Changes on Storage of Peanut Oils Containing High Levels of Tocopherols and β-Carotene

Jan POKORNÝ[1], Lucie PARKÁNYIOVÁ[1], Zuzana RÉBLOVÁ[1], Ludmila TROJÁKOVÁ[1], Hidetoshi SAKURAI[2], Tatsuo UEMATSU[2], Mitsuyoshi MIYAHARA[2] and Tomohiro YANO[2]

[1]Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Czech Republic; [2]Department of Agriculture and Biological Chemistry, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

Abstract

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We compared changes of tocopherols and β-carotene in a traditional peanut oil (cultivar Virginia, 30.5% linoleic acid) with a modified high-oleic peanut oil (cultivar SunOleic, 2.7% linoleic acid), developed in Florida, USA. The initial contents of tocopherols and trace lipid oxidation products, including hydroperoxides, were of the same order in both oils. The stability against oxidation was tested under the conditions of the Schaal Oven Test at 40 and 60°C, in emulsion, using AOM, Rancimat, and the apparatus Oxipres at 100°C. Tocopherols were determined using HPLC with an electrochemical detection (without previous saponification). The high-oleic peanut oil SunOleic was about 4–8 times more stable against oxidation than the traditional peanut oil Virginia. The contents of total tocopherols were 303 mg/kg in Virginia oil and 426 mg/kg in SunOleic oil, respectively. Ratios of α-:γ-:δ-tocopherols were rather similar in both oils. Thus, the observed differences in the oxidative stabilities cannot be due to tocopherols only. The decomposition of tocopherols in peanut oils, containing an addition of 500 mg/kg γ-tocopherol, on storage was substantially slower in high-oleic SunOleic peanut oil than in Virginia peanut oil. Very similar results were observed in the case of the additions of 50 mg/kg β-carotene to peanut oil. The vitamin value was much better preserved in high-oleic peanut oil than in traditional peanut oil.

Keywords: antioxidants; autoxidation; carotene; peanut oil; storage; tocopherols

Obesity belongs to very frequent lifestyle diseases (Frisby 1999), similarly as cardiovascular diseases, diabetes or osteoporosis. Food and pharmaceutical industries have developed many foods and medical preparations for the prevention of these diseases. They belong to the category of "special dietary foods" or to subcategories of medical (Bell *et al*. 1998) or functional (wellness) foods.

Functional foods should resemble the conventional foods to be acceptable by the consumers. The only difference is in that functional foods contain some nutrients in substantially higher amounts than do conventional foods. As they are sold in food super or hypermarkets, they should compete with traditional foods, and the consumers' logic should be observed for their marketing (Wennstrom 2000). Both high quality raw materials and unconventional food materials or by-products may be used (Wiseman *et al*. 2000) for the production of functional foods. They may have high contents of specific nutrients or they may be enriched by respective highly nutritive preparations. Special flavourings, which have been developed as addition to functional foods, should be sensorily acceptable, and the eventual off-flavours have to be masked (Milo-Ohr 1999).

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In diets destined for the weight reduction, low-fat foods were most often used but consumers are now more interested in low-energy foods fortified with physiologically important micronutrients, such as vitamins, trace elements or antioxidants (Sloan 2000). The best way of the product development is to combine both aspects, i.e. to add vitamins and antioxidants to low-fat foods. If the fat content is lower than 20% energy (about 10% weight), the absorption of liposoluble vitamins may be impaired. Therefore, the product should be enriched with vitamins and/or provitamins above the common level. In such a way, the body obtains sufficient vitamin supply in spite of lower availability.

The daily consumption of functional foods is usually lower than that of conventional foods, and thus the functional food products are often stored in the household in open packages for a longer time than conventional foods. More stable lipid fractions are thus more preferable in functional foods than in conventional foods. Olive oil, especially virgin olive oil, is the first choice but it is rather expensive in comparison with other vegetable oils. Therefore, soya, sunflower, and peanuts were modified by breeding so that the oil fraction in the seeds consists mostly of oleic acid, while the linoleic acid content becomes only low (Pokorný & Sakurai 2000). Such oils are more stable against autoxidation on storage or heating than are conventional oils (Parkányiová *et al*. 2000). The contents of essential fatty acids are, of course, much lower than in the original oils but both n-6 and n-3 polyenoic fatty acids may be added in the relatively stable encapsulated form (Roche 1999). Vitamins E and A (or its provitamins) have pronounced antioxidant activites and, naturally, more polar antioxidants of plant origin may be added, too.

