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Stability and Degradation Kinetics of Lycopene in Vegetable Oils

Antonio Zuorro, Roberto Lavecchia

Abstract – *The stability of lycopene in sunflower seed oil (SSO), grape seed oil (GSO) and rice bran oil (RBO) was investigated in the temperature range of 10-40 °C. Kinetic analysis of lycopene degradation showed that the process is first-order with respect to the carotenoid concentration, with apparent activation energies between 50.8 kJ mol^{-1} (RBO) and 70.0 kJ mol^{-1} (GSO). The half-life of lycopene in the oils was dependent on the storage temperature and varied from 303 to 530 days, at 4 °C, and from 86 to 104 days, at 20 °C. Below 15-20 °C, stability increased in the order: RBO < GSO < SSO, while at higher temperatures such differences tended to disappear. This is likely due to the combined effects of endogenous antioxidants and unsaturated triacylglycerols in the oils on the degradation pathway of lycopene.* ***Copyright © 2011 Praise Worthy Prize S.r.l. - All rights reserved.***

Keywords: *Degradation Kinetics, Functional Food Products, Lycopene, Storage Stability, Vegetable Oils*

I. Introduction

The health benefits of the Mediterranean diet, a dietary habit characterized by the use of olive oil as the main added fat and the consumption of fresh fruits and vegetables, are mostly attributed to the high intake of antioxidant components [1], [2]. Oxidative stress is, in fact, increasingly recognized as an important causative factor of cardiovascular disease [3] and cancer [4].

Lycopene, the carotenoid responsible for the deep red colour of ripe tomatoes and tomato products, is one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol [5]. Chemically, lycopene (ψ,ψ–carotene) is an acyclic tetraterpenic hydrocarbon with 13 carbon–carbon double bonds, 11 of which are conjugated (Fig. 1).

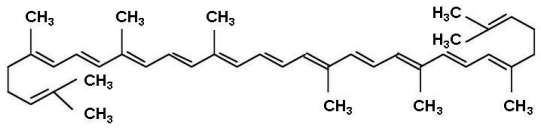

Fig. 1. Chemical structure of lycopene

Lycopene is the predominant carotenoid in human plasma, where it is found at concentrations of about 0.2–1 nmol/mL [6]. Since lycopene is not synthesized in the human body, its presence in the organism is exclusively related to the intake of lycopene-containing foods, particularly tomatoes and tomato products. Several studies have shown that an inverse relationship exists between plasma lycopene levels and the incidence of prostate, lung, and stomach cancer [7]-[9].

Furthermore, it has been clinically demonstrated that dietary consumption of tomato products reduces cellular DNA damage and biomarkers of lipid oxidation in healthy subjects, smokers, and type 2 diabetics [10]. These beneficial effects are believed to result from the antioxidant properties of lycopene and from the presence of other tomato phytochemicals, such as β-carotene, phytoene and phytofluene, which exhibit synergistic activity [11]. For all these reasons, there is currently much interest in recovering lycopene from natural sources [12]-[14] and in fortifying foods by the addition of lycopene [15].

In a recent paper it has been shown that incorporation of processed tomato peels or tomato puree in a vegetable oil can lead to the obtainment of functional products with an average lycopene content of 1.99 mg/100 g and an average β-carotene content of 1.07 mg/100 g [16]. While these products may offer great health benefits and new opportunities for the food industry, there are a number of issues that still must be addressed. One of the most important is the poor stability of lycopene in a lipid environment at ordinary temperatures [17], [18]. In fact, while the native membrane-bound carotenoid is relatively stable [19], it undergoes rapid isomerization and oxidation when released into a nonpolar environment [20].

In order to provide information useful for the formulation of lycopene-containing food products, we investigated the kinetics of lycopene degradation in some vegetable oils at different storage temperatures. We focused on sunflower seed oil (SSO), grape seed oil (GSO) and rice bran oil (RBO) because they are promising candidates for the preparation of functional food products. The objectives of the study were twofold:

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(1) to estimate the kinetic parameters controlling the stability of lycopene in the three oils and (2) to assess whether and to what extent the type and composition of the oil might affect the carotenoid stability.

II. Experimental

II.1. Materials

Fresh ripe tomatoes of the commercial variety "Roma" were obtained from a local market and stored at 4 °C for a maximum of 2 days before use. SSO and RBO were purchased from a grocery store. GSO was from ACEF (Fiorenzuola d'Arda, PC, Italy). The oils were kept at room temperature in the dark and used without further treatment or purification.

II.2. Preparation of Lycopene-Enriched Oil Samples

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

Lycopene-containing oil samples 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 (20 ± 2 °C) in the dark for 10-12 h. After this time the content of each flask was centrifuged at 5000 rpm × 15 min to allow separation of the oil from the plant material. The oil was then analysed for lycopene content.

II.3. Lycopene Assay

Lycopene concentration in the vegetable oils was determined spectrophotometrically. Absorption spectra were recorded at room temperature 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. Absorption spectra of the enriched oils were all similar in shape and showed the three characteristic peaks of lycopene at around 456, 483 and 518 nm (Fig. 2). To minimise interference from other carotenoids, measurements were made at the longest wavelength [21].

II.4. Thermal Stability Studies

Thermal degradation experiments were carried out in magnetically stirred jacketed glass vessels (60-mL working volume) connected to a circulating water bath whose temperature was controlled within ± 0.1 °C.

In a typical run, about 30 mL of pure vegetable oil were charged into the vessel and allowed to equilibrate at the desired temperature (10, 25 or 40 °C). Then an appropriate amount (about 2 to 6 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 made at least in duplicate and the results were averaged.

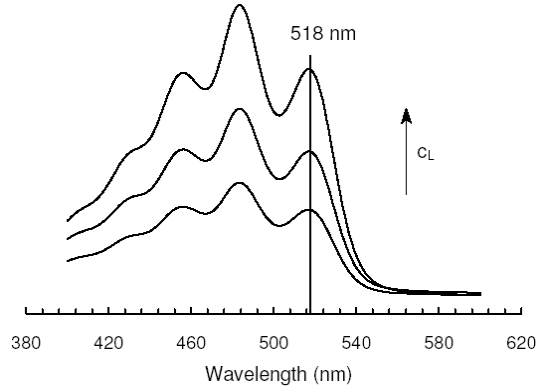

Fig. 2. Absorption spectra of lycopene-enriched GSO at increasing lycopene concentrations (c_L)

III. Results and Discussion

For all the systems investigated, a linear decrease in the absorbance at 518 nm was observed over the entire time course (up to about 200 days). Some representative plots are displayed in Fig. 3.

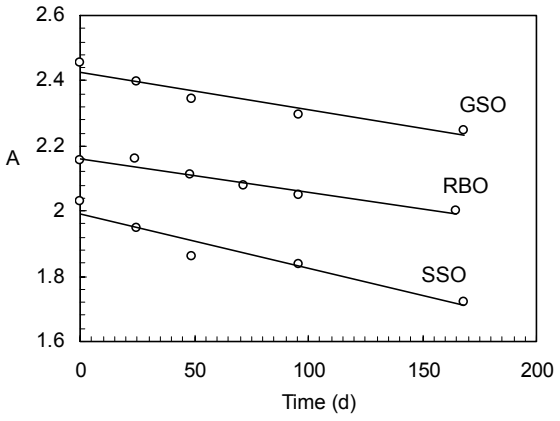

Fig. 3. Effect of storage time at 25 °C on the stability of lycopene in the three oils. *A* is the absorbance at 518 nm

From the slope of the absorbance-time plots, the experimental degradation rates at each temperature and lycopene concentration were determined. The latter was evaluated by assuming a molar extinction coefficient of $1.303\ 10^5\ M^{-1}cm^{-1}$ [22].

Plotting the degradation rates against the average lycopene concentrations at the temperatures studied gave the results shown in Fig. 4.

The linear dependence of the degradation rate (r_d) on concentration (c) is indicative of a first-order kinetics:

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$$r_d = k_d c \tag{1}$$

where k_d is the rate constant of degradation. The values of k_d were determined by least-squares regression analysis, yielding the estimates listed in Table I. The agreement between experimental and calculated degradation rates was very satisfactory (see Fig. 5), the average absolute error being 0.0108 μM d^{-1}.

Examination of the values of the first-order rate constant in the three oils shows that lycopene stability increases in the order RBO < SSO < GSO. Furthermore, temperature has a pronounced effect on stability, at least in the temperature range considered.

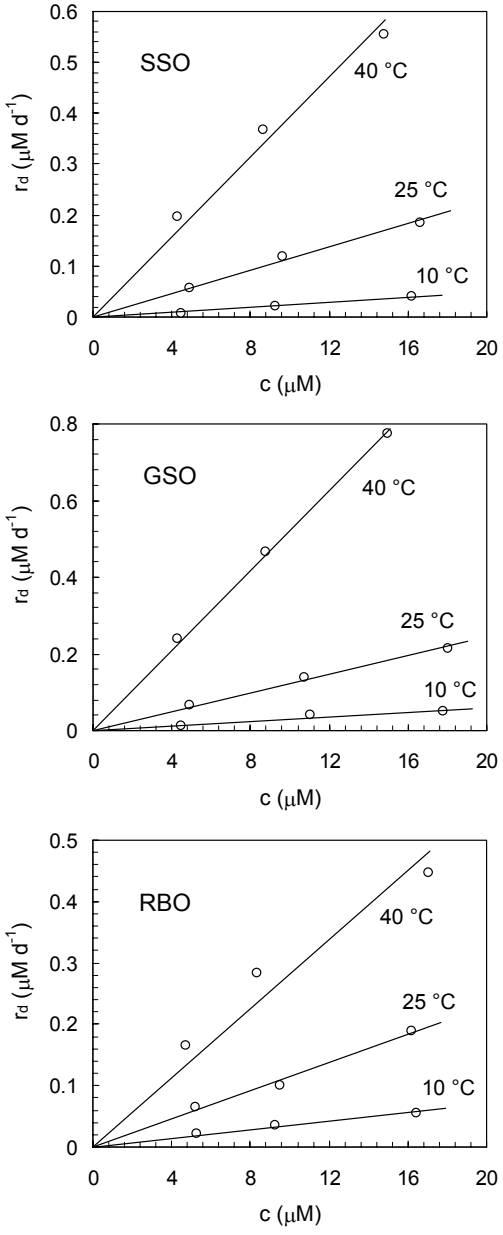

Fig. 4. Kinetic plots showing the dependence of the degradation rate (r_d) on lycopene concentration (c) in the three oils

TABLE I
KINETIC PARAMETERS FOR LYCOPENE DEGRADATION IN THE OILS

Vegetable oil	T / °C	k_d / d^{-1}	E_a / kJ mol^{-1}
SSO	10	(2.391 ± 0.128) 10^{-3}	68.86 ± 2.76
	25	(1.146 ± 0.031) 10^{-2}	
	40	(3.926 ± 0.187) 10^{-2}	
GSO	10	(3.014 ± 0.260) 10^{-3}	70.04 ± 2.89
	25	(1.221 ± 0.031) 10^{-2}	
	40	(5.229 ± 0.073) 10^{-2}	
RBO	10	(3.565 ± 0.175) 10^{-3}	50.86 ± 2.32
	25	(1.145 ± 0.036) 10^{-2}	
	40	(2.816 ± 0.246) 10^{-2}	

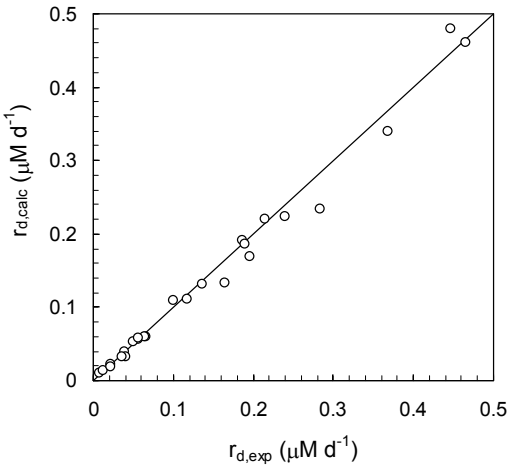

Fig. 5. Experimental ($r_{d,exp}$) and calculated ($r_{d,calc}$) lycopene degradation rates

To describe the temperature dependence of k_d we used the Arrhenius equation:

$$k_d(T) = k_0 \, exp\left(\frac{-E_a}{RT}\right) \tag{2}$$

where k_0 is the pre-exponential factor and E_a is the apparent activation energy. An example of the resulting Arrhenius plots is displayed in Fig. 6, while the activation energies obtained from these plots are given in Table I.

The estimated activation energies varied from approximately 50 to 70 kJ mol^{-1}, suggesting that the nature of the oil medium has some effect on the susceptibility of lycopene to degradation. More particularly, temperature sensitivity increased as follows: RBO < SSO < GSO.

To further validate the kinetic analysis, we performed additional long-term experiments (408 days at 10 °C) in SSO and GSO. The experimentally determined lycopene degradation rates were compared with the model predictions.

The results are summarized in Table II, from which it can be seen that the assumed first-order relationship for the degradation kinetics provides an accurate description of the systems under consideration (average percentage error: 1.59%).

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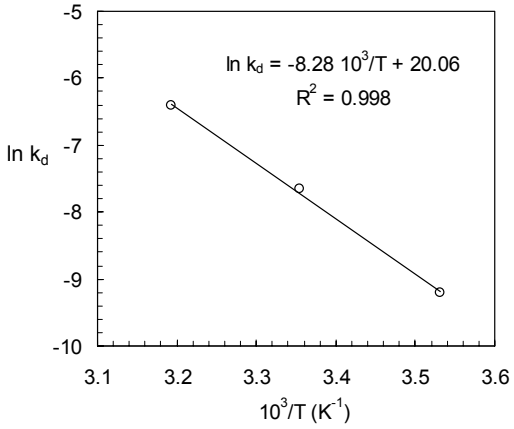

Fig. 6. Arrhenius plot for lycopene degradation in GSO

TABLE II
KINETIC MODEL VALIDATION

Vegetable oil	c_0 / μM	$c_{t,exp}$ / μM	$c_{t,calc}$ / μM	ε%
SSO	4.54	4.45	4.36	2.09
	9.33	8.95	8.95	–0.05
	16.39	15.56	15.74	–1.14
GSO	4.54	4.48	4.32	3.75
	11.22	10.87	10.66	1.98
	17.98	17.17	17.08	0.52

c_0: initial lycopene concentration; $c_{t,exp}$, $c_{t,calc}$: experimental and calculated lycopene concentrations after storage at 10 °C for 408 days, ε%: percentage error of prediction

Finally, in order to quantify the stability of the enriched oil products under typical storage conditions we evaluated the apparent lycopene half-life ($t_{1/2}$), *i.e.*, the time required for half of the initial amount of lycopene to disappear.

For first-order kinetics, $t_{1/2}$ depends only on temperature and can be calculated as:

$$t_{1/2} = \frac{ln2}{k_d(T)} \tag{3}$$

In the temperature range of 10 to 40 °C, $t_{1/2}$ varied from 18 to 290 days in SSO, from 13 to 230 days in GSO and from 25 to 194 days in RBO.

Using the estimates of the parameters k_0 and E_a we determined the half-life of lycopene in the three oils between 0 and 40 °C.

The resulting trends are presented in Fig. 7, where two significant storage temperatures, 4 and 20 °C, are also represented.

We note that at lower temperatures (approximately below 15–20 °C) the half-life curves are well separated and $t_{1/2}$ increases in the order RBO < GSO < SSO. Above 20 °C, however, differences in stability in the three oils tend to disappear.

This behavior must be indicative of some variations in the degradation pattern of lycopene caused by the different composition of the oils. The main characteristics of the three vegetable oils, including their fatty acid composition and the antioxidant content, are reported in Table III.

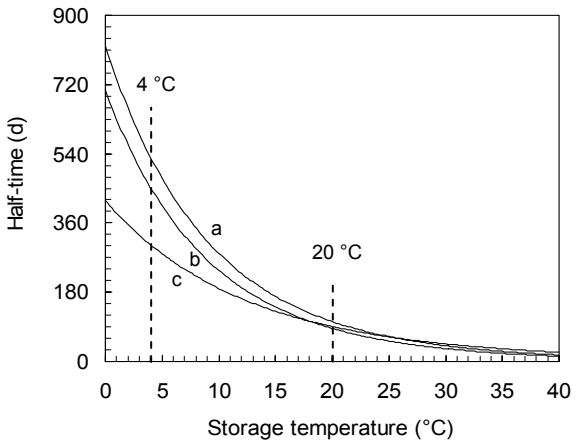

Fig. 7. Calculated effect of storage temperature on the half-life of lycopene in: (a) SSO, (b) GSO and (c) RBO

TABLE III
FATTY ACID COMPOSITION AND ANTIOXIDANT CONTENT OF SSO, GSO AND RBO [23]

Vegetable oil	Fatty acid composition / wt%		
	SFA	MUFA	PUFA
SSO	12	21	67
GSO	12	16	72
RBO	20	45	35
	Antioxidant content / mg/100 g		
	TCPs	TCTs	γ-oryzanol
SSO	40.3–102	–	–
GSO	1.6–20.4	37.3–72.5	–
RBO	25.6–64.8	24.4–104.1	400–1000

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TCPs: tocopherols, TCTs: tocotrienols

It can be seen that SSO, GSO and RBO differ both in fatty acid composition and in the type and level of endogenous antioxidants. In particular, RBO is the oil with the lowest unsaturation degree (80 wt% unsaturated fatty acids). In GSO and SSO, unsaturated compounds are present in equal amounts (88 wt%), but they are differently distributed in the mono- and polyunsaturated classes.

It is generally accepted that the higher the degree of unsaturation of a vegetable oil, the faster is its degradation by oxidative modifications [24], [25]. Oxidation of lipids leads to the formation of highly reactive species, such as alkyl and peroxyl radicals, which may, in turn, promote the degradation of easily oxidizable compounds, as is lycopene in our case [26]. Accordingly, lycopene in RBO should deteriorate less than in SSO and GSO. Stability, however, is also affected by the presence of endogenous antioxidants, such as tocopherols and tocotrienols. Studies performed in nonpolar environments have shown that tocopherols can protect lycopene from oxidative damage [27]. Similar results were obtained for β-carotene [28], [29]. The underlying hypothesis is that tocopherols can regenerate the biologically active carotenoid molecules by an electron transfer mechanism [30]. On the other

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hand, it is also known that tocopherols at high concentrations may exert a pro-oxidant activity and that this activity is temperature-dependent [31], [32].

With regard to the oils of interest, we note that SSO contains the highest levels of tocopherols (up to about 100 mg/100 g), even though GSO and RBO have some additional amounts of tocotrienols and, in the case of RBO, γ-oryzanol. The latter consists of a mixture of at least 10 ferulic acid esters of triterpene alcohols and sterols [33], whose main mechanism of action is to inhibit the formation of iron-driven hydroxyl radicals [34].

From the above considerations, we can speculate that at lower temperatures lycopene degradation proceeds more slowly in SSO than in GSO and RBO because of the higher tocopherol content of the former. As the temperature is increased, pro-oxidant effects may become important, particularly in SSO, due to its higher concentration of tocopherols and their possible competition with unsaturated fatty acids [35]-[36], leading to a reduced lycopene stability. Although the precise mechanisms involved in this process remain to be elucidated, 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. So, at 4 °C differences in half-life as high as 226 days were observed, while at 20 °C they reduced to less than 13 days.

IV. Conclusion

The results obtained in the present study indicate that thermal degradation of lycopene in vegetable oils follows first-order kinetics, with an apparent half-life depending on temperature and oil type. At temperatures around 20 °C the average half-life of lycopene in the oils examined was of about three months, while at 4 °C values between 10 and 17 months were observed.

Overall, the findings from this research support the possibility of enriching vegetable oils with lycopene and storing them for an adequate period of time. The fact that at lower storage temperatures the stability of lycopene is more sensitive to the features of the oil environment suggests that stability issues, in addition to nutritional value, should be taken into account when formulating functional oil products enriched or fortified with lycopene.

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