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Thermal Stability Of Tomato Lycopene In Vegetable Oils



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ABSTRACT

The thermal stability of lycopene in vegetable oil extracts was investigated in the temperature range 60-75°C. Extracts were prepared by contacting ripe tomato skins with three commercial vegetable oils: rice bran oil (RBO), grape seed oil (GSO) and sunflower seed oil (SSO). The results obtained indicate that the stability of lycopene in all three oils is extremely low. A kinetic analysis of degradation rate data showed that thermal degradation follows first-order kinetics, with apparent activation energies varying from 59.6 kJ mol⁻¹ (in SSO) to 108.9 kJ mol⁻¹ (in RBO). At 60°C lycopene degrades more slowly in RBO, the oil with the lower degree of unsaturation and the higher content of antioxidants, while at higher temperatures differences among RBO, GSO and SSO tend to disappear. This could be the result of the combined influence of unsaturated triacylglycerols, tocopherols and tocotrienols on the anti-oxidant/pro-oxidant properties of the oil. © 2006 Trade Science Inc. - INDIA

KEYWORDS

Lycopene;
Carotenoids;
Vegetable oils;
Degradation kinetics;
Activation energy;
Thermal stability.

INTRODUCTION

In recent years lycopene, the major carotenoid pigment found in ripe tomato fruits and responsible for their characteristic red colour, has been the focus of considerable attention for its potential health benefits^[1]. Results from *in-vitro* studies and a growing body of clinical evidence support the view that

lycopene may provide protection against cardiovascular disease and certain types of cancer^[2,3]. Beneficial effects are thought to be primarily related to its powerful antioxidant properties, but other mechanisms such as modulation of intercellular gap-junctional communication, hormonal and immune system have also been invoked^[4,5].

Structurally, lycopene (Ψ, Ψ -carotene) is a highly

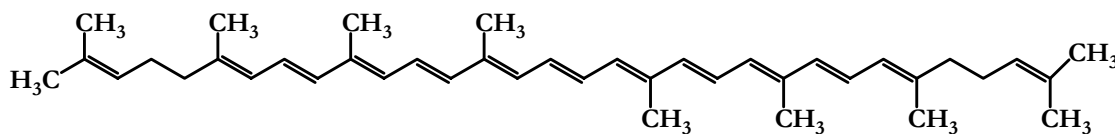


Figure 1: Molecular structure of lycopene

unsaturated aliphatic hydrocarbon containing 13 carbon-carbon double bonds, 11 of which are conjugated (Figure 1). In nearly all fruits and vegetables lycopene occurs in the all-trans configuration, the most thermodynamically stable form. The extensive conjugation is the key to its antioxidant activity as well as to its limited stability. Light, heat, oxygen, extremes of pH and metallic ions are the main factors responsible for degradation. In their presence lycopene undergoes trans-to-cis isomerization and oxidation, both causing an activity loss^[6,7].

Available evidence seems to indicate that, as long as lycopene is retained inside the cell structures, degradation is significantly reduced. The protection provided by the cellular environment helps explain why during typical food processing of tomatoes and related products the lycopene content remains essentially unchanged^[8-10]. By contrast, when lycopene is released from the cell interior degradation proceeds at a faster rate^[11].

Vegetable oils have been proposed as effective means to favour extraction of lycopene from tomatoes. Vasapollo et al.^[12] showed that about 60% of the total lycopene could be extracted using supercritical CO₂ at 65-70°C and hazelnut oil as co-solvent. Similar results were obtained by Shi^[13], who subjected a mixture of tomato powder and soybean oil to supercritical CO₂ extraction. In another process, tomato-based raw materials were treated with hot steam in the presence of sunflower oil, and the extract was saponified to yield a lycopene-rich food additive^[14]. A multiphase mixture consisting of water, soybean oil and ethanol was used by Kagan and Braun^[15] to extract lycopene from tomatoes. From this process, operating at temperatures ranging from 60 to 70°C, a product with increased bioavailability and adequate for human consumption was obtained.

