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An investigation of influence of solvent on the degradation kinetics of carotenoids in oil extracts of *Calendula officinalis*

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Abstract: The stability of carotenoids was studied in marigold oil extracts prepared with following solvents: Myritol 312®, paraffin oil, almond oil, olive oil, sunflower oil, grape seed oil, and soybean oil. The concentration of the carotenoids was determined by spectroscopic measurement at 450 nm. Degradation rate showed a first order dependence on the concentration of carotenoids with a faster first stage (which lasted 35–50 days, depending on the solvent) and a slower second stage. The highest degradation rates were observed in extracts prepared with linoleic acid rich solvents (sunflower oil, soybean oil and grape seed oil), while the lowest were found in oil with saturated fatty acids (Myritol 312®) and paraffin oil. These results confirm the connection between the degradation of carotenoids and lipid autoxidation, and suggest that the influence of the oil solvents on the stability of oil extracts of *Calendula officinalis* is a factor that must be considered when selecting a solvent for the production of marigold oil extracts.

Keywords: *Calenula officinalis*, oil extract, carotenoids.

INTRODUCTION

Marigold (*Calendula officinalis*) has been used as a folk medicine since ancient times. Extracts of this herb are important ingredients of a number of pharmaceutical preparations for the treatment of poorly healing wounds, burns, cuts, bruises and eczema because of their anti-inflammatory activity and promotion of the formation of granulation tissue.[1]

Calendula extracts are also used in cosmetics because of their emollient, protective, antiiritant and antiseptic properties. They are important ingredient of bath preparations, hand care products, sunscreens, products for irritated skins and baby toiletries. Besides cosmetic and medical usage, Calendula flowers have culinary use as substitutes for saffron in salads and omlettes and as colorants for cheese and butter.[2,3]

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Marigold has beneficial properties since it contains wide range of bioactive compounds. Carotenoids, especially β-carotene, are very abundant and active. Carotenoids are multifunctional, naturally occurring, red, yellow and orange pigments. They are widely used in pharmaceutical and food industry as natural colorants and as sources of provitamin A. Lately, interest in carotenoids has increased because they function as chain breaking antioxidants, and protect cells and other body components from free radical attack. They also protect the skin from redness and damage following exposure to UV radiation. The results of these studies caused an increased usage of supplementary carotenoids in pharmaceutical and cosmetic products, in the form of pure chemicals or various herbal extracts that contain carotenoids. Of these two forms, extracts are less expensive and often more efficient because of the synergistic activity of other compounds with carotenoids.

The stability of carotenoids and the kinetics of their degradation in various food systems have been extensively studied,[4–10] but there is a lack of such investigations for pharmaceutical and cosmetic products. The general conclusion of these studies in the field of food chemistry was that the kinetics of degradation are influenced by factors such as reaction medium, temperature, physical state and type of carotenoid, as well as environmental conditions. The majority of studies to data have focused on synthetic supplementary carotenoids in low-moisture and solvent-based model systems[4–8] while investigations of the stability of carotenoids in oil systems are very rare. Investigations of oil systems (liposomes, methyl linoleate and natural oils) with supplementary carotenoids were mostly concentrated on the determination of the antioxidant activity of carotenoids, while the kinetics of their degradation were not determined.[9–11] The only investigation of the degradation kinetics was performed by Henry *et al.*.[12] They reported that thermal and oxidative degradation of lycopene, lutein and β-carotene in safflower oil follows first order kinetics. Investigations of the stability of natural mixtures of carotenoids in natural oils have not been reported yet. The results obtained in a pure oil model system may not be directly transposed to those observed in more complex food matrices because other compounds may act as cooxidants and promote oxidation or may have a protective effect.

Therefore, the objective of this research was to determine a kinetic model of the degradation of complex mixture of carotenoids in marigold oil extracts (prepared with various oil solvents) during storage and to determine the influence of the solvent on the rate of degradation in order to chose the best solvent for the production of marigold oil extracts.

