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Analytical Methods

Effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology

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Abstract

Skin, rich in lycopene, is an important component of waste originating from tomato paste manufacturing plants. A central composite design with five independent variables, namely solvent/meal ratio (20:1, 30:1, 40:1, 50:1, and 60:1 v/w); number of extractions (1, 2, 3, 4 and 5); temperature (20, 30, 40, 50 and 60 °C); particle size (0.05, 0.15, 0.25, 0.35 and 0.43 mm); extraction time (4, 8, 12, 16 and 20 min) was used to study their effects on lycopene extraction. The experimental values of lycopene ranged between 0.639 and 1.98 mg/100 g. The second order model obtained for extracted lycopene revealed a coefficient of determination (R^2) of 0.99 and a standard error of 0.03. Maximum lycopene (1.98 mg/100 g) was extracted when the solvent/meal ratio, number of extractions, temperature, particle size and extraction time were 30:1 v/w, 4, 50 °C, 0.15 mm and 8 min, respectively.

Keywords: Tomato waste pomace; Skin; Response surface methodology; Lycopene

1. Introduction

Tomato (*Lycopersicon esculentum*) is one of the most popular of vegetables, used as a salad, in food preparations, and as juice, soup, puree, ketchup or paste. Lycopene is the principal carotenoid, causing the characteristic red hue of tomatoes (Clinton, 1998; Shi & Le Maguer, 2000). Tomato lycopene content varies considerably, reflecting the influence of variety (generally genetic factors), maturity, and both agronomic and environmental conditions during growing (George, Kaur, Khurdiya, & Kapoor, 2004; Kaur, Sharma, Wani, Gill, & Sogi, 2006; Martinez-Valverde, Periago, Provan, & Chesson, 2002; Shi & Le Maguer, 2000). Processed tomatoes additionally appear to increase the lycopene absorption by body tissues, due to enhanced bioavailability attributed to geometric isomer variation during processing (*cis*-isomers are more available) and to changes in the composition and structure of the food, which may increase the release of lycopene from the tomato tissue matrix (Shi & Le Maguer, 2000). Several epidemiological studies report that lycopene-rich diets have beneficial effects on human health (Arab & Steck, 2000; Sharoni, Danilenko, & Levy, 2000). A possible role has been suggested for tomatoes and tomato products in preventing cardiovascular disease and protecting against some types of cancer (based on lycopene content) (Willcox, Catignani, & Lazarus, 2003).

Commercial processing of tomato produces a large amount of waste at various stages. Tomato pomace constitutes the major part of the waste that comes from the pulper. The wet pomace contains 33% seed, 27% skin and 40% pulp while the dried pomace contains 44% seed and 56% pulp and skin (Sogi & Bawa, 1998). Pomace consists of skin that could be utilized for extracting lycopene (Chandler & Schwartz, 1987; Heinonen, Ollilainen, Linkola, Varo, & Koivistoinen, 1989; Sadler, Davis, & Dezman, 1990; Sogi & Bawa, 1998). Skin can be separated by a floatation-cum-sedimentation technique, from other

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constituent seed and fibrous matter, to facilitate better pigment extraction (Kaur, Sogi, Garg, & Bawa, 2005). Most of the lycopene is associated with the water-insoluble fraction and the skin (Sharma & Maguer, 1996). Results indicate that 72–92% of the lycopene is associated with the water insoluble fraction and the skin. Therefore, skin extracts are especially rich in lycopene. Baysal, Ersus and Starmans (2000) reported that a large quantity of carotenoids is lost as waste in tomato processing. The fresh skin has a high moisture content that makes it susceptible to microbial proliferation and spoilage. Therefore, skin can be preserved by drying and then used for lycopene extraction (Kaur, Wani, Sogi, & Shivhare, 2006).

