of regression coefficients (β): the intercept (β_0), linear (β_1, β_2, β_3), interaction (β_{12}, β_{13}, β_{23}) and quadratic coefficients (β_{11}, β_{22}, β_{33}). The "Design expert" (Trial version 7) was used for regression and graphical analyses of the obtained data.

$$y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

The results of tests performed on tomato waste samples are shown in table 1. From the left, respectively, the first column is the number of trials, the second column is sample, the third column is sonicator time, the fourth column is the amount of enzyme activity and last column is extracted lycopene. The change portion of 16

predetermined tests was designed based on the statistical model by used software and results of different responses including the amount of sample, ultrasound time and enzyme activity defined as a quantity index was obtained in certain circumstances. Therefore, favorable conditions for obtaining lycopene with minimum damage and economic conditions were obtained by the analysis of data obtained from statistical relationships and response surface methodology and with regard to the lack of lycopene degradation.

Table 1: Arrangement of the Central Composite Design for the three independent variables used in the present study.

Lycopene Content (ug/g)	Enzyme Con. (U) (C)	Sonic time(min) (B)	Sample (g) (A)	Run
6.17	20	0	0.5	1
16.99	20	0	1.5	2
12.26	20	60	0.5	3
21.19	20	60	1.5	4
4.43	60	0	0.5	5
13.77	60	0	1.5	6
11.45	60	60	0.5	7
20.14	60	60	1.5	8
13.09	40	30	0.5	9
23.57	40	30	1.5	10
10.61	40	0	1	11
17.42	40	60	1	12
17.68	20	30	1	13
17.68	60	30	1	14
19.3	40	30	1	15
19.3	40	30	1	16

RESULTS AND DISCUSSIONS

Results of Analysis of Variance:

The results of analysis of variance of data and amount of the lack of fit for each model are presented in Table 2. Statistical analysis shows that the three parameters of the sample amount, sonication time and enzyme activity have positive effects and the effects of the sample amount and sonication time parameters are significant ($P \leq 0.05$).The corresponding variables would be more significant whether the absolute F value becomes greater and the p-value becomes smaller. It can be seen that the three independent variables, that is, Sample weight (A) and sonic time (B) with the largest effect on the response ($p < 0.001$) were the linear terms. The non-significant value of lack of fit (more than 0.05) showed that the quadratic model was valid for the present study.

Table 2: Analysis of variance (ANOVA) of RSM for lycopene extraction.

P	F	MS	df	SS	Factors
< 0.0001	92.879	47.652	9	428.864	Model
< 0.0001	453.65	232.75	1	232.75	Sample (A)
< 0.0001	181.31	93.02	1	93.02	Sonication time (B)
0.0237	9.05	4.65	1	4.65	Enzyme concentration (C)
					Interaction
0.2587	1.556	0.798	1	0.798	AB
0.4316	0.711	0.365	1	0.365	AC
0.1764	2.347	1.204	1	1.204	BC
					Square
0.6742	0.195	0.100	1	0.100	A^2
< 0.0001	104.581	53.656	1	53.656	B^2
0.1046	3.650	1.873	1	1.873	C^2
		0.513	6	3.078	Residual
		0.616	5	3.078	Lack of fit
		0	1	0.000	Pure error
			15	431.942	The total correction

Response surfaces and contour plots:

Determination of the response surface model is performed based on the impact of linear and quadratic response, and interaction effect of the independent variables of the regression. Regression model for estimating the amount of extracted lycopene based on actual values is presented in equation (2).

$$Y=-1.92+12.69A+0.39B+0.136C-0.021AB-0.021AC+0.0006BC-0.779A^2-0.005B^2-0.002C^2 \quad \text{Equation} \quad (2)$$

Correlation coefficient and corrected correlation coefficient of fitting the experimental results with equations were 0.993 and 0.982, respectively. Correlation coefficients indicate the confidence of fitting the experimental results with quadratic mathematical equations. Therefore, these empirical equations exactly indicate the relationships between the independent variables and the responses. Also, the variation coefficient is

68.4%. Variation coefficient is reported as a percentage of the ratio of the standard error to the experimental data mean. If the variance coefficient is less than 10%, it indicates the reproducibility of the model.

Amounts of lycopene extracted in performed tests versus the values predicted by the model are presented in Figure 1. The distribution of data around the fitted line indicates good compliance of data and with regard to the figure, it is observed that there is little difference between these two values**.**

Figure 2 shows the effect of the amount of sample and sonication time on the amount of lycopene extracted in a constant concentration of the enzyme of 40 units. Extracted lycopene linearly increases with the increasing amount of sample and the amount of extracted lycopene increases by increasing the sonication time period up to 30 minutes but with more increasing the sonication time the amount of lycopene reduces because more increase in sonication time resulted in the destruction and degradation of extracted lycopene [27].

