JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Detection of Key Factors in the Extraction and Quantification of Lycopene from Tomato and Tomato Products

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The analytical process of lycopene extraction and photometrical determination was critically examined for raw tomato and processed tomato products by means of a 2_{IV}^{15-10} Plackett–Burman experimental design in order to identify the key factors (KFs) involved. Fifteen apparent key factors (AKFs) reported in the literature were selected: sample weight (X_1); volume of extraction solution (X_2); antioxidant concentration (BHT, X_3); neutralizing agent concentration ($MgCO_3$, X_4); light presence during lycopene extraction (X_5), homogenization velocity (X_6) and time (X_7), agitation time (X_8), and temperature (X_9) during the extraction process; water volume for separation of polar/nonpolar phases (X_{11}); presence of inert atmosphere throughout the process (X_{12}); time (X_{13}), temperature (X_{14}), and light presence (X_{10}) during separation of phases and time delay for reading (X_{15}). In general, higher lycopene concentrations in samples led to a higher number of key factors (KF). Thus, for raw tomato (lycopene range 1.22–2.29 mg/100 g) no KF were found, whereas for tomato sauce (lycopene range from 5.80 to 8.60 mg/100 g) one KF (X_4) and for tomato paste (lycopene range from 35.80 to 51.27 mg/100 g) five KFs (X_1, X_2, X_4, X_{11}, and X_{12}) were detected. For lycopene paste, X_1 and X_2 were identified as the KFs with the greatest impact on results, although in fact the X_1/X_2 ratio was the real cause. The results suggest that, with increased processing, the physical and chemical structure of lycopene becomes less important since the identified KFs explain almost 90% of variability in tomato paste but only 32% in raw tomato.

KEYWORDS: Lycopene; tomato; tomato products; Plackett–Burman experimental design

INTRODUCTION

Lycopene is the major carotenoid present in tomatoes and is recognized a major dietary antioxidant with beneficial effects for human health. The role of lycopene in the prevention of cardiovascular diseases and several types of cancer has been widely reported in the literature (*1–3*). The tomato industry is therefore particularly interested in planting tomato varieties with higher lycopene content, in developing industrial processes to increase lycopene content in tomato products, and in including lycopene content as an added value on labels for products such as tomato sauce, juice, soup, and ketchup. Lycopene content varies considerably as a function of tomato variety (generally due to genetic factors), maturity, and both agricultural and weather conditions during growth (*4–6*): food-industry control quality departments thus need to implement an analytical method for checking lycopene content in order to provide true information on product labels. Since HPLC-based methods for carotenoid analysis are both expensive and time-consuming, faster analytical methods have been developed on the basis of certain color parameters of tomato and tomato products. Although rapid, inexpensive, and requiring no hazardous chemicals, these methods are not accurate. An alternative technique is spectrophotometric analysis of lycopene, which is faster and less expensive than chromatographic methods and more sensitive that color parameter based methods (*2, 7*).

A previous paper has addressed the optimization of the solvent mixture for extracting lycopene from tomato and tomato products (*7*). In addition to the need for an optimal extraction mixture, several factors appear to affect considerably the extraction and determination of lycopene; as a result, a number of general recommendations have been made for working with carotenoids. Detrimental effects, especially trans/cis isomerization, should be minimzed by working under a vacuum or with an inert gas (such as nitrogen or argon) instead of a normal atmosphere. All analytical operations should be carried out at room temperature or below and in diffuse daylight or subdued

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10.1021/jf0705623 CCC: $37.00
Published on Web 10/09/2007

Table 1. Moisture, Soluble Solids, and pH in Raw Tomatoes, Tomato Sauce, and Tomato Paste[a]

sample	moisture (%)	SS[b] (%)	pH
raw tomato	94.5	4	4.24
tomato sauce	82.97	13	4.25
tomato paste	76.21	29	4.14

[a] Mean values of three measurements. [b] Soluble solids.

artificial light. The addition of antioxidants and neutralizing agents may be advantageous (*8*). On the basis of this information, 15 apparent key factors (AKFs) were identified that might influence lycopene extraction and determination. However, published analytical techniques vary with regard to the specification of these AKFs. Standardization of these methods would appear useful in enabling comparison of results obtained in different laboratories.