In the processes described above lycopene, once extracted and solubilized in the oil, is exposed to

potentially harmful temperatures without the protection of the plant tissue. In such conditions degradation is expected to be large, because it occurs in an environment where the reactivity of the pigment is maximal. This is indeed what is suggested by the limited experimental information available^[7,16-19]. Studies, however, were mainly performed on model systems, using lycopene of chemical grade and vegetable oils free of endogenous antioxidants. Under real process conditions the situation may be quite different, due to the presence of other tomato components, such as β -carotene, phytosterols and tocopherols, and to the fact that vegetable oils usually contain antioxidants. All of these components could, in principle, affect the pathway of degradation and, hence, the stability of the pigment.

In view of the above considerations, we investigated the thermal behaviour of lycopene in tomato extracts obtained using vegetable oils as solvents. The purpose of this study was twofold: (1) to provide a quantitative kinetic description of lycopene degradation at temperatures close to those used in the industrial practice; and (2) to highlight the effect of the type of vegetable oil on the stability of lycopene.

Attention was focused on three commercial oils: rice bran oil (RBO), grape seed oil (GSO) and sunflower seed oil (SSO). They were selected for their differences in composition and for the interest in their potential use as a base for preparing lycopene-enriched functional foods. Lycopene extracts were obtained from ripe tomato skins, the richest natural source of lycopene and a waste byproduct of the tomato processing industry.

EXPERIMENTAL

Materials

Fresh ripe tomatoes of the commercial variety

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Roma were obtained from a local market and stored at 4°C for a maximum of 2 days before use.

RBO and SSO were purchased from a grocery store. GSO was from ACEF (Italy). The three oils were stored at room temperature in the dark and used without further treatment or purification.

Preparation of vegetable oil extracts

Lycopene was extracted from the skin of tomatoes. After removal of damaged parts and washing, whole tomato fruits were immersed in boiling water for 1-2 min. Then they were cooled under tap water and hand peeled. The peels were left in air to dry for a few hours and stored at 4°C.

Extracts were prepared by contacting 10 g of the partially dehydrated tomato peels with 100 mL of the vegetable oil into magnetically stirred flasks. The flasks were maintained at room temperature in the dark for 24-48 h. After this time the content of each flask was centrifuged at 5000 rpm × 15 min to allow separation of the oil from the residual plant material. The liquid was then analysed for lycopene content. Oil extracts were used as such or diluted with appropriate amounts of pure vegetable oil.

Lycopene assay

Lycopene concentration in the oil extracts was determined spectrophotometrically. Absorption spectra were recorded in the wavelength range 350-600 nm using a double-beam UV-VIS spectrophotometer (Perkin-Elmer Lambda 25) and quartz cells of 1-cm path length. For quantitative determinations, a molar extinction coefficient of $1.585 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ was assumed^[20].

Thermal stability studies

Thermal degradation experiments were carried out in magnetically stirred jacketed glass vessels (60-mL working volume) connected to a circulating water thermostat. The temperature was controlled within $\pm 0.1^\circ\text{C}$.

In a typical run about 40 mL of pure vegetable oil were charged into the vessel and allowed to equilibrate at the desired temperature. Then a small amount (2-5 mL) of the lycopene-containing oil extract was added to the thermally conditioned oil. At selected times, aliquots were withdrawn and

analysed for lycopene content. All measurements were performed at least in duplicate and the results were averaged.

The temperatures investigated were 60, 70 and 75°C, and the initial lycopene concentration was varied between about 1 and 10 mM.

RESULTS AND DISCUSSION

Visible absorption spectra of tomato extracts in sunflower, rice bran and grape seed oils were all similar in shape and showed the three characteristic peaks of lycopene in the 400-550 nm range (Figure 2).