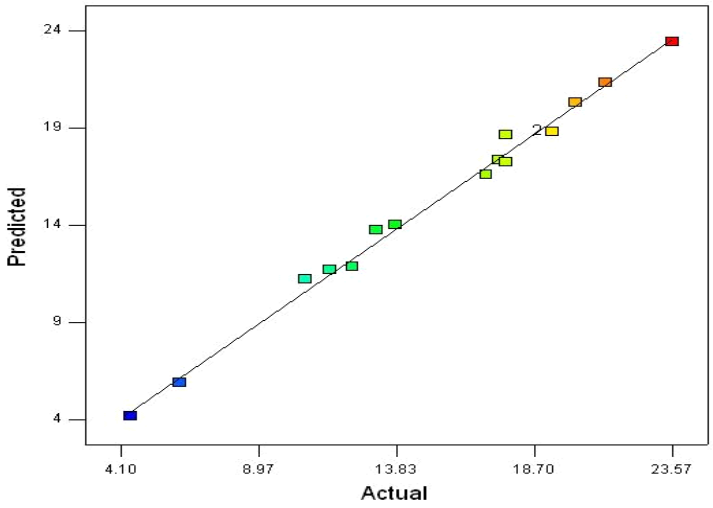

Fig. 1: The predicted values by the model versus actual measured value.

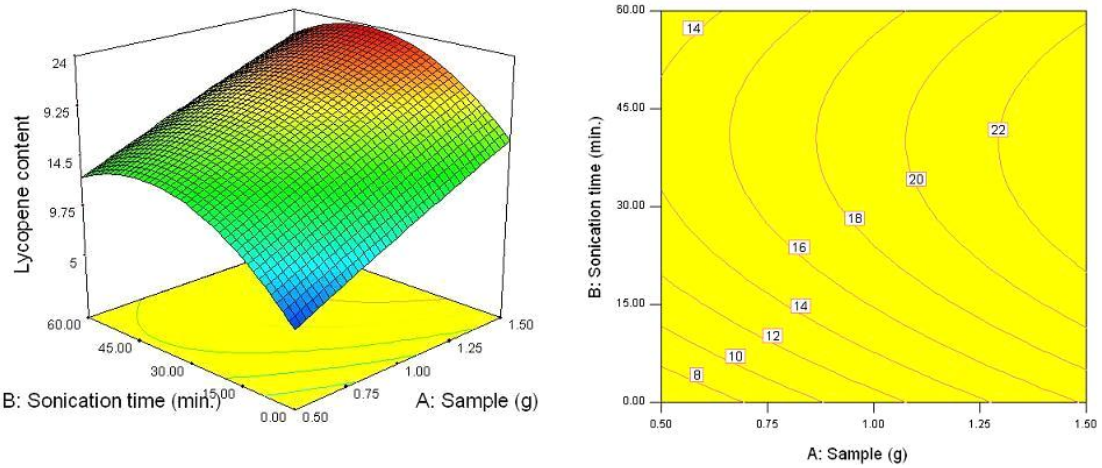

Fig. 2: Response surface and contour plots for the effect of ultrasonic time and weight of sample on the lycopene extraction, (concentration of the enzyme =40 units)

In Figure 3 is examined effect of samples amount and enzyme activity on the amount of extracted lycopene at constant sonication time of 30 minutes. About the effect of enzyme activity and the amount of sample grams on the extraction efficiency of lycopene from tomato waste in constant time of ultrasound can be said that the effect of sample amount is much more intuitive and are almost incremental. But there is a more uniform level of enzyme activity, in the other word, the incremental process is very uniform in the activity of 20 to 60. These statements are deduced from the low slope of enzyme activity curve or vertical curve.

The effect of the ultrasound time amount and enzyme activity on lycopene extraction in a constant concentration of sample of 0.5 g was shown in Figure 4. As the figure suggests, in integration of extraction with enzymatic and ultrasonic waves processes, both parameter (ultrasonic time of enzyme activity) have a significant impact on the extraction but the effect of ultrasonic time is very remarkable. In the case of the effect

of ultrasonic time parameter can be stated that this incremental process is until about 50 minutes and then, it has been followed by a decreasing trend. However, surface of the curve of enzyme activity is uniform and slope of the curve is the reason of these statements. Similar results were found by Sivakumar et al. [16].

Optimum condition for ultrasonic - enzymatic extraction:

Optimum conditions for lycopene extraction from tomato waste using ultrasonic - enzymatic processes was determined to obtain maximum lycopene content. Second order polynomial models obtained in this study were utilized for each response to determine the specified optimum conditions. The sequential quadratic programming in Design-Expert D×6 6.0.10 is used to solve the second degree polynomial regression equation 2. The optimum values obtained by substituting the respective coded values of variables are 1.5 g, 30 min, 40 U. At this point, the maximum yield of lycopene extraction was calculated as 23.57 µg/g.