The lycopene extraction and quantification method outlined here consists of two consecutive steps: AKFs screening, followed by optimization of AKFs found to have a significant effect on sensitive analysis. The AKFs that are really important to the analytical procedure are called key factors (KFs). These are critical not only for the efficient extraction of lycopene from the food matrix but also for sensitive and efficient quantification. In a previous experiment, $2^{(k-p)}$ fold-over Placket–Burman experimental designs were found to be effective for screening analytical methods and procedures (*10*), in that they determine exactly which of the apparent factors actually have a significant effect on the dependent variable or response in question (*9*). In the second step, once KFs have been identified, lycopene extraction and quantification can be optimized using response surface methodology (RSM), a collection of statistical and mathematical techniques for developing, improving, and optimizing processes (*11*). This second step will be addressed by future research and discussed in a later paper. The aim of the present study was to screen apparent key factors (AKFs) and to determine the key factors (KFs) which might affect lycopene extraction and quantification in tomato and tomato products, using a $2^{(k-p)}$ fold-over Placket–Burman experimental design.

MATERIALS AND METHODS

Materials. Samples used were raw tomatoes (commercial variety: Canario, size 57/67, first class), tomato sauce, and tomato paste, all purchased in a local supermarket. On the day of purchase, raw tomatoes were cleaned, homogenized, and stored at −80 °C in plastic bottles until analysis. Common brands of tomato sauce and tomato paste were sampled directly from the containers. Prior to lycopene analysis, soluble solids, moisture, and pH were analyzed in all samples (**Table 1**).

Methods. *Experimental Design.* Previous research has shown that Placket–Burman experimental designs provide an effective way of screening analytical methods and procedures (*10*), identifying the best experimental approach using a minimum number of experimental runs, based on Hadamard matrices, in which the number of experimental runs is a multiple of four. For lycopene extraction and determination, a 2_{III}^{15-11} Plackett–Burman experimental design (*11*) was used, enhancing design resolution via fold-over (2_{IV}^{15-10}) following Box and Hunter's criteria (*12*). By obtaining resolution IV designs, main effects are no longer confused with the two-way interaction, but some two-way interactions are aliased with other two-way interactions.

According to published data and methods for lycopene determination, 15 potential or apparent key factors (AKFs) were identified, and coded X_i, where $i = 1$–15 (**Table 2**). Transformation of natural variables is advisable, and the coded variables are usually defined as dimensionless with mean zero and the same spread or standard deviation (*11*). The AKFs selected were as follows: sample weight (X_1); volume of extraction solution (X_2); antioxidant concentration (BHT, X_3); neutralizing agent concentration ($MgCO_3$, X_4); light presence during lycopene extraction (X_5), homogenization velocity (X_6) and time (X_7), agitation time (X_8), and temperature (X_9) during extraction process; water volume for separation of polar/nonpolar phases (X_{11}); presence of inert atmosphere throughout the process (X_{12}); time (X_{13}), temperature (X_{14}), and light presence (X_{10}) during separation of phases; and time delay for reading (X_{15}).

Table 2. Apparent Key Factors (AKFs) Considered for the Two-Level Fold-Over Experimental Design at Resolution IV, Codes, and Experimental Ranges

		levels	
AKF	code	−1	+1
sample weight (g)	X_1	1	2
volume of extraction solution (mL)	X_2	50	100
BHT concentration (%, w/v)	X_3	2.5	5
$MgCO_3$ concentration (%, w/v)	X_4	5	10
presence of light during extraction	X_5	no	yes
homogenization velocity (rpm)	X_6	3000	6000
homogenization time (s)	X_7	30	60
extraction time (min)	X_8	30	60
extraction temperature (°C)	X_9	10	22
presence of light during phase separation	X_{10}	no	yes
water volume (mL)	X_{11}	10	20
inert atmosphere with N_2	X_{12}	no	yes
phase separation time (min)	X_{13}	5	10
separation temperature (°C)	X_{14}	10	20
time delay for reading (min)	X_{15}	5	30

Table 2 shows the codes for each of the AKFs considered and their experimental design levels. Trials were run in a random order (trial order) to avoid erroneous conclusions due to extraneous sources of variability introduced by the experimenter (*13, 14*). The random order of the runs is shown in **Table 3**.