Upon heating, a progressive reduction in

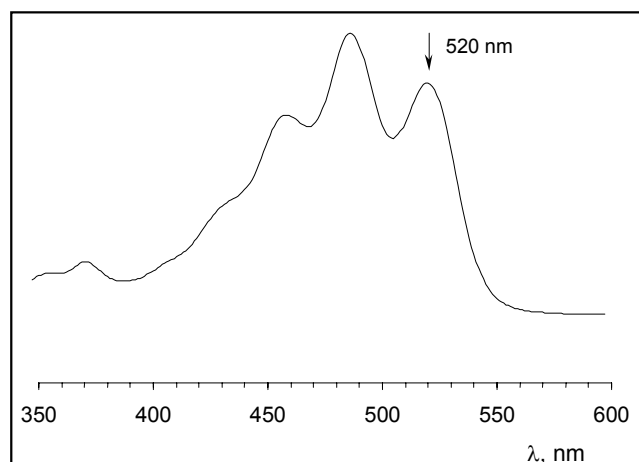


Figure 2: Absorption spectrum of lycopene in GSO

absorbance was observed. The changes were largely irreversible and were attributed to thermal degradation of lycopene^[6]. To follow the kinetics of this phenomenon we monitored, at constant temperature, the absorbance decay at 520 nm. We selected this wavelength, although not associated with the highest peak, to minimise interference from other carotenoids^[21]. Data points were taken within the first minutes, and initial rates were calculated from the slope of the absorbance-time plots.

For all three oils, degradation rates were found to be linearly related to the concentration of undegraded lycopene, and increased as the temperature was raised (Figure 3). Accordingly, the kinetic data were interpreted by a first-order rate expression:

$$r = kc \quad (1)$$

where r is the degradation rate, k is the first-order

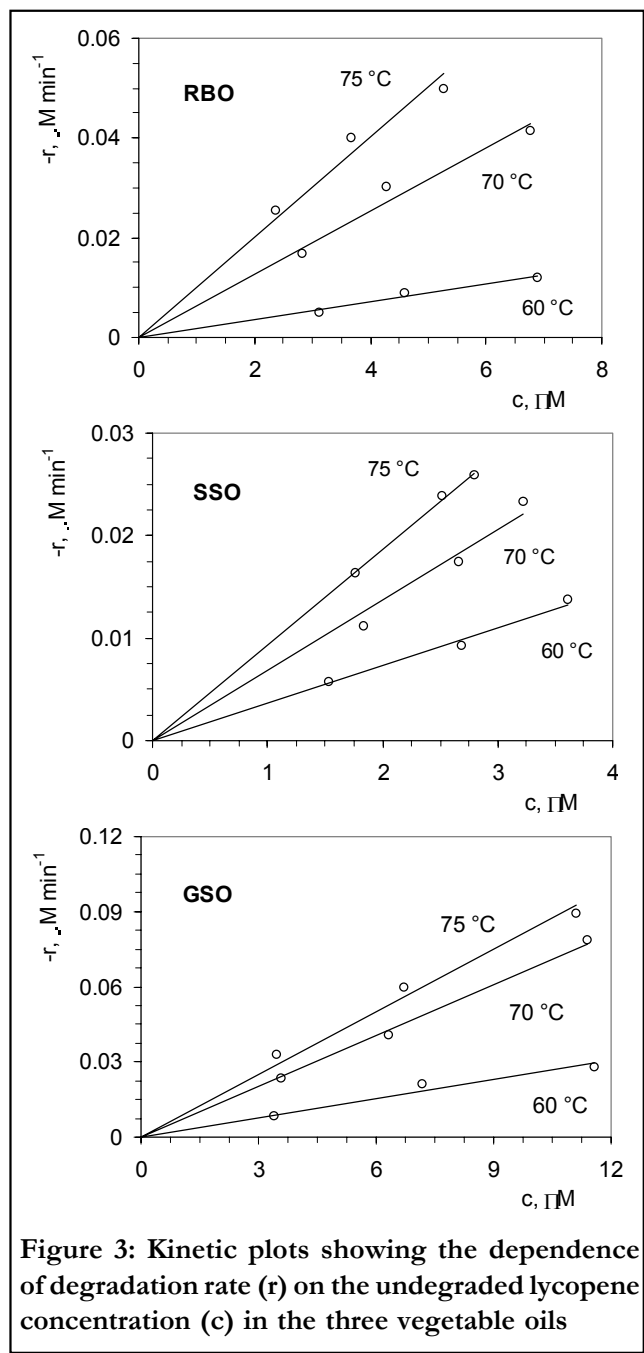


Figure 3: Kinetic plots showing the dependence of degradation rate (r) on the undegraded lycopene concentration (c) in the three vegetable oils

rate constant and c is the lycopene concentration. Rate constants were determined by least squares regression analysis. The results are presented in TABLE 1.