Recent studies have described a lycopene extraction process based on supercritical CO_2, which allows the extraction of over 60% of the lycopene from tomato waste (Baysal et al., 2000; Rozzi, Singh, Vierling, & Watkins, 2002; Sabio et al., 2003). However, because lycopene is fat-soluble, it is more commonly extracted with organic solvents, such as ethanol, acetone, petroleum ether, hexane, benzene and chloroform, prior to chemical analysis for quantitative determination (Al-Wandawi, Abul-Rahman, & Al-Shaikhly, 1985; Lin & Chen, 2003; Sadler et al., 1990; Sharma & Maguer, 1996). A mixture of hexane with acetone and ethanol or methanol is often used (Shi & Le Maguer, 2000; Van den Berg et al., 2000) because other components, such as diethyl ether and tetrahydrofuran, may contain peroxides that react with carotenoids (Van den Berg et al., 2000), and the stability of lycopene extracts obtained with hexane/acetone or hexane/ethanol is higher than that of extracts obtained with other organic solvents, such as chloroform, methanol or dichloromethane (Taungbodhitham, Jones, Wahlqvist, & Briggs, 1998).

A statistical design of experiments (DOE), a well-established concept for the planning and execution of informative experiments was employed to standardize the parameters (solvent/meal ratio, number of extractions, temperature, particle size and time) for maximum extraction of lycopene from dehydrated tomato waste skin.

2. Material and methods

2.1. Material

Tomato pomace was obtained from a tomato paste manufacturing unit (Nijjer Agro Industries) located in Amritsar, Punjab (India).

2.2. Sample preparation

Skin was separated from pomace by a continuous floatation-cum-sedimentation system (Kaur et al., 2005). The separated skin was dried in a cabinet dryer (La Parmigiana, Italy) according to the methods of Kaur et al. (2006). The dried skin was ground in a mixer (Sujata, India) and then passed through different sieves (0.05, 0.15, 0.25, 0.35 and 0.43 mm).

2.3. Proximate analysis

Moisture, ash, crude protein, crude fibre and crude fat content were determined according to AOAC (1990). Carbohydrates were computed by subtracting percent content of all the above components from one hundred.

2.4. Pigment extraction

Sample (1 g) was extracted using solvent (hexane:acetone:alcohol 2:1:1) containing 0.05% (w/v) butylated hydroxytoluene (BHT). Cold distilled water (15 ml) was added and the suspension was agitated. The solution was then allowed to stand for 15 min for separation of polar and non-polar layers. The polar layer, containing lycopene was obtained and the absorbance was measured using a UV visible spectrophotometer (Shimadzu Co., Ltd., Japan) at 472 nm and expressed as mg/100 g using an extinction coefficient of 17.2×10^4 mol cm^{-1} (Sadler et al., 1990).

2.5. Experimental design

The effects of five independent variables X_1 (solvent/meal ratio), X_2 (number of extractions), X_3 (temperature), X_4 (particle size) and X_5 (time), at five levels, on extracted lycopene yield (dependent variable) were investigated using central composite design (Table 1). Thirty two combinations of the independent variables, selected per experimental design for five parameters, are shown in Table 2. (Gomez & Gomez, 1984). Skin, separated from pomace by a continuous floatation-cum-sedimentation system and dried in a cabinet dryer (La Parmigiana, Italy) was ground in a mixer (Sujata, India). The ground skin was passed through standard sieves (0.05, 0.15, 0.25, 0.35 and 0.43 mm) to get a product of desired particle size. Data pertaining to five independent, and one response, variable were analyzed to get a multiple regression equation

$$Y = b_0 + \sum_{n=1}^{5} b_n X_n + \sum_{n=1}^{5} b_{mn} X_n^2 + \sum_{n<m}^{5} b_{nm} X_n X_m \quad (1)$$

where b_0 is the value for the fixed response at the central point of the experiment; and b_n, b_m and b_{nm} are the linear, quadratic and cross product coefficients, respectively.