Lycopene Extraction. A sample amount (X_1, 1 or 2 g) of raw tomatoes, tomato sauce, and tomato paste was weighed into a 125 mL flask. Then $MgCO_3$ (X_4) was added to give final concentrations of 5% or 10% according to the appropriate volume of the extraction mixture (X_2, 50 or 100 mL), which was also added. The optimal proportions of hexane, acetone, and ethanol in the extraction mixture had previously been determined for each type of sample (*7*) and were as follows: hexane/acetone/ethanol 47.8/15.1/37.1 (v/v/v) for raw tomato, 59.1/6.1/34.8 (v/v/v) for tomato sauce, and 58.7/18.1/23.1 (v/v/v) for tomato paste. The respective mixtures were prepared with butylated hydroxytoluene (BHT, X_3) as antioxidant, in two concentrations (2.5% and 5% w/v) to prevent oxidation processes during analysis. Samples were homogenized with an Omni-mixer (Giralt, International Waterbury, CT) at different speeds (X_6, 3000 or 6000 rpm) and times (X_7, 30 or 60 s). Mixtures were then shaken for 30 or 60 min (X_8) with a magnetic stirrer at 5000 rpm at different temperatures (X_9, 10 and 22 °C) in the presence or absence of light (X_5) to extract lycopene from the food matrix. Volumes of distilled water (X_{11}, 10 or 20 mL) were added, and solutions were left to separate into a polar and a nonpolar layer for 5 or 10 min (X_{13}) at different temperatures (X_{14}, 10 or 22 °C) in the presence or absence of light (X_{10}). The nonpolar layer containing lycopene was collected, and total lycopene content was obtained by measuring absorbance at 472 nm (*15, 16*), considering as AKF the time delay until reading (X_{15}, 5 or 30 min). Atmospheric air was replaced by N_2 in the trials with $X_{12} = +1$ to determine the effect of aerial oxygen. Lycopene concentration was determined using the molar extinction coefficient of lycopene in hexane at 472 nm ($E^{1\%}$1cm 3450). All analyses were performed in triplicate.

Sample Chemical Parameters. Moisture content was analyzed in all samples by oven-drying at 105 °C to constant weight. pH was determined using a Crisson 2000 pH meter (Barcelona, Spain), and soluble solids were quantified in homogenized samples using a Leica Abbe Mark II refractometer (Buffalo, NY) (*17*).

Analysis of Lycopene Isomers by HPLC. The lycopene content of the tomato samples was analyzed as described previously (*18*) with a C_{30} column (250 × 4.6 mm, 5 μm, Trentec, Gerlingen, Germany) at 17 °C and with the diode array detector set at 450 nm (*19*). The lycopene isomer content of the samples was quantified comparing peaks

Table 3. Experiment 2^{15-10}IV and Responses Values for Raw (R_1), Salse (R_2), and Concentrated (R_3) as mg of Lycopene/100 g Sample[a]