The agreement between experimental and calculated degradation rates was very good (see Figure 3), with an average error of 5.8 % and a standard deviation of 3.5 %.

To assess the statistical significance of the results we calculated the model residuals:

TABLE 1: Kinetic parameters for lycopene degradation in the three vegetable oils. Parameters are expressed as mean \pm standard deviation. Numbers in parentheses are the determination coefficients

Vegetable oil	T (°C)	k (10^2 min^{-1})	E_a (kJ mol^{-1})
RBO	60	0.188 ± 0.019 (0.780)	108.9 ± 6.0 (0.997)
	70	0.636 ± 0.030 (0.957)	-
	75	1.006 ± 0.048 (0.927)	-
GSO	60	0.251 ± 0.020 (0.923)	80.1 ± 4.5 (0.975)
	70	0.680 ± 0.016 (0.994)	-
	75	0.838 ± 0.039 (0.964)	-
SSO	60	0.369 ± 0.013 (0.976)	59.6 ± 0.8 (0.999)
	70	0.683 ± 0.030 (0.948)	-
	75	0.934 ± 0.006 (0.998)	-

$$\rho_i = r_{i,\text{exp}} - r_{i,\text{calc}} \quad (2)$$

which represent the differences between experimental degradation rates and model responses. In order to ensure that each data had the same statistical weight, we evaluated the following normalised residuals:

$$\Gamma_i = \frac{\rho_i}{\left[\frac{1}{N-P} \sum_{i=1}^N \rho_i^2 \right]^{0.5}} \quad (3)$$

where N and P are the number of data and parameters, respectively (in our case: $N = 27$, $P = 1$). It can be shown^[22] that, for Gaussian errors, 95 % of the normalised residuals should be in the range ± 2 . Calculation of residuals gave the results displayed in figure 4, with all data points between -2 and $+2$ and no appreciable temperature effect.

The ordered residuals (r_i) were then plotted against the corresponding normal-order statistics medians, defined as:

$$\Phi_i = F^{-1} \left(\frac{i}{N+1} \right) \quad (4)$$

where F is the standard normal cumulative

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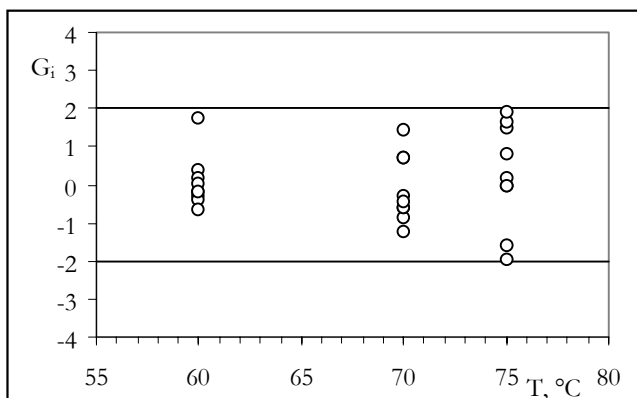


Figure 4: Normalised model residuals (G_i) as a function of temperature. The horizontal lines ($G = \pm 2$) delimit the 95 % confidence region