In this study, we compared the stability of vitamin E and β-carotene in a conventional and in a modified – low-linoleic and high-oleic – peanut oils.

MATERIALS AND METHODS

Materials. Two peanut cultivars were used in our experiments, i.e. high-oleic low-linoleic peanuts from the cultivar SunOleic developed in Florida (Gorbet *et al*. 1995; Knauft & Gorbet 1997) and harvested in Southern Japan, and conventional peanuts, cultivar Virginia, harvested in China. Their moisture content was 6.40% and 6.30%, respectively, and the oil content (on the dry matter basis) was 42.24% and 44.04%, respectively. Samples of deshelled peanuts were stored in a refrigerator before the extraction of lipids.

Tocopherols were produced by Calbiochem, Biosciences, Inc., La Jolla, CA, USA, and β-carotene by E. Merck, Germany.

Analytical methods. The peroxide value was determined iodometrically and expressed as meq/kg (IUPAC 1987). The acid value was determined by alkalimetric titration and expressed in mg KOH/g (IUPAC 1987). The content of conjugated dienes was determined by ultraviolet spectrophotometry at 234 nm, and expressed in p. c. (IUPAC 1987).

Total sterols were determined using the standard procedure (IUPAC 1987), the contents of monoacyl and diacyl glycerols using thin layer chromatography (TLC) after AOCS (1998).

Tocopherols were determined by high-performance liquid chromatography (HPLC) without previous saponification after Andrikopoulos *et al*. (1991) with the following modifications: A column (250 mm × 4 mm) packed with Separon SGX-RPS (5 μm particle size), manufactured by Tessek, Prague, CR, the micropump LCP 4000.11 (Ecom, Prague, CR) and an electrochemical detector HP 1049 A provided with a carbon cell (500 μA); a mixture of acetonitrile and methanol (1:1 v/v) with dissolved salts – 5 mmol/l NaCl and 20 mmol/l $LiClO_4$ was used as the mobile phase; sample injection 20 μl; flow rate 1 ml/min (Trojáková 1999).

The oil discoloration on heating was measured at 430 nm and at 452 nm in a chloroform solution.

The content of β-carotene was measured spectrophotometrically at 452 nm, and the results were expressed as absorbance in 10 mm cells.

Phospholipids were extracted after Folch *et al*. (1957), modified after Melton *et al*. (1979), and further analyzed by HPLC (Yeo & Horrocks 1988) in the modification published as the IUPAC standard method No. 5.302, employing: 250 mm × 4 mm column packed with Separon SGX (particle size 5 μm, Tessek, Prague, CR); injection of 10 μl of a hexane solution; mobile phase: a mixture of hexane, 2-propanol and an acetate buffer (pH = 4.2) in the ratio of 8:8:1 (v/v/v); ultraviolet detection at 206 nm (detector HP 1050).

Triacylglycerols and polar lipids were determined by HPLC, after the authors' modification: The sample was dried with anhydrous sodium sulphate and extracted with the mobile phase to obtain a 3% solution; 50 ml of the solution were injected (Réblová *et al*. 1999) using an LCP 4000.11

micropump (Ecom, Prague, CR) an autosampler HP 1050 (Hewlett-Packard), a Separon SGX-C18 (5 μm particle size) column (250 mm × 4 mm) manufactured by Tessek, Prague, CR, a mixture of acetone, acetonitrile, and methanol (Merck, HPLC grade) in the ratio of 4:2:1 (v/v/v) as the mobile phase, flow rate 0.8 ml/min, and a refractometric detector (HP 1047A, provided with an automatic cleaning after each analysis). Peaks of triacylglycerols fractions are characterized by their ECN (Equivalent Chain Number) values which are the sum of carbon atoms of fatty acids bound in the triacylglycerol molecule diminished by twice the number of double bonds. Triacylglycerols containing polyunsaturated fatty acids have a lower ECN (40–46) than triacylglycerols containing only monounsaturated and saturated fatty acids (more than 46). More detailed information on the composition of individual fractions can be obtained from Pánek (2000).