Table 1
Independent variables and their levels used for central composite rotatable design

Independent variables	Symbol	Coded variable levels				
		−2	−1	0	+1	+2
Solvent/meal ratio (v/w)	X_1	20	30	40	50	60
Number of extractions	X_2	1	2	3	4	5
Temperature (°C)	X_3	20	30	40	50	60
Particle Size (mm)	X_4	0.05	0.15	0.25	0.35	0.43
Time (min)	X_5	4	8	12	16	20

Table 2
Central composite arrangement for independent variables X_1 (solvent/meal ratio, v/w), X_2 (number of extractions), X_3 (temperature, °C), X_4 (particle size, mm) and X_5 (time, min) and their response (lycopene yield, mg/100 g)

Run	Variables levels (uncoded)					Lycopene yield (mg/100 g)	
	X_1 (solvent/meal ratio, v/w)	X_2 (no. of extractions)	X_3 (temperature, °C)	X_4 (particle size, mm)	X_5 (time, min)	Experimental	Predicted
1	−1(30)	−1(2)	−1(30)	−1(0.35)	1(16)	1.19	1.18
2	1(50)	−1(2)	−1(30)	−1(0.35)	−1(8)	1.19	1.18
3	−1(30)	1(4)	−1(30)	−1(0.35)	−1(8)	1.13	1.13
4	1(50)	1(4)	−1(30)	−1(0.35)	1(16)	1.32	1.33
5	−1(30)	−1(2)	1(50)	−1(0.35)	−1(8)	1.04	1.04
6	1(50)	−1(2)	1(50)	−1(0.35)	1(16)	1.06	1.02
7	−1(30)	1(4)	1(50)	−1(0.35)	1(16)	1.65	1.64
8	1(50)	1(4)	1(50)	−1(0.35)	−1(8)	1.17	1.16
9	−1(30)	−1(2)	−1(30)	1(0.15)	−1(8)	0.951	0.949
10	1(50)	−1(2)	−1(30)	1(0.15)	1(16)	1.44	1.44
11	−1(30)	1(4)	−1(30)	1(0.15)	1(16)	1.38	1.40
12	1(50)	1(4)	−1(30)	1(0.15)	−1(8)	0.936	0.954
13	−1(30)	−1(2)	1(50)	1(0.15)	1(16)	1.23	1.21
14	1(50)	−1(2)	1(50)	1(0.15)	−1(8)	1.35	1.33
15	−1(30)	1(4)	1(50)	1(0.15)	−1(8)	1.98	1.97
16	1(50)	1(4)	1(50)	1(0.15)	1(16)	1.48	1.48
17	−2(20)	0(3)	0(40)	0(0.25)	0(12)	1.40	1.42
18	2(60)	0(3)	0(40)	0(0.25)	0(12)	1.25	1.26
19	0(40)	−2(1)	0(40)	0(0.25)	0(12)	1.06	1.11
20	0(40)	2(5)	0(40)	0(0.25)	0(12)	1.56	1.54
21	0(40)	0(3)	−2(20)	0(0.25)	0(12)	1.31	1.28
22	0(40)	0(3)	2(60)	0(0.25)	0(12)	1.55	1.60
23	0(40)	0(3)	0(40)	−2(0.25)	0(12)	0.639	0.677
24	0(40)	0(3)	0(40)	2(0.05)	0(12)	0.962	0.945
25	0(40)	0(3)	0(40)	0(0.43)	−2(4)	0.907	0.925
26	0(40)	0(3)	0(40)	0(0.25)	2(20)	1.16	1.17
27	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.921	0.862
28	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.892	0.862
29	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.832	0.862
30	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.862	0.862
31	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.847	0.862
32	0(40)	0(3)	0(40)	0(0.25)	0(12)	0.847	0.862

The predicted values were obtained from the regression equation and analyzed for coefficient of determination (R^2), standard error (SE) and residual plot. Analysis of the coefficients of regression models was carried out using an ANOVA technique to find the significance of each coefficient.