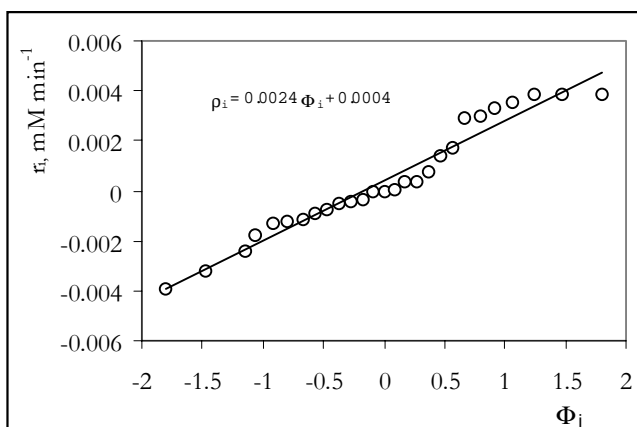


Figure 5: Normal probability plot showing the dependence of ordered residuals (r_i) against normal-order statistics medians (F_i)

distribution function. Data plotted in such a way should yield a straight line, with the intercept and slope representing, respectively, the location and scale parameters of the normal distribution^[23].

As evidenced in figure 5, a highly linear pattern was attained, with small deviations in the upper region. From the regression line equation, the following estimates were derived for the location and scale parameters: 0.0004 and 0.0024 mM min⁻¹. The correlation coefficient was 0.98. Since it is greater than the critical value for the normal probability distribution ($R_c = 0.96$, at the 5% significance level^[24]) the assumption that the data belong to a population with a normal distribution can not be rejected, i.e. the interpretation of degradation rate data by the first-order kinetic model is statistically

correct.

To describe the temperature dependence of k we assumed the Arrhenius equation:

$$k(T) = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

where k_0 is the frequency factor and E_a is the apparent activation energy. The resulting plots are shown in figure 6, while the estimated activation energies are listed in TABLE 1. E_a varies from 59.6 ± 0.8 kJ mol⁻¹ to 108.9 ± 6.0 kJ mol⁻¹. A comparison with literature data (TABLE 2) shows that these values are very close to those determined in safflower oil and in a model system consisting of a thin vacuum-deposited film of lycopene. On the contrary, smaller activation energies are reported for oil-in-water emulsions and for organic solvents, suggesting that lycopene degradation may follow different kinetic pathways, depending on the properties of the surrounding medium. In an oil medium, namely, an environment closely resembling the natural matrix

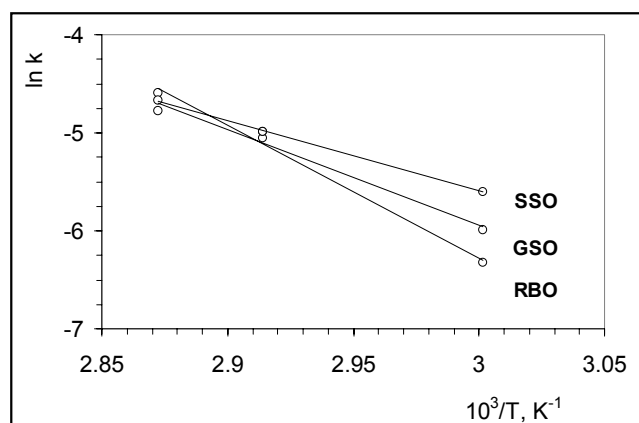


Figure 6: Arrhenius plots for lycopene degradation in the three vegetable oils

TABLE 2: Literature values for the activation energy of lycopene degradation in different media

Medium	E_a (kJ mol ⁻¹)	Reference
Safflower oil	82.9	[17]
Soybean oil	93.1	[43]
Vacuum-deposited film of lycopene	61.0	[7]
Oil-in-water emulsions		
Oxygen free	23.0	[18]
Oxygen saturation	18.0	[18]
Dioxane	30.0	[44]
Heptane	30.0	[44]

in which the pigment is embedded, degradation seems to be energetically disfavoured.