Procedures. Peanuts were crushed using a Moulinex mill and extracted with light petroleum (equal parts of crushed peanuts and light petroleum were mixed, stirred, and left overnight at room temperature, the solution was removed, and the resulting material was extracted again; the procedure was repeated three times in the same way). The combined light petroleum solutions were evaporated in a rotating evaporator under a reduced pressure, and the extract was kept under vacuum until the solvent was removed. The extracts were not refined; as the crushed material was not conditioned before the extraction, the composition was similar to that of virgin oil.

The Schaal Oven Test was carried out at 40°C and at 60°C using 100 ml beakers and 25 g samples of peanut oil, and the oxidation was monitored by weighing (Pokorný *et al*. 1985). The induction period (expressed in days) was calculated by the tangent method from the changes of the slope in the time: weight plots.

The accelerated stability tests were performed using the Active Oxygen Method (AOM) at 100°C after the standard procedure (AOCS 1997), the Rancimat method (Hasenhuettl & Wan 1992), and the ML Oxipres apparatus (Mikrolab Oxipres, Aarhus, Denmark). The principle of the last method is heating of a 5 g sample to 100°C under the oxygen pressure of 0.5 MPa. The end of the induction period was determined (Trojáková *et al*. 1999) from the change of the oxygen pressure and it was expressed in hours. The oxidative stability in emulsion was determined at 40°C in the presence of copper salts under continuous shaking and at a free access of air (Zainuddin *et al*. 2002).

RESULTS AND DISCUSSION

Differences in the composition between the two peanut oil samples

The composition and properties of oils isolated from the two peanut cultivars studied (Virginia and SunOleic) were determined only to explain the differences between their oxidation. The results should not be generalized as they do not include the seasonal or locality factors. The differences are, however, sufficiently great to enable some general conclusions. The simplified fatty acid compositions (only the most important fatty acids are included) of the two samples of peanut oil are given in Table 1. It is interesting that the high oleic cultivar SunOleic contained lesser amounts of saturated fatty acids than the conventional (more unsaturated) Virginia cultivar (13.0 and 19.8%, respectively).

Differences in the contents of linoleic and oleic acids in the two samples, respectively, are reflected in the differences in the triacylglycerol (TAG) contents (Table 2). Triacylglycerols containing at least one linoleoyl residue (ECN 40–46) were present in very small amounts in SunOleic peanut oil while in Virginia peanut oil they prevailed.

The contents of α-tocopherol were not substantially different in the two oils (Table 3), while the contents of γ- and δ-tocopherols, which have higher antioxidant activities in bulk edible oils than α-tocopherol (Kamal-Eldin & Appelqvist 1996), were present in moderately higher amounts in SunOleic peanut oil. Differences in the γ- and δ-tocopherol contents would not be sufficient to explain the differences in the stabilities against oxidation as

Table 1. Major fatty acids of peanut oils

Fatty acid (%)	Virginia peanut oil	SunOleic peanut oil
Palmitic	12.2	6.2
Stearic	3.1	1.9
Saturated C20–C26	4.5	4.9
Oleic	48.4	81.7
Linoleic	30.5	2.7
Eicosenoic	0.9	2.2
Trace fatty acids	0.4	0.4

Table 2. Main triacylglycerol fractions of peanut oils

Triacylglycerols (% total triacylglycerols)	Virginia peanut oil	SunOleic peanut oil
ECN 40	0.35	trace
ECN 42	2.40	trace
ECN 44	17.75	1.32
ECN 46	30.20	7.27
ECB 48 and more	44.30	86.60

Table 3. Tocopherol content in peanut oils

Tocopherol (mg/kg)	Virginia peanut oil	SunOleic peanut oil
Alpha	162	201
Gamma	134	214
Delta	6	11
Total	303	426

at the concentrations higher than 200 mg/kg the effect of further additions is only small.