The process was optimized using response surface methodology for two independent variables at a time. The rest of the three parameters were fixed at zero level. The surface graphs gave values of independent variables where the response variable is the maximum, considering that all the independent variable conditions can be identified for optimum lycopene yield. The experiment is run again at the optimum level of independent variable for confirmation of the results.

2.6. Statistical analysis

A central composite design for variables was selected and the co-ordinate was given by factorial design (Cochran & Cox, 1957). Regression coefficients and ANOVA tables were computed using Minitab-11.12 (Mini Tab Inc., USA) software. Surface graphs were plotted for the predicted value of lycopene obtained from the models against two different process variables.

3. Results and discussion

3.1. Proximate analysis

The dried skin contained 5.74% moisture, 14.3% protein, 3.72% crude fat, 1.28% ash, 71.3% crude fibre and 3.46% carbohydrate. Earlier studies have reported 6.69–10% moisture, 10–10.7% crude protein, 1.7–3.96% crude fat, 1.13–5.6% ash, 46.1–55.9% crude fibre and 26.7% carbohydrate (Lazos & Kalathenos, 1988; Sogi & Bawa, 1998; Tsatsaronis & Boskou, 1975). The moisture, ash and crude fat values for the present study were in the range of previously reported values. The lycopene content of the tomato waste skin was 1.98 mg/100 g. In common varieties of tomatoes, lycopene is found at a concentration of 4–14.3 mg/100 g (Kaur et al., 2006; Nguyen & Schwartz, 1999). The lycopene content of the skin separated from

processing waste was less because it was produced from the hot break process.

3.2. Lycopene extraction

The dehydrated tomato waste skin was used for the extraction of lycopene. The extraction process was standardized for the maximum recovery of the pigment, using response surface methodology. Five independent variables X_1 (solvent/meal ratio), X_2 (number of extractions), X_3 (temperature), X_4 (particle size) and X_5 (time) were studied and their levels were selected by rotatable central composite design (Table 2). The solvent used for lycopene extraction was hexane:acetone:ethanol, in 2:1:1 ratio, containing 0.05% BHT. The experimental values of lycopene obtained with different combinations of independent variables varied from 0.639 to 1.98 mg/100 g (Table 2). Nunes and Mercadante (2004) also worked on lycopene extractions from tomato skin using four independent variables, solvent type (methanol and ethanol), volume of solvent (30, 45 and 60 ml), number of extractions (2, 3 and 4) and temperature (30, 45 and 60 °C) and their experimental values ranged from 2.55 to 9.85 mg/100 g, respectively. The lower experimental values in the present study were due to lower lycopene content of the waste skin. Sadler et al. (1990) used hexane:acetone:ethanol in 2:1:1 ratio for the extraction of carotenoids from tomato paste and pink grapefruit homogenates. The average lycopene content was 15.8 mg/l00 g for tomato paste and 3.32 mg/l00 g for grapefruit pulp. Rao, Waseem and Aggarwal (1998) extracted lycopene from tomato skin and tomato pulp with hexane:methanol:acetone in 2:1:1 ratio containing 2.5% BHT and reported lycopene recovery of 14.1 mg/100 g and 6.94 mg/100 g, respectively, on a fresh weight basis. Tan and Soderstrom (1988) recovered 25.4 mg/100 g of lycopene from tomato paste with 95% ethanol and low boiling petroleum ether (40–60 °C). These studies showed that extraction variable had an impact on lycopene recovery (Gomez-Prieto, Caja, Heerraiz, & Mariaa, 2003).