run	trial	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	R_1	R_2	R_3
13	1	−1	−1	1	1	1	−1	−1	−1	−1	1	1	1	−1	−1	1	1.99	7.06	40.08
22	2	−1	1	−1	1	1	−1	1	1	−1	1	1	−1	1	−1	−1	2.01	7.03	47.8
2	3	1	−1	−1	−1	−1	−1	−1	1	1	1	1	1	1	−1	−1	1.6	5.8	37.48
5	4	−1	−1	1	−1	1	−1	1	−1	1	−1	1	−1	1	1	−1	1.6	6.32	42.34
28	5	−1	−1	1	−1	−1	1	−1	1	−1	1	1	−1	1	1	1	1.52	6.33	43.38
4	6	1	1	−1	−1	1	−1	−1	−1	−1	1	−1	−1	1	1	1	2.29	6.52	44.12
12	7	1	1	−1	1	1	−1	1	−1	1	−1	−1	1	−1	−1	−1	1.89	6.62	41.69
20	8	−1	−1	1	1	−1	1	1	1	1	−1	1	1	−1	−1	−1	1.96	6.22	39.02
29	9	1	1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	1	−1	2.02	6.72	43.27
32	10	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1.65	6.28	43.58
17	11	1	1	1	1	−1	−1	−1	−1	−1	−1	1	1	1	1	−1	1.68	6.49	39.86
9	12	−1	−1	−1	1	1	1	−1	1	−1	−1	−1	1	1	1	−1	1.76	6.01	43.02
3	13	−1	1	−1	−1	−1	1	1	−1	−1	1	1	1	−1	1	−1	1.25	6.43	49.91
15	14	−1	1	1	1	−1	−1	−1	1	1	1	−1	−1	−1	1	−1	1.85	6.76	51.27
1	15	−1	−1	−1	−1	1	1	1	1	1	1	−1	−1	−1	−1	1	1.79	6.39	41.38
7	16	−1	1	1	−1	−1	−1	1	1	−1	−1	−1	1	1	−1	1	1.69	6.69	49.4
21	17	1	1	−1	1	−1	1	−1	1	−1	1	−1	1	−1	−1	1	1.22	6.51	41.54
25	18	1	1	1	−1	−1	−1	1	−1	1	1	1	−1	−1	−1	1	1.56	6.49	41.18
30	19	−1	1	−1	−1	1	−1	−1	1	1	−1	1	1	−1	1	1	1.53	6.46	45.76
6	20	1	−1	1	−1	−1	1	−1	−1	1	−1	−1	1	−1	1	1	1.54	6.05	39.48
16	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.43	6.36	37.64
23	22	1	−1	−1	1	1	1	−1	−1	1	1	1	−1	−1	1	−1	1.54	6.26	38.13
27	23	1	−1	1	−1	1	−1	1	1	−1	1	−1	1	−1	1	−1	1.43	6.16	37.89
31	24	1	−1	−1	−1	1	1	1	−1	−1	−1	1	1	1	−1	1	1.98	6.28	37.9
8	25	1	1	1	−1	1	1	−1	1	−1	−1	1	−1	−1	−1	−1	1.38	7.56	41.97
11	26	−1	1	−1	1	−1	1	−1	−1	1	−1	1	−1	1	−1	1	1.39	8.21	44.66
18	27	−1	1	1	1	1	1	1	−1	−1	−1	−1	−1	−1	1	1	1.63	8.29	48.24
10	28	1	−1	−1	1	−1	−1	1	1	−1	−1	1	−1	−1	1	1	1.96	7.6	35.8
24	29	−1	−1	−1	1	−1	−1	1	−1	1	1	−1	1	1	1	1	2.02	8.03	39.97
14	30	1	−1	1	1	−1	1	1	−1	−1	1	−1	−1	1	−1	−1	1.55	7.56	37.38
19	31	1	−1	1	1	1	−1	−1	1	1	−1	−1	−1	1	−1	1	1.24	7.19	40
26	32	−1	1	1	−1	1	1	−1	−1	1	1	−1	1	1	−1	−1	1.42	8.6	48.16

[a] Mean values of three measurements.

to those of authentic standard (*all-E*)-lycopene (Carotene Nature, Switzerland). As lycopene isomer standards were not available, (*Z*)-lycopene isomer peaks were quantified by comparison with (*all-E*)-lycopene peaks.

RESULTS AND DISCUSSION

Table 3 shows the full 2^{15-10}IV experiment design and the amounts of lycopene extracted (expressed as mg/100 g fresh matter) from raw tomatoes, tomato sauce, and tomato paste for each of the 32 experimental conditions assayed. The "trial" column shows the randomized order in which the experiments were carried out, while the "run" column shows the formal or systematic order used to obtain the experimental design. Randomization by this means is essential to ensure that the average influence of noise factors, such as environmental factors, is lessened (*14*).