Pronounced differences were observed in the phospholipid fraction (Table 4), as Virginia peanut oil was richer in phosphatidylcholine (PC) while SunOleic peanut oil was richer in acidic phospholipids such as phosphatidic acids and phosphatidylinositol. Phosphatidylcholine has a higher antioxidant activity than acidic phospholipids (Pokorný & Korczak 2001), but the acidic phospholipids have higher activities in binding heavy metals into inactive complex salts (Pokorný *et al*. 1986). Both influences are only moderate so that neither effect would satisfactorily explain the differences in the stabilities against oxidation between the respective oils.

Table 4. Phospholipid composition of peanut oils

Phospholipid class	Virginia peanut oil (%)	SunOleic peanut oil (%)
Phosphatidylcholine	65.2	46.2
Phosphatidylethanolamine	4.0	5.0
Phosphatidic acids	8.7	15.6
Phosphatidylinositol	12.9	23.3
Phosphatidylserine	9.2	9.9

Table 5. Minor substances in peanut oils

Characteristic	Virginia peanut oil	SunOleic peanut oil
Peroxide value (meq/kg)	1.46	0.05
Conjugated dienes (%)	2.13	1.36
Polar lipids (% total triacylglycerols)	2.10	1.68
Monoacylglycerols (%)	0.10	0.10
Diacylglycerols (%)	0.02	0.05
Free fatty acids (%)	0.72	0.69
Phytosterols (%)	0.70	0.56
Acid value (mg KOH/g)	1.48	1.37

The peroxide value was much lower in SunOleic peanut oils than in Virginia peanut oil (Table 5) which is explainable from the different linoleic acid levels in the two oils. Nevertheless, even the peroxide value of Virginia oil was fully acceptable according to Czech regulations of refined or virgin oils for edible uses (10 and 15 meq/kg, respectively). The content of dienoic conjugated fatty acids, which are good markers of lipid oxidation, was also higher in Virginia oil than in SunOleic oil. This difference is understandable as conjugated dienoic acids are formed mainly from linoleic acid by the action of lipoxygenases. The absorbances at 430 nm at the end of the induction period of peanut oil heated to 60°C were 0.123 and 0.028 in the case of Virginia and SunOleic oils, respectively, which is in agreement with the respective contents of linoleic acid.

The acid value and the contents of partial glycerol esters were nearly the same in the two oils studied which signifies that the two brands of peanuts were harvested approximately at the same stage of ripeness. The contents of polar lipids also confirmed a similar quality of the two oils. Contents of carotenoids and chlorophylls were negligible in both oils.

Differences in the oxidative stabilities between the two peanut oils

The oxidative stability showed great differences between the two oils. The SunOleic peanut oil was several times more stable than the conventional peanut oil both at 40°C and 60°C under the conditions of the Schaal Oven Test (Figure 1) which

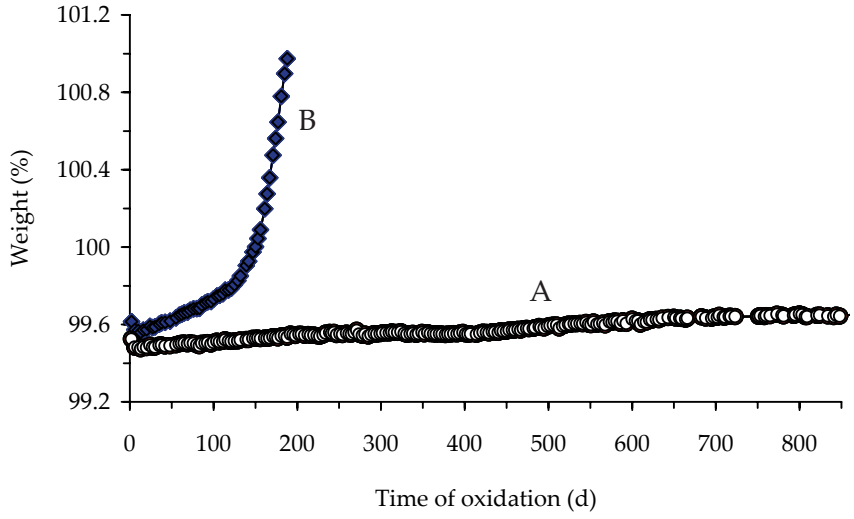

Figure 1. Oxidative stability of peanut oil after Schaal Oven Test at 40°C (A = SunOleic oil, B = Virginia oil)