EXPERIMENTAL

Method for production of the extracts

Marigold oil extracts were prepared by the digestion method at a temperature of 70 ºC for 10 h. The marigold-oil ratio was 1:6. The mixture of flowers and oil was light protected. Marigold flowers

were provided by the Institute for Medicinal Plant Research Josif Pancic (Belgrade, Serbia and Montenegro). For the production of the extracts, the following oil solvents were used : sunflower oil (Vital; Vrbas, Serbia and Montenegro), olive oil (Monini; Spoleto, Italy), almond oil (Sinefarm; Vrsac, Serbia and Montenegro), soybean oil (Oroli; Monchiero, Italy), Muritol 312® (Henkel; Düsseldorf, Germany), paraffin oil (Hansen & Rosenthal; Hamburg, Germany) and grape seed oil (Collaborative Laboratories Inc.; New York, USA). The extracts were kept in the dark at 22 ºC in sealed vessels for at least 100 days.

Concentration measurement

The concentration of carotenoids in the extracts was determined by spectroscopic measurement at 450 nm.[13] The oil extracts were saponified previous to the spectroscopic measurement. As a blind sample, oil solvent, which had undergone the same treatment as the extracts, was used. Authentic standards of β-carotene for calibration curves were provided by Merck, Darmstadt, Germany. The concentrations of carotenoids are expressed as concentrations of β-carotene. Each experiment was carried out in duplicate. The average values of the concentrations are presented.

Measurement of the degree of oxidation

The degree of oxidation of the oil extracts was determined as a peroxide value.[13] In order to monitor the antioxidative properties of the extracts, the peroxide values of the oil solvents, which were exposed to the same experimental conditions, were also monitored and compared with the peroxide values of the extracts.

Kinetic model of carotenoid degradation

After inspection of previous reports regarding the stability of carotenoids and the mechanisms of their degradation, four kinetic model hypotheses were considered: a first-order dependence on carotenoids, zero-order kinetics, overall second-order kinetics (first-order with respect to carotenoids and first-order with respect to peroxides), and a first-order dependence on peroxides. The third and the fourth model take into account the effects of the peroxide concentration. In several studies it was postulated that the most important mechanism of the autioxidative activity of carotenoids is the formation of an adduct radical with peroxides.[14] Therefore, the eventual influence of the concentration of peroxides on the rate of degradation of carotenoids was investigated. The concentration of peroxides was monitored during storage and the c_p–t dependence was modeled with various functions. The best fit was obtained with a Boltzman's function:

$$c_p = (A_1 - A_2)/(1 + \exp((t - t_0)/\Delta t)) + A_2 \quad (1)$$

where A_1, A_2, Δt, and t_0 are model constants.

1. The first-order dependence on carotenoids is described by the differential equation:

$$-\mathrm{d}c_c/\mathrm{d}t = k_1 c_c \quad (2)$$

which can be transformed into the following equation:

$$\ln c_c = \ln c_{c0} - k_1 t \quad (3)$$

where c_c is concentrations of carotenoids at time t, c_{c0} the initial concentration of carotenoids, and k_1 the rate constant.

2. Zero-order model is described by the following expressions:

$$-\mathrm{d}c_c/\mathrm{d}t = k_0 \quad (4)$$

$$c_c = c_{c0} - k_0 t \quad (5)$$

3. The second-order model is described by the following diferential equation:

$$-\mathrm{d}c_c/\mathrm{d}t = k_2 c_c c_p \tag{6}$$

where c_p is the concentration of peroxides. After replacing the concentration of peroxides with the Boltzman's equation, the following c_c–t model was obtained:

$$\ln(c_c/c_{c0}) = k_2[(A_1-A_2)\Delta t \ln(1 + \exp((t-t_0)/\Delta t))-A_1 t] - k_2(A_1-A_2) \times \\ \times \Delta t \ln(1 + \exp(-t_0/\Delta t)) \tag{7}$$

where c_{c0} is the initial concentration of carotenoids.