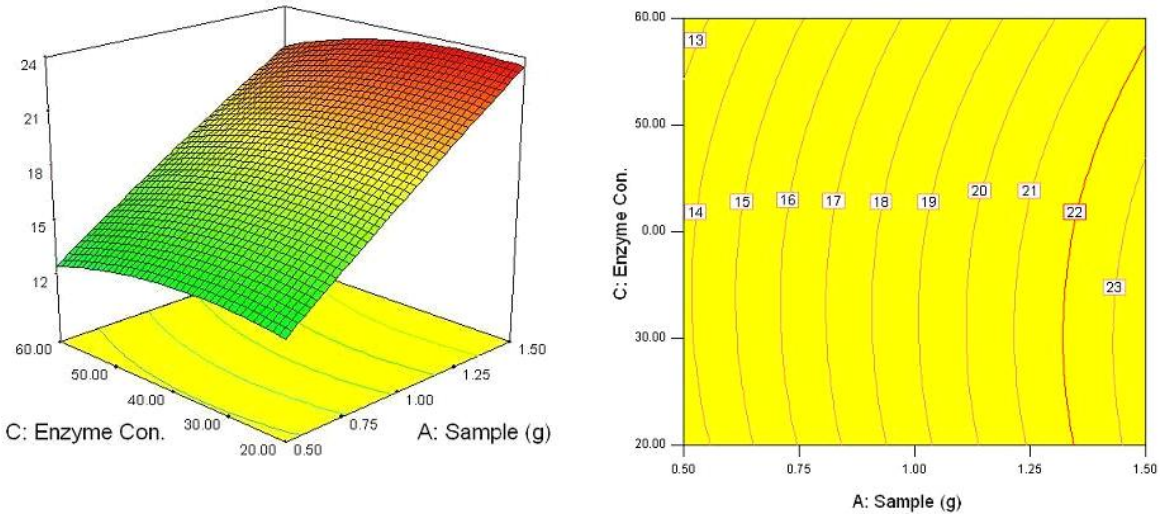

Fig. 3: Response surface and contour plots for the effect of enzyme activity and weight of sample on the lycopene extraction, (sonication time =30 minutes)

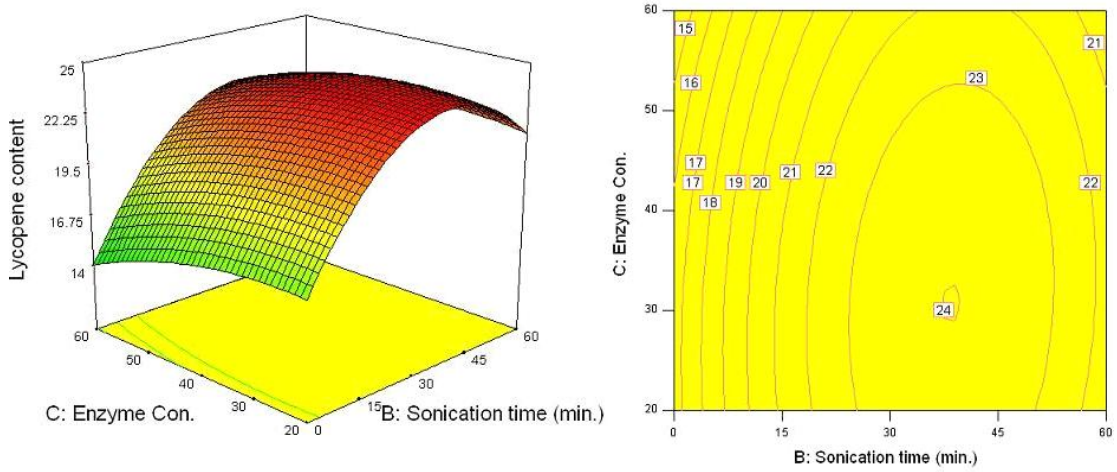

Fig. 4: Response surface and contour plots for the effect of enzyme activity and ultrasonic time on the lycopene extraction, (weight of sample=0.5 g)

Conclusion:

In this present study, experimentation has been done to lycopene extraction from tomato waste using ultrasonic - enzymatic processes. Response surface methodology have been used to generate the model on yield of lycopene from tomato waste. Weight of the sample, sonication time, and enzyme concentration markedly influenced the lycopene extraction from tomato waste. Response surface plots gave a good interaction between the three variables and the response and the resulting model for the lycopene extraction from tomato waste explained 99.29% variance (R^2= 0.9929).

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