Lycopene concentrations increased with tomato processing, being significantly higher in tomato paste than tomato sauce and raw tomato. This behavior is widely known since heat processing of tomato is characterized by water reduction and an increase in total solids (**Table 1**). The results obtained ranged from 1.22 to 2.29 mg of lycopene/100 g in raw tomato, from 5.80 to 8.60 mg of lycopene/100 g in tomato sauce, and from 35.80 to 51.27 mg of lycopene/100 g in tomato paste (**Table 3**).

The effect of AKFs on lycopene extraction, and their statistical significance, are listed in **Table 4**. As previously established (*10*), a conventional criterion used successfully in the screening phase is to consider that the AKF has a strong effect when $p \leq 0.150$; this criterion was therefore used to identify the real KFs. Interestingly, as the lycopene concentration in samples increased, and industrial processing was more intense, the number of key factors (KF) detected was higher.

Table 4. Effects of Coded Factors on Each Response in Terms of Lycopene Extracted and Determined for Raw Tomato (R_1), Tomato Sauce (R_2), and Tomato Paste (R_3), and Statistical Significance Obtained for Each Value[a]

	responses					
	R_1		R_2		R_3	
factor	effect	$p^{(2)}$	effect	$p^{(2)}$	effect	$p^{(2)}$
X_1	−0.431	0.672	−1.188	*0.252*	−7.972	0.000
X_2	−0.511	0.616	1.491	0.155	7.683	0.000
X_3	−1.396	0.181	0.716	0.484	0.123	0.903
X_4	0.499	0.624	1.712	0.106	−2.035	0.058
X_5	0.258	0.799	0.226	0.824	−0.102	0.919
X_6	−1.499	0.156	0.548	0.591	−0.303	0.766
X_7	1.246	0.230	0.264	0.794	−1.126	0.276
X_8	−0.339	0.739	−1.370	0.189	−0.006	0.995
X_9	−0.350	0.730	−0.077	0.939	−1.007	0.329
X_{10}	−0.247	0.808	−0.168	0.686	0.127	0.900
X_{11}	−0.350	0.730	−0.837	0.415	−2.651	0.017
X_{12}	−0.347	0.739	−1.380	0.186	−1.514	0.149
X_{13}	0.592	0.562	0.721	0.481	−0.052	0.959
X_{14}	0.419	0.680	−0.889	0.386	0.662	0.517
X_{15}	0.109	0.914	0.875	0.394	−1.181	0.254
R^2	0.322		0.468		0.898	

[a] AKFs with $p < 0.150$ were considered significant and therefore declared as KFs.

For raw tomato (lycopene range 1.22–2.29 mg/100 g) no KFs were found. Therefore, in raw samples (R_1, **Table 4**) analysis may be performed using any value included in the experimental range considered for each AKF (**Table 2**) with no significant differences in results. For tomato sauce (lycopene range from 5.80 to 8.60 mg/100 g), one KF (X_4) was identified, while for tomato paste (lycopene range from 35.80 to 51.27 mg/100 g), five KFs (X_1, X_2, X_4, X_{11}, and X_{12}) were detected.

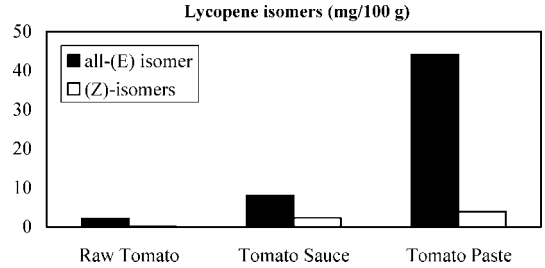

Figure 1. Content of (*all-E*)-lycopene and (*Z*)-lycopene in raw tomato, tomato sauce, and tomato paste, measured by HPLC and expressed as mg/100 g sample.