To quantify the influence of the oil nature on the stability of lycopene we evaluated the apparent half life, i.e. the time required for half of the original amount of lycopene to disappear. For a first-order kinetics this quantity depends only on temperature and can be calculated as:

$$\tau = \frac{\ln 2}{k(T)} \quad (6)$$

Substitution of the appropriate rate constants in eqn. (6) gave the results presented in figure 7. Estimated half lives were between 1.15 and 6.14 h, the value being dependent on the temperature and the vegetable oil considered. These results are in close agreement with those of Henry et al.^[17] who performed the only, to our knowledge, kinetic study on lycopene degradation in vegetable oils. The authors used safflower oil as the solvent, obtaining first-order rate constants ranging from 0.109 to 0.518 h⁻¹ as the temperature was varied between 75 and 95 °C. From these values, half lives of the order of 1 to 6 h can be estimated.

A closer examination of our results reveals that at 70 and 75°C stability is only marginally influenced by the oil nature, while at 60°C differences are more evident. At this temperature the following stability order can be established:

RBO > GSO > SSO

To interpret these results and clarify the role of solvent on stability, attention should be focused on

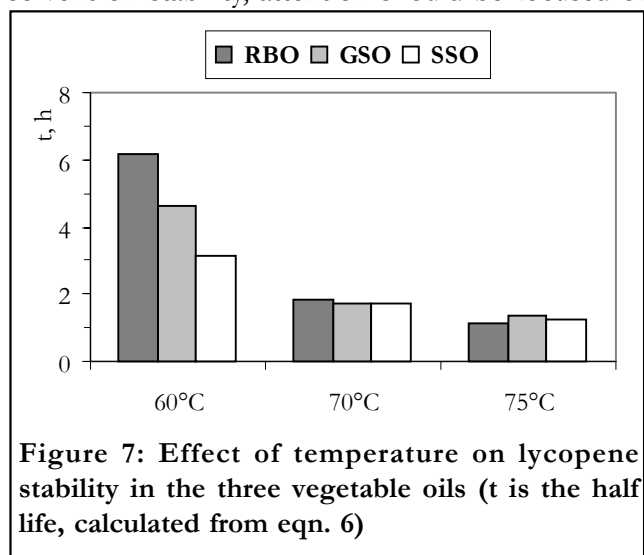


Figure 7: Effect of temperature on lycopene stability in the three vegetable oils (t is the half life, calculated from eqn. 6)

two key factors: the fatty acid composition of the oil and the type and content of antioxidants.

The strong influence of fatty acid composition on the stability of carotenoids was clearly evidenced by Bezbradica et al.^[25], who studied the degradation of complex mixtures of carotenoids in mineral and vegetable oil extracts of marigold flowers. The most significant result of this study was the recognition of a relationship between the degradation rate of carotenoids and the degree of unsaturation of the oil. In particular, oils with a high content of unsaturated fatty acids, such as sunflower, soybean and grape seed oils, resulted in faster degradation. The lowest degradation rates were measured in Myritol 312® and paraffin oil, containing only saturated compounds, and an intermediate behaviour was observed for almond and olive oils. A reasonable explanation of these results can be found in the phenomenon of lipid autoxidation, which leads to the formation of highly reactive species, such as alkyl and peroxy radicals, when molecular oxygen reacts with polyunsaturated lipids^[26,27]. Carotenoids can interact with free radicals and other reactive oxygen species and undergo cleavage^[28]. In the case of lycopene, apo-lycopenals have been identified as the main degradation products^[29,30]. They are very similar to those formed upon oxidation of β -carotene or treatment with radical initiators^[31,32].

Stability of carotenoids can also be affected by the presence of endogenous antioxidants (such as tocopherols and tocotrienols). Studies performed in non-polar environments have shown that tocopherols can protect β -carotene from oxidative damage^[28,33,34]. Similar results were obtained by Mortensen and Skibsted^[35] using lycopene. One hypothesis for such protection is that tocopherols can regenerate the biologically active carotenoid molecules by an electron transfer mechanism^[36].