3.3. Extraction optimization

The data pertaining to the independent and response variables were analyzed to get a regression equation with linear, square and interaction coefficients as follows:

$$\begin{aligned} Y = {} & 0.8615 - 0.0384X_1 + 0.1078X_2 + 0.0805X_3 \\ & + 0.0669X_4 + 0.0619X_5 + 0.11920011X_1^2 \\ & + 0.1155X_2^2 + 0.1452X_3^2 - 0.0127X_4^2 + 0.0468X_5^2 \\ & - 0.1133X_1X_2 - 0.0687X_1X_3 - 0.0019X_1X_4 \\ & + 0.0204X_1X_5 + 0.0985X_2X_3 + 0.0019X_2X_4 \\ & + 0.0167X_2X_5 + 0.0761X_3X_4 - 0.0798X_3X_5 \\ & - 0.0204X_4X_5. \end{aligned} \quad (2)$$

The predicted values of lycopene content were calculated using the regression model and compared with experimental values. The value for the coefficient of determination (R^2) was 0.99 which indicates the adequacy of the applied model. The statistical analysis of data revealed that linear, quadratic and interaction coefficients were significant (Table 3). The ANOVA also showed that there was a non-significant ($p > 0.05$) lack of fit which further validates the model. The scattered plot between the experimental values and difference between the experimental and predicted value did not show a pattern that further indicated the adequacy of the model (Fig. 1). The levels of independent variables for optimal extraction conditions of lycopene content were determined using response surface graphs plotted between two independent variables while remaining independent variables were kept at zero level (Table 3). Variation in number of extractions showed an increase in lycopene recovery; however, increase in the solvent/meal ratio did not show any significant change in lycopene yield (Fig. 2). Maximum lycopene was obtained at a 20:1 v/w solvent/meal ratio with five extractions. The effect of solvent/meal ratio and particle size on extraction of lycopene revealed that, with increase in particle size, lycopene yield increased slightly; however, change in the solvent/meal ratio did not give any pattern while temperature, number of extractions and extraction time were fixed at 40 °C, 3 and 12 min, respectively (Fig. 3).

Table 3
Analysis of variance of independent (ANOVA) variables for extraction of lycopene from tomato waste skin

Source	Degree of freedom	*F*-value	Probability
Regression	20	69.92	0.000
Linear	5	72.22	0.000
Square	5	137.70	0.000
Interaction	10	34.88	0.000
Lack of fit	6	2.22	0.200
Pure error	5		
Total error	31		

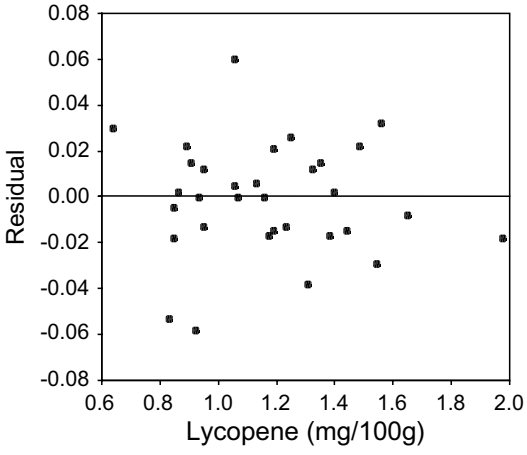

Fig. 1. Plot of residual fit of regression model for lycopene yield extracted from tomato skin.

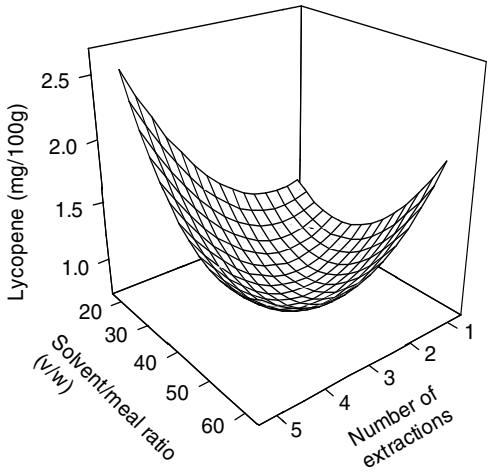

Fig. 2. Effect of solvent/meal ratio and number of extractions on lycopene yield extracted from tomato skin at 40 °C for 12 min and having particle size of 0.25 mm.