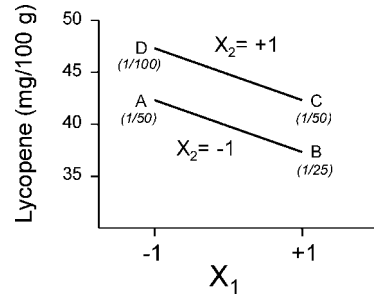

Figure 2. Lycopene responses measured in tomato paste as a function of both sample weight (X_1) and volume of extraction solution (X_2). Letters represent lycopene concentrations at different ratios of X_1/X_2 of 42.38, 37.22, 42.20, and 47.36 mg/100 g for points A, B, C, and D, respectively. The X_1/X_2 ratio is specified below the letter.

The addition of $MgCO_3$ (X_4) was the only KF identified for both tomato sauce and tomato paste but was not found to be significant for raw tomato. The addition of $MgCO_3$ is recommended to neutralize the fruit acids released during homogenization of raw tomato. These fruit acids are reported to have general detrimental effects on lycopene; for example, they have been found to catalyze the furanoid rearrangement of epoxids (*8*). Interestingly, addition of $MgCO_3$ did not influence lycopene extraction from raw tomato, the sample type with the highest organic acid content. Moreover, for tomato sauce (R_2, **Table 4**), $MgCO_3$ (X_4) addition had a positive effect (1.712, $p = 0.106$). This means that more lycopene was extracted with 10% $MgCO_3$ than with 5% $MgCO_3$. By contrast, in tomato paste samples (R_3, **Table 4**) the effect was similar in intensity but negative (-2.035, $p = 0.058$), indicating better extractability of lycopene with 5% than with 10% $MgCO_3$. These apparently contradictory results may be accounted for by the different chemical forms of lycopene in the samples investigated here.

Homogenization and heat treatment prompt changes in the chemical and physical structure of lycopene and govern the intra- or extracellular location of lycopene in the food matrix. The current view is that heat treatment promotes (all-*E*/*Z*)-isomerization, increasing the proportion of lycopene (*Z*)-isomers. A significant conversion from (all-*E*)-lycopene to (*Z*)-isomers has been reported (*20–23*), the kinetics increasing with rising temperatures and processing times (*2*). The proportion of (all-*E*)-lycopene in tomato foodstuffs varies from 96% in preserved tomato paste to 35% in a long-term cooked spaghetti sauce prepared from canned tomato (*24*). Furthermore, (*Z*)-isomers are more readily oxidized than (all-*E*)-lycopene (*25*). In addition, a very low moisture content and low water activity seem to favor oxidative degradation in tomato products (*26*).

The tomato sauce and tomato paste were produced using different raw materials and industrial processes and therefore had different chemical compositions (**Table 1**). Tomato sauce had higher water content (**Table 1**), contained sunflower oil, corn starch, and salt, and was processed by heat sterilization. However, the only ingredient in tomato paste was tomato concentrated by evaporation to reduce the initial water content of raw tomato by one-fifth: moisture content was therefore lower (**Table 1**). Variations in (*Z*)-isomers and oxidation products of lycopene are to be expected in products that have undergone different processing times and temperatures during heat treatment.

To determine the chemical forms of lycopene in the various samples tested, total lycopene isomer content (*all-E* and *Z*) was measured by HPLC in three samples (**Figure 1**). Lycopene from raw tomatoes was largely in the form of (all-*E*)-lycopene, whereas tomato sauce and tomato paste displayed higher (*Z*)-isomer content. The content for the two chemical forms was 2.1 and 0.06 mg/100 g for raw tomato, 8.03 and 2.25 mg/100 g for tomato sauce, and 44.86 and 3.92 mg/100 g for tomato paste. The proportion of (*Z*)-isomers was roughly 3%, 28%, and 9% for raw tomato, tomato sauce, and tomato paste, respectively. Addition of $MgCO_3$ would appear to have a positive effect on lycopene extraction in samples with higher (*Z*)-isomer content, preventing greater oxidation, since (*Z*)-isomers are usually more sensitive to the oxidative process than (*all-E*)-lycopene (*8, 25*).

Addition of $MgCO_3$ to the sample modifies the pH of the extraction solution, showing that pH appears to play a major role during the lycopene extraction process. This hypothesis is borne out by the fact that this is the only KF determined in two of the three samples. For this reason, the effect of pH changes on lycopene extraction will be tested in future research (particularly in the optimization phase).