4. The first-order model with respect to the peroxide concentration can be described by the differential equation:

$$-\mathrm{d}c_c/\mathrm{d}t = k_1 c_p \tag{8}$$

After same procedure as in case of the second-order kinetics, the following c_c–t model was obtained:

$$c_c - c_{c0} = k_1[(A_1-A_2)\ \Delta t \ln(1 + \exp((t-t_0)/\ \Delta t))-A_1 t] - k_1(A_1-A_2) \times \\ \times \Delta t \ln\ (1 + \exp(-t_0/\Delta t)) \tag{9}$$

RESULTS

Kinetics of carotenoids degradation was investigated in extracts prepared with sunflower oil, olive oil, almond oil, soybean oil, Myrotol 312®, paraffin oil and grape seed oil. Sunflower, almond, grape seed, olive, and soybean oil consist almost entirely of unsaturated fatty acids, while paraffin oil and Myritol 312® contain only saturated compounds. In addition, the main fatty acid in compositions enabled the determination of the relation between the fatty acid sunflower and soybean oil is linoleic acid, while in almond and olive oil the most abundant is oleic acid. This selection of oils having very different fatty acid composition of the oils and the rate of carotenoids degradation. The concentrations of carotenoids and peroxide values were monitored during storage. Each experiment was carried out in duplicate and average values are presented.

The measured concentrations of carotenoids with time are presented in Fig. 1

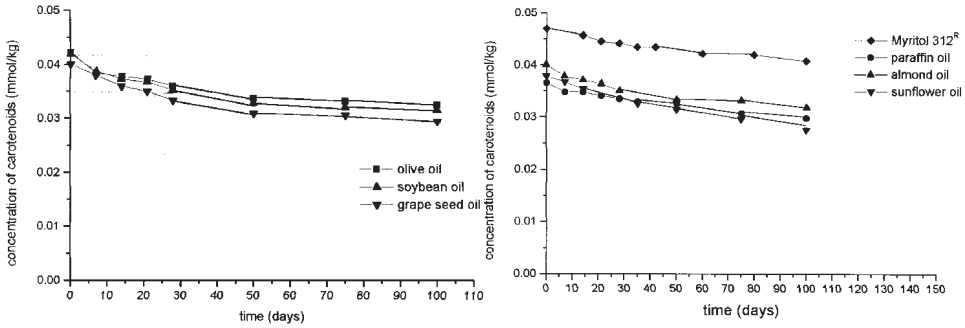

Fig. 1. Stability plots.

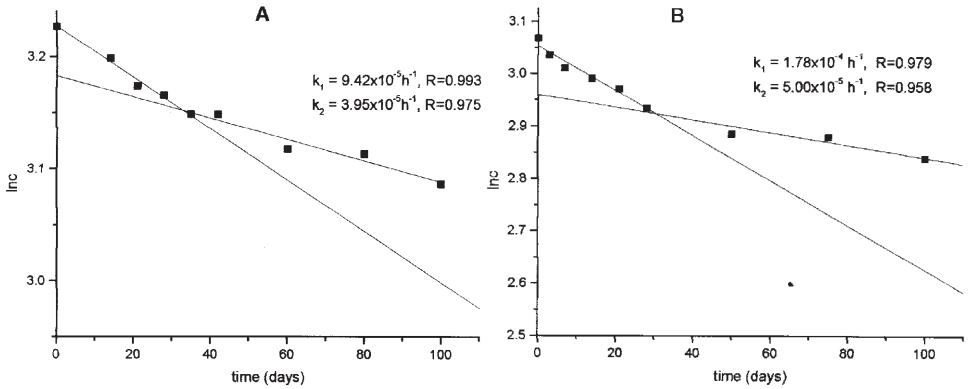

Fig. 2. Two stage first-order kinetic model: (A) extract in Myritol 312^{R}, (B) almond oil extracts.

and the obtained correlation coefficients for the suggested models are listed in Table I. The highest correlation coefficients were obtained with model 1, which indicates that the degradation of the carotenoids follows first-order kinetics with respect to carotenoids. However, the obtained experimental data in this study imply a more complex first-order kinetics that can be divided into two stages. The experimental data in the representative groups and the corresponding broken line regression fits are illustrated in Fig. 2. In all experimental groups, the degradation follows first-order kinetics during both stages but the rate constants of degradation are larger in the first stage. The first stage lasted for the first 35 to 50 days of storage (depending on the type of oil solvent). The rate constants for the degradation of carotenoids are listed in Table II. The rate of degradation of carotenoids increased in the order Myritol 312® < paraffin oil < olive oil < almond oil < sunflower<soybean oil.