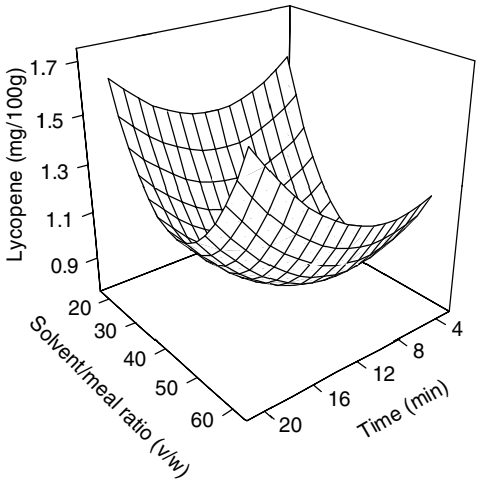

Fig. 4. Effect of extraction time and solvent/meal ratio on lycopene yield extracted from tomato skin run for three extractions at 40 °C and having particle size 0.25 mm.

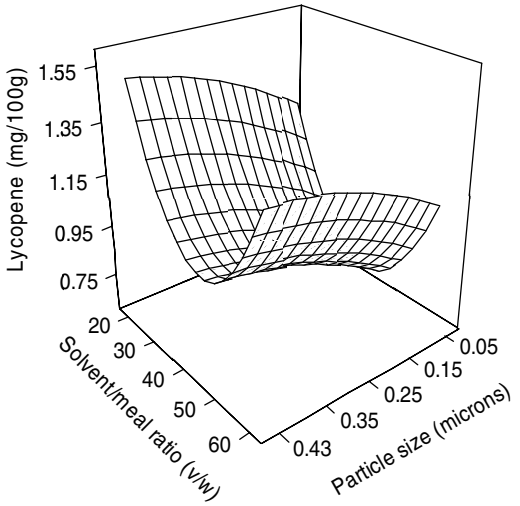

Fig. 3. Effect of solvent/meal ratio and particle size on lycopene yield extracted from tomato skin run for three extractions for 12 min at 40 °C.

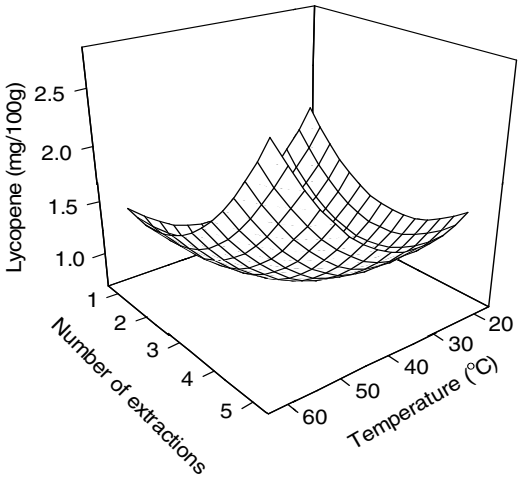

Fig. 5. Effect of number of extractions and temperature on lycopene yield extracted from tomato skin having 40:1 v/w solvent/meal ratio, 0.25 mm particle size and 12 min extraction time.

Variation in extraction time and solvent/meal ratio for lycopene extraction revealed that maximum lycopene recovery was at 20 min extraction time and a 20:1 v/w solvent/meal ratio while temperature, number of extractions and particle size were kept at 40 °C, 3 and 0.25 mm, respectively (Fig. 4). The effect of temperature and number of extractions revealed that, with increase in number of extractions and temperature, the lycopene yield increased significantly ($p < 0.05$), while solvent/meal ratio, particle size and extraction time were kept at 40:1, 0.25 mm and 12 min, respectively (Fig. 5).