The most important KFs for tomato paste (R_3, **Table 4**) were sample weight (X_1) and volume of extraction mixture (X_2). The effects obtained for these two variables were quantitatively similar, but with opposite signs (values of -7.972 and 7.683, respectively, **Table 4**). The simple conclusion of an antagonist effect and hence a relationship between the two factors has to be rejected, however, as it is not supported by the results, which show no interaction (**Figure 2**). The true KF is, therefore, the linear combination of both KFs, and more precisely the X_1/X_2 ratio, given that similar ratios of sample weight to volume of extraction mixture (points A and C in **Figure 2**) produced similar results for lycopene, while a lower X_1/X_2 ratio (1:100, point D in **Figure 2**) yielded the highest lycopene value. The results showed that the extraction rate was simultaneously dependent on X_1 and X_2, since extraction yield increased with decreasing sample weight (X_1) and increasing solvent volume (X_2). However, this effect was only observed for tomato paste, so X_1 and X_2 are only considered to be critical in samples with high lycopene content. This might be due to the fact that lycopene is almost insoluble in the ethanol forming part of the extraction mixture, so a greater volume of extraction mixture might facilitate the solubility of high lycopene concentrations. However, these findings are in contrast to those described by Van den Berg et al. (*27*), who reported that a smaller volume of organic solvents is preferable for the extraction of carotenoids from plant foods, although no reasons are given.

The other KFs identified for lycopene extraction from tomato paste were the volume of water added to facilitate phase separation (X_{11}) and the use of N_2 (X_{12}). The effect was in both cases negative (values of -2.651 and -1.514, respectively). So, a higher volume of water reduces the lycopene concentration, probably because oxidized products of lycopene exhibit slightly hydrophilic properties and could thus move to the polar phase containing ethanol, acetone, and water, which is discarded. The negative effect of N_2 may be ascribed to the chemical appearance of lycopene in tomato paste, with a considerable amount

of (*Z*)-isomers. Thus, an inert atmosphere becomes less important in the extraction of lycopene from processed tomato products.

The responses obtained prompt the hypothesis that as the degree of processing increases, the extraction of lycopene depends increasingly less on the physical and chemical structure of lycopene, since the identified KFs account for almost 90% of the variation in the results in tomato paste, 47% in tomato sauce, and only 32% in raw tomato.

In raw tomato, lycopene is located in the chromoplasts (*27*, *28*) where it appears as crystals, needlelike structures, or oily droplets, depending on the tomato variety or cultivar (*18*, *25*). The chromoplast membrane is intact, and extraction and solubilization of lycopene thus depend on the physical form of lycopene (*25*, *27*). But this natural physical structure is modified by processing; cellular structures are destroyed, and as well as (all-*E*)-lycopene *Z*-isomers and oxidation products of lycopene also appear. Processing (e.g., homogenization) ruptures cell structures and releases lycopene (*28*), which enhances lycopene extraction from the food matrix. According to Seybold et al. (*18*), the pressure of 70 bar leads to destruction of carotenoid–protein complexes and chromoplast membranes, leading to carotenoid degradation by oxygen and high temperatures. Intensified temperature and time conditions facilitate extraction. and the isomers and oxidized forms thereby occurring are better soluble in solvents (*2*). Thus physical form becomes less important.

It may be concluded that, of all the AKFs studied, sample weight, neutralizing agent concentration ($MgCO_3$), and water volume for separation of polar/nonpolar phases can be considered KFs in the extraction and spectrophotometric quantification of lycopene in raw tomato and tomato products. These factors will therefore be considered in future research to optimize the analytical procedure using response surface methodology.

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Received for review February 26, 2007. Revised manuscript received August 28, 2007. Accepted September 6, 2007. The authors are grateful for the financial support of the projects LYCOCARD-2006-016213 (VI FP EU) and CGL2006-26965-E/ALI (Spanish Government) and to the "Fundación Seneca", Murcia Regional Government, for the grant for K. Jacob.

JF0705623