are close to the storage conditions. The SunOleic peanut oil was substantially more stable even at higher temperatures used in the Active Oxygen Method, in the Rancimat apparatus (Table 6), and in the Oxipres apparatus (Figure 2), which simulate frying conditions. This indicates a much better stability of low-linoleic SunOleic peanut oil both under storage and under frying conditions. This better stability is not due to tocopherols or phospholipids as they would not cause such a big difference in stabilities but it is due to the different contents of polyunsaturated fatty acids or the respective triacylglycerols. Triacylglycerols containing bound linoleic acid were oxidized several times faster than triacylglycerols containing only oleic and saturated fatty acids (Parkányiová *et al*. 2000). The contents of 2,4-decadienals in oxidized oils corresponded to the original linoleic acid contents as decadienals belong to major oxidation products of linoleic acid, especially at higher temperatures (Pokorný 1989). On the contrary, the stability of SunOleic peanut oil was only moderately higher in emulsion as the oxidation was catalyzed by the addition of copper ions.

Table 6. Oxidative stability of peanut oils

Method	Virginia peanut oil	SunOleic peanut oil
Schaal Test at 40°C (days)	120	> 850
Schaal Test at 60°C (days)	30	220
Oxipres (h)	11.9	79.8
Rancimat (min)	465	618
AOM (h)	3.74	> 48
Emulsion (h)	229	337
Decadienal content (mg/kg)	38	2

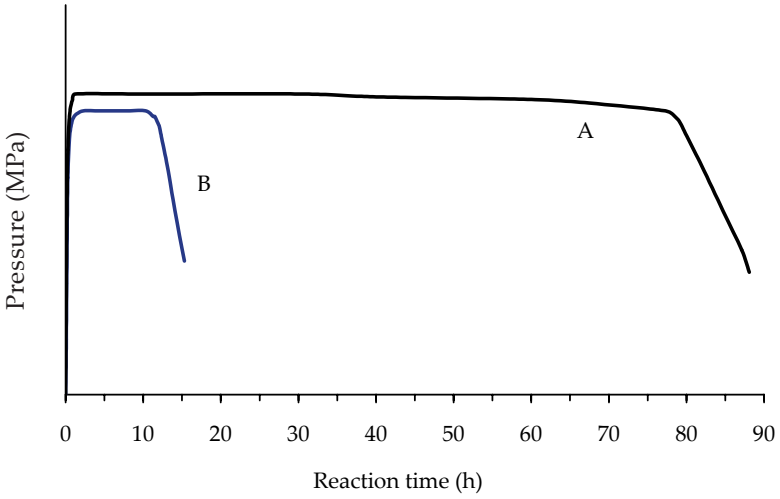

Figure 2. Oxidative stability of peanut oil using Oxipres apparatus at 100°C and oxygen pressure 0.5 MPa (A = SunOleic oil, B = Virginia oil)

Decomposition of tocopherols and carotene in peanut oil during storage

Both types of peanut oil were stored after the addition of 500 mg γ-tocopherol per kg at 6°C under the conditions of the Schaal Oven Test. Peanut oils contained their natural levels of all tocopherols before the addition of γ-tocopherol. Changes of α-tocopherol (initially present in the natural amount) and γ-tocopherol (the sum of the natural content and the added amount, i.e. 634 mg/kg and 704 mg/kg in Virginia and SunOleic oils, respectively) are shown in Figure 3. It is evident that both tocopherols were substantially more stable in SunOleic peanut oil than in Virginia peanut oil. The rate of α-tocopherol destruction was reported to be higher than that of γ-tocopherol destruction under the storage conditions (Nogala-Kalucka & Gogolewski 2000) but the half life-times of both α- and γ-tocopherols were not much different in our experiments (Table 7), which could be explained by a great difference between the initial concentrations of the tocopherols. The relatively small amount of α-tocopherol naturally present cannot efficiently protect the substantially larger excess of γ-tocopherol against oxidation.