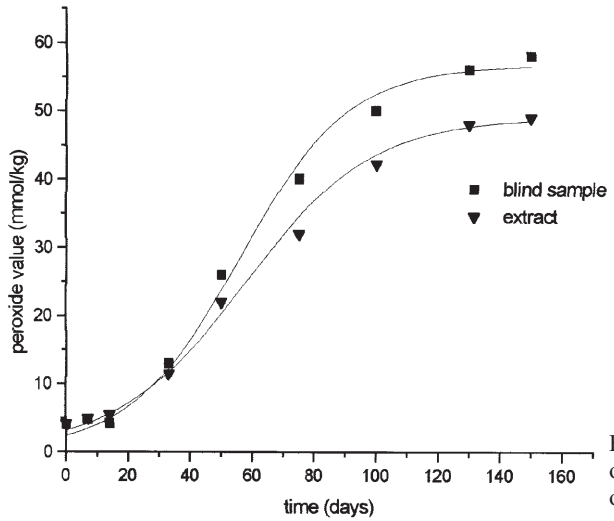

Fig. 3. Illustration of the degree of oxidation of the extract in sunflower oil.

TABLE I. Correlation coefficients of the proposed kinetic models

Solvent	Correlation coefficient for $r = kc_c$ model	Correlation coefficient for $r = kc_cc_p$ model	Correlation coefficient for $r = kc_p$ model	Correlation coefficient for $r = k$ model
Myritol 312®	0.983	0.881	0.874	0.931
Paraffin oil	0.961	0.832	0.826	0.918
Sunflower oil	0.992	0.875	0.884	0.925
Olive oil	0.952	0.736	0.723	0.862
Soybean oil	0.971	0.796	0.772	0.918
Garape seed oil	0.952	0.768	0.743	0.898
Almond oil	0.948	0.762	0.758	0.935

Comparison of the peroxide values of the samples and the blind samples for the representative groups is illustrated in Fig. 3. Complete results (as the constants in model (1)) for each of the examined oils are presented in Table III.

DISCUSSION

Degradation kinetics

First-order kinetics of carotenoids degradation has been reported by several authors who performed experiments in low-moisture, solvent-based model systems and oil systems.[4,8,12] Although simple first-order kinetics is predominant in the reports, two-stage first-order kinetics was also observed by Desobry *et al.*[6] who examined the stability of β-carotene encapsulated in maltodextrin. Comparison of Figs. 2 and 3 shows that turning point of the degradation coincides with the change of the antioxidative properties of extracts. The existence of a turning point for the rate of degradation and oxidative properties indicates a change in the composition of the experimental system.

Theobserved pro-oxidative properties of marigold oil extracts in the first stage of storage are a somewhat surprising because of the recognized antioxidative properties of carotenoids and some of other components of *Calendula* (for example flavonoids). However, there are several reports of pro-oxidative properties of carotenoids. Warner and Frankel[15] reported this kind of action of carotenoids during the heating of soybean oil at 60 ºC in the absence of light, which is similar to the conditions of marigold oil production. The pro-oxidative effect may be related to the degradation of the stable adduct radical which gives rise to carotenoid-5,6-epoxide and a highly reactive alkoxy radical.[11] These radicals had probably been accumulated during the extraction and they caused an acceleration of the chain reaction of lipid autoxidation in the first stage of storage because they react faster with lipids than (normaly present) alkyl radicals. Carotenoids degrade faster in these reactions so their consumption is also accelerated due to the presence of alkoxy radicals. After all the alkoxy radicals have been quenched, carotenoids can react only

with low-energy radicals, which is macroscopically manifested as the second "slower" stage of carotenoids degradation.