The effect of particle size and number of extractions on lycopene recovery revealed that, with increase in particle size, lycopene yield did not vary significantly, while increase in number of extractions significantly ($p < 0.05$) increased lycopene yield when temperature, extraction time and solvent/meal ratio were kept at 40 °C, 12 min and 40:1 v/w, respectively (Fig. 6). Baysal et al. (2000) reported that, with increase in temperature from 35 to 65 °C during supercritical extraction, the lycopene yield increased from 7.85% to 21.9%. Similar results for lycopene extraction at higher temperatures have been reported in the literature (Yapinga et al., 2002). Variation in number of extractions and extraction time showed that lycopene recovery increased with increase in extraction time and number of extractions (Fig. 7).

Variation of temperature and extraction time revealed that lycopene recovery was maximum when temperature and extraction time were 20 °C and 20 min, respectively (Fig. 8). Tanugbodhitham, Jones, Wahlqvist and Briggs (1998) reported that an acetone/hexane mixture (4:6, v/v) was as efficient as an ethanol/hexane mixture (4:3, v/v)

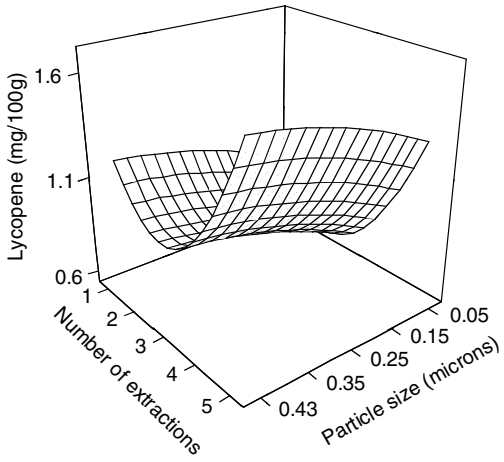

Fig. 6. Effect of number of extractions and particle size on lycopene yield extracted from tomato skin having 40:1 v/w solvent/meal ratio at 40 °C for 12 min.

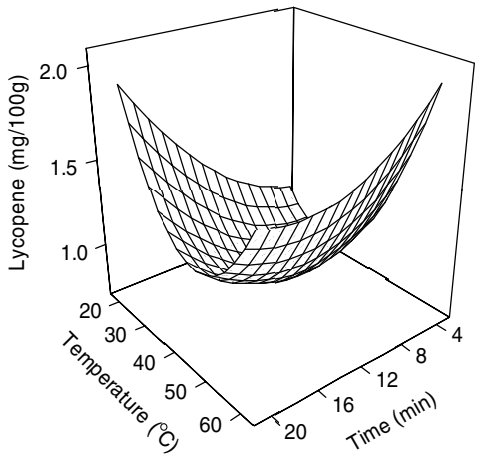

Fig. 8. Effect of temperature and extraction time on lycopene yield extracted from tomato skin run for three extractions having 40:1 v/w solvent/meal ratio and having 0.25 mm particle size.

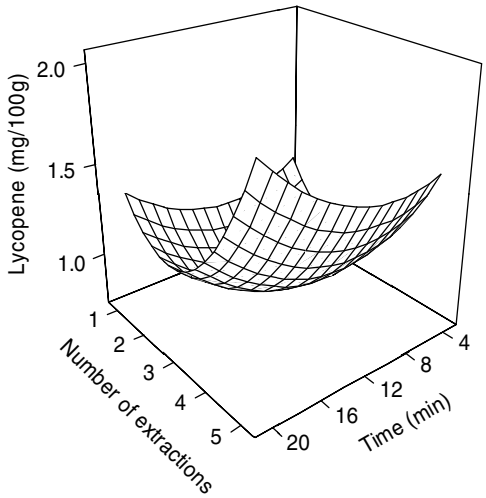

Fig. 7. Effect of number of extractions and extraction time on lycopene yield extracted from tomato skin having 40:1 v/w solvent/meal ratio at 40 °C and having 0.25 mm particle size.