In tocopherol-enriched oils, tocopherols were fully decomposed after 35 d storage in the case of Virginia oil, and after 190 d in the case of SunOleic oil, respectively. These values correspond to the induction periods obtained by the Schaal Oven Test at the same temperature of 60°C.

In another experiment, 50 mg of β-carotene was added to both peanut oils. This amount corresponds to its content in some raw vegetable oils, e.g. rapeseed oil from rape grown in the Czech Republic (own unpublished results). The oils were stored again at 60°C under the conditions of the Schaal Oven Test, and the changes of β-carotene were evaluated on the basis of spectrophotometric measurements at 452 nm. The results are shown

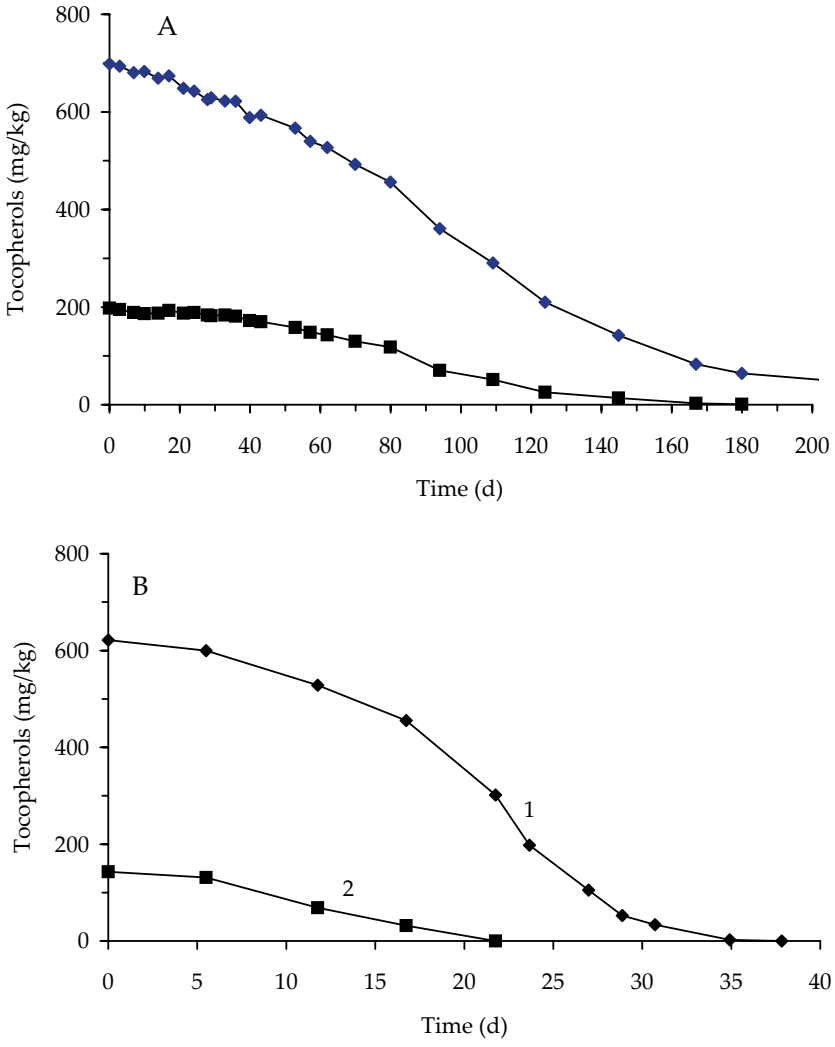

Figure 3. Degradation of tocopherols in peanut oil during storage at 60°C (1 = γ-tocopherol, 2 = α-tocopherol; A = SunOleic oil, B = Virginia oil)