Turning now to our results, we report in TABLE 3 some characteristics of the oils used. As can be seen, RBO is the oil with the lowest unsaturation degree. In GSO and SSO unsaturated compounds are present at nearly equal proportions, but they are differently distributed in the mono- and poly-unsaturated classes. We also note that RBO contains the highest amount of antioxidants. They include tocopherols,

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TABLE 3: Fatty acid composition and antioxidant content of vegetable oils^[45] (SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids)

Vegetable oil	SFA (%)	MUFA (%)	PUFA (%)	Tocopherols (mg/100 g)	Tocotrienols (mg/100 g)	g-oryzanol (mg/100 g)
RBO	20	45	35	25.6-64.8	24.4-104.1	400-1000
GSO	12	16	72	1.6-20.4	37.3-72.5	-
SSO	12	21	67	40.3-102	-	-

tocotrienols and γ -oryzanol, a group of sterol ferulates with significant antioxidant activity and synergistic effects with tocopherols^[37]. Tocopherols in GSO are less abundant than in SSO, but the former displays high levels of tocotrienols, whose antioxidant activity generally exceeds those of tocopherols. According to Serbinova et al.^[38] α -tocotrienol may have 6.5-60 times greater activity than α -tocopherol. Moreover, in GSO a relevant fraction of tocopherols is made of γ -tocopherol, while in SSO they consist almost entirely of α -tocopherol^[39]. This is an important point to emphasise, since γ -tocopherol oxidises to compounds that are all effective antioxidants, while one out of the α -tocopherol degradation products has antioxidant activity^[40].

From the above considerations it can be argued that at 60°C (and probably below) lycopene degrades more slowly in RBO because of the lower degree of unsaturation and higher content of antioxidants of this oil. For the other two oils, showing a similar degree of unsaturation, the main contribution to stability should come from endogenous antioxidants. In particular, the presence of tocotrienols and the high percentage of γ -tocopherol would make GSO more resistant to oxidation and lycopene less susceptible to degradation. The disappearance of differences among the three oils at 70 and 75°C (see Figure 7) suggests that, as the temperature is increased, the protection offered by RBO reduces much more than that exerted by GSO and SSO. This could be a reflection of a temperature-induced perturbation of the balance between pro- and anti-oxidant properties of the oil, a phenomenon observed at high concentrations of tocopherols^[39,41]. Another possible, but perhaps less likely, explanation is that tocopherols degrade faster in RBO than in GSO and SSO because of the inverse relationship between the high-temperature degradation of antioxidants and the degree of unsaturation of the lipid matrix^[42].

Whatever the cause, it is clear that above a certain temperature there no longer seems to be an appreciable influence of the vegetable oil properties on lycopene stability. This should be taken into account when selecting a suitable oil for lycopene extraction. If the temperature is not too high and/or the extraction time is short, oils with a low degree of unsaturation and a high content of antioxidants should be preferred. Under more severe conditions, the oil nature becomes progressively less significant, unless the process is aimed at producing a lycopene-enriched oil product. In this case the vegetable oil composition is of utmost importance, being responsible for the functional properties and the shelf life of the product, the two main factors determining its value and quality.

CONCLUSIONS

The results obtained in the present study indicate that the stability of tomato lycopene in vegetable oil extracts is extremely low. In the temperature range 60-75°C half lives are of the order of a few hours and scarcely sensitive to temperature. This could be the consequence of the high affinity of lycopene for vegetable oils which, on the one hand, enhances its reactivity and, on the other, makes it more susceptible to denaturation.

For all three oils, thermal degradation follows first-order kinetics, with activation energy values higher than those found in oil-in-water emulsions and in organic solvents.

As regards the effect of oil type, the most significant result is that at lower temperatures lycopene degrades more slowly in RBO, the oil with the lower degree of unsaturation and the higher content of antioxidants. At higher temperatures, differences among RBO, GSO and SSO tend to vanish, probably because of the combined influence

of unsaturated triacylglycerols, tocopherols and tocotrienols on the anti-oxidant/pro-oxidant properties of the oil.

Although further research is needed to clarify these points, the fact that lycopene undergoes a rapid degradation when dissolved in a vegetable oil medium must be carefully considered when developing processes based on the use of vegetable oils as extracting agents.

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