TABLE II. Two-stage first-order rate constant and corresponding correlation coefficients

Solvent	$k_1 \times 10^4$ / h^{-1}	R_1	$k_2 \times 10^4$ / h^{-1}	R_2
Sunflower oil	1.88	0.999	1.15	0.995
Olive oil	1.66	0.952	0.329	0.990
Almond oil	1.78	0.956	0.5	0.958
Grape seed oil	2.51	0.994	0.354	0.956
Paraffin oil	1.07	0.968	0.688	0.989
Myritol 312®	0.942	0.993	0.395	0.975
Soybean oil	1.89	0.972	0.338	0.997

In conclusion, the existence of the first faster stage of the degradation of carotenoids is the result of subsequent or extended action of the high temperature of extraction. This effect is "transmited" by compounds accumulated during that process. After the removal of these compounds from the system, in which carotenoids and other compounds with antioxidative properties were involved, the slower stage of degradation commences. The peroxide value-storage time curves in Fig. 3 and the model constants in Table III confirm the antioxidative properties of marigold extracts. The values of the model constant A_2, which represents the concentration of peroxide in equilibrium conditions, indicate that carotenoids exhibit an inhibitory effect in all of the examined oil extracts. The presence of carotenoids (and other compounds with antioxidative properties) in extracts of *C. officinalis* leads to a significant reduction (12 – 28 %) of the equilibrium concentration of peroxides compared with values measured in the corresponding blind samples.

The effect of solvent on the stability of carotenoids

The lowest values of the degradation rate constants were measured in extracts prepared with Myritol 312® and paraffin oil, both of which contain only saturated compounds. On the other hand, the highest degradation rates were noticed in extracts prepared with solvents in which linoleic acid is the most abundant fatty acid (sunflower oil, soybean oil and grape seed oil). These results indicate the strong influence of the fatty acid composition of the solvent on the rate of the degradation of carotenoids. In addition, a connection between the degradation of carotenodis and lipid autoxidation is implied since it was reported that triglycerides with a high content of linoleic acid are the most susceptible to oxidation.[16] It was found that the slowest (rate controlling) step of this process is the homolitic splitting of the C–H bond in the lipid molecule and this reaction is faster for triglycerides with a high content of linoleic acid because a resonance stabilized bis-allyl radical is formed. On the other hand, extracts prepared with oils containing high amounts of oleic acid (olive oil and almond oil) exhibited medium stability of carotenoids

among the studied experimental groups. This result is also in agreement with the theory of lipid autoxidation since autooxidation occurs *via* an allyl radical which contains less energy than an alkyl radical (formed in extracts in Myritol 312® and paraffin oil), but more than a bis-allyl radical.

TABLE III. Calculated values of constants in model (1)

		A_1	A_2	t_0	Δt
Sunflower oil	Blind sample	5.04	55.76	42.47	12.06
	Extract	4.81	49.12	39.12	6.85
Olive oil	Blind sample	5.12	56.15	42.47	14.14
	Extract	5.04	47.86	38.72	9.28
Almond oil	Blind sample	5.71	55.21	57.77	5.08
	Extract	3.51	46.38	57.96	12.97
Grape seed oil	Blind sample	10.21	67.21	44.23	12.22
	Extract	11.73	51.08	43.83	9.63
Soybean oil	Blind sample	4.23	50.12	48.10	10.56
	Extract	5.72	41.56	50.54	8.87
Paraffin oil	Blind sample	1.76	15.12	48.72	14.60
	Extract	1.12	12.35	46.56	15.11
Myritol 312®	Blind sample	1.25	11.46	48.93	12.99
	Extract	1.74	8.23	58.33	18.81

In the second stage of storage, the differences between rate constants in the different solvents decreased and the relative order of magnitude was changed. These results suggest that degradation of carotenoids in the two stages differed not only in rates but also in the mechanism. Carotenoids degradation is only related to lipid autoxidation in the first stage while during the second stage it probably occurs in reactions which are independent of lipid autoxidation.

In conclusion, the presented experimental results suggest that the influence of oil solvent on the stability of oil extracts of *Calendula officinalis* is a factor which has to be carefully considered in the selection of a solvent for the production of marigold oil extracts. The most important property of the solvent is its fatty acid composition. Extracts prepared with oils that contain only saturated compounds showed carotenoids with the highest stability while the carotenoids degraded with the highest rate in oils with high content of linoleic acid. Therefore, the usage of linoleic acid rich oils as solvents must be reconsidered, although this acid is benificial as an essential fatty acid. The degradation rate of carotenoids showed first order dependence on the concentration of carotenoids in two stages. Furtunately, after about 40 days of storage, a slower stage of commences with rate constants 40–80 % lower than during the first faster stage.