whereas Lin and Chen (2003) found that a acetone/hexane mixture (3:5, v/v) led to lower lycopene extraction rates in tomato juice. A positive synergistic secondary effect was found for a hexane/ethanol (4:3, v/v) mixture which increased lycopene extraction in tomatoes; however, comparison of samples showed that the lycopene content increased significantly as a function of heat processing (Lin & Chen, 2003; Tanugbodhitham et al., 1998). Khachick (1992), observed that the total carotenoid content of raw tomato, after stewing the raw tomatoes, remained unchanged, whereas heat processing applied during the manufacture of tomato paste led to an increase in total carotenoids due to concentration; however, the qualitative distribution remained identical for raw and stewed tomatoes. Heat processing leads to a breakdown of tomato cell wall structures, disrupting chromoplast membranes and reducing cellular integrity, thus rendering several phytochemicals, including lycopene, more accessible to extraction (Shi & Le Maguer, 2000; Van Den Berg et al., 2000). The effect of particle size and extraction time revealed that maximum lycopene was obtained at 0.05 mm particle size and 20 min extraction time while solvent/meal ratio, temperature and number of extractions were 40:1 v/w, 40 °C and 3, respectively (Fig. 9).

By taking all the responses into account, it is proved that solvent/meal ratio, temperature and number of extractions had the greatest effects on the lycopene extraction from skin. Maximum lycopene was recovered using a 30:1 v/w solvent/meal ratio, four extractions, 50 °C temperature, 8 min extraction time and 0.15 mm particle size. Nunes and Mercadante (2004), optimized a method, through factorial experimental design for the lycopene extraction by

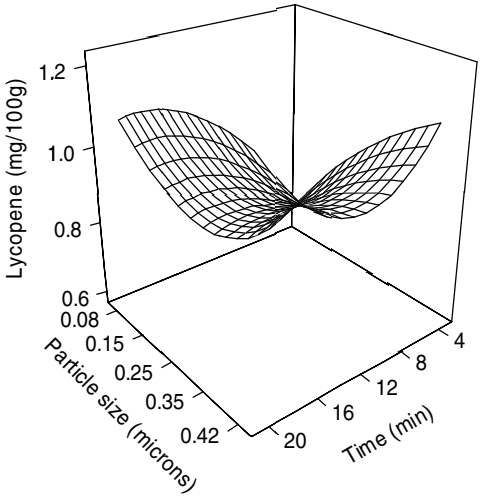

Fig. 9. Effect of particle size and extraction time on lycopene yield extracted from tomato skin run for three extractions having 40:1 v/w solvent/meal ratio at 40 °C.

four extractions of 120 min with ethyl acetate, and each extraction with a mass/volume ratio of 1:0.7. Evidently, the present study showed better conditions for extraction, with short extraction time, while the number of extractions was the same.

3.4. Confirmatory studies

The experiment was run at the optimum conditions of solvent/meal ratio 30:1 v/w, number of extractions four, temperature 50 °C, particle size 0.15 mm and extraction time 8 min. The solvent used for lycopene extraction was hexane:acetone:ethanol in 2:1:1 ratio, containing 0.05% BHT. The experimental lycopene yield at the optimum level was 1.99 mg/100 g skin and the calculated amount of lycopene yield with these parameters using the regression model was 1.97 mg/100 g. This confirmed that these conditions were optimal for lycopene extraction.

4. Conclusion

Lycopene was extracted from tomato skin following thirty two selected combinations of solvent/meal ratio, number of extractions, temperature, particle size and extraction time. The experimental value of lycopene yield (response variable) varied from 0.639 to 1.98 mg/100 g. The second order model developed for lycopene content exhibited non-significant lack of fit and a high value for the coefficient of determination (0.99). The surface graphs indicated that maximum lycopene yield was obtained by extracting tomato skin with a 30:1 v/w solvent/meal ratio, four extractions, 50 °C temperature, 0.15 mm particle size and 8 min extraction time.

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