Notation:

c_c – Concentration of carotenoids, mmol kg^{-1}
c_p – Concentration of peroxides, mmol kg^{-1}
c_{c0} – Initial concentration of carotenoids, mmol kg^{-1}
t – Time, h
k_0 – Rate constant, mmol kg^{-1} h^{-1}
k_1 – Rate constant, h^{-1}
k_2 – Rate constant, kg mmol^{-1} h^{-1}
A_1 – Model constant, mmol kg^{-1}
A_2 – Model constant, mmol kg^{-1}
t_0 – Model constant, h
Δt – Model constant, h

ИЗВОД

ИСПИТИВАЊЕ УТИЦАЈА ЕКСТРАГЕНСА НА КИНЕТИКУ РАЗГРАДЊЕ КАРОТЕНОИДА У УЉАНИМ ЕКСТРАКТИМА НЕВЕНА

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У овом раду испитана је стабилност каротеноида у екстрактима невена у: Myritol 312®, парафинском уљу, бадемовом уљу, маслиновом уљу, сунцокретовом уљу, уљу коштица грожђа и сојином уљу. Значајна разлика у масно-киселинском саставу испитиваних уља омогућује одређивање везе између брзине разградње каротеноида и масно-киселинског састава. Установљено је да се разградња каротеноида одвија у складу са кинетиком првог реда по каротеноидима са две фазе: првом, која траје 35–50 дана, у току које је разградња бржа, и другом фазом за коју су карактеристичне мање брзине разградње. Највеће брзине разградње су измерене у екстрактима произведеним са екстрагенсима богатим линолном киселином (сунцокретово уље, сојино уље и уље коштиц агрожђа), а најмање у онима који су припремљени са растварачима који садрже засићена једињења (парафинско уље и Myritol 312®). Ови резултати показују да на брзину разградње каротеноида у уљаним екстрактима значајно утиче процес аутооксидације липида и да утицај врсте уља на стабилност каротеноида у уљаним екстрактима *Calendula officinalis* мора бити узет у обзир приликом избора екстрагенса.

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REFERENCES

1. R. Della Loggia, A. Tubaro, S. Sosa, H. Becker, S. Saar, O. Isaac, *Planta Med.* **60** (1994) 516
2. K. J. Kemper, Calendula Clinical Information Summary, http://www.mcp.edu/herbal/default.htm 1999
3. P. Tisnes, A. M. Moulet-Scott, *Actifs et additifs en cosmetologie*, Tec&Doc and Editions medicales internationales, Paris, 1999, pp. 8–10
4. M. I. Minguezmosquera, M. Jarengalan, *J . Sci. Food Agric.* **67** (1995) 153
5. B. H. Chen, Y. C. Tang, *J. Agric. Food Chem.* **46** (1998) 2312
6. S. A. Desobry, F. M. Netto, T. P. Labuza, *J. Food Sci.* **62** (1997) 1158
7. A. H. El-Tinay, C. O. Chichester, *J. Org. Chem.* **35** (1970) 2290
8. H. Chou, W. Breene, *J. Food Sci.* **37** (1972) 66

9. T. Anguelova, J. Warthesen, *J. Food Sci.* **65** (2000) 71
10. M. J. Goulson, J. J. Warthesen, *J. Food Sci.* **64** (1999) 996
11. K. Haila, *Academic dissertation*, University of Helsinki 1999, p. 20–30
12. L. K. Henry, G. L. Catignani, S. J. Schwartz, *J. Am. Oil Chem. Soc.* **75** (1998) 823
13. H. Pardun, in *Handbuch der Lebensmittelchemie – Fette und Lipoide*, W. Heimann, Ed., Springer-Verlag, Berlin, 1969, p. 797–800
14. G. W. Burton, K. U. Ingold, *Science* **224** (1984) 569
15. K. Warner, E. N. Frankel, *J. Am. Oil Chem. Soc.* **64** (1987) 213
16. O. R. Fennema, *Food Chemistry*, 2nd edn., Marcel Dekker Inc., New York, 1985, pp. 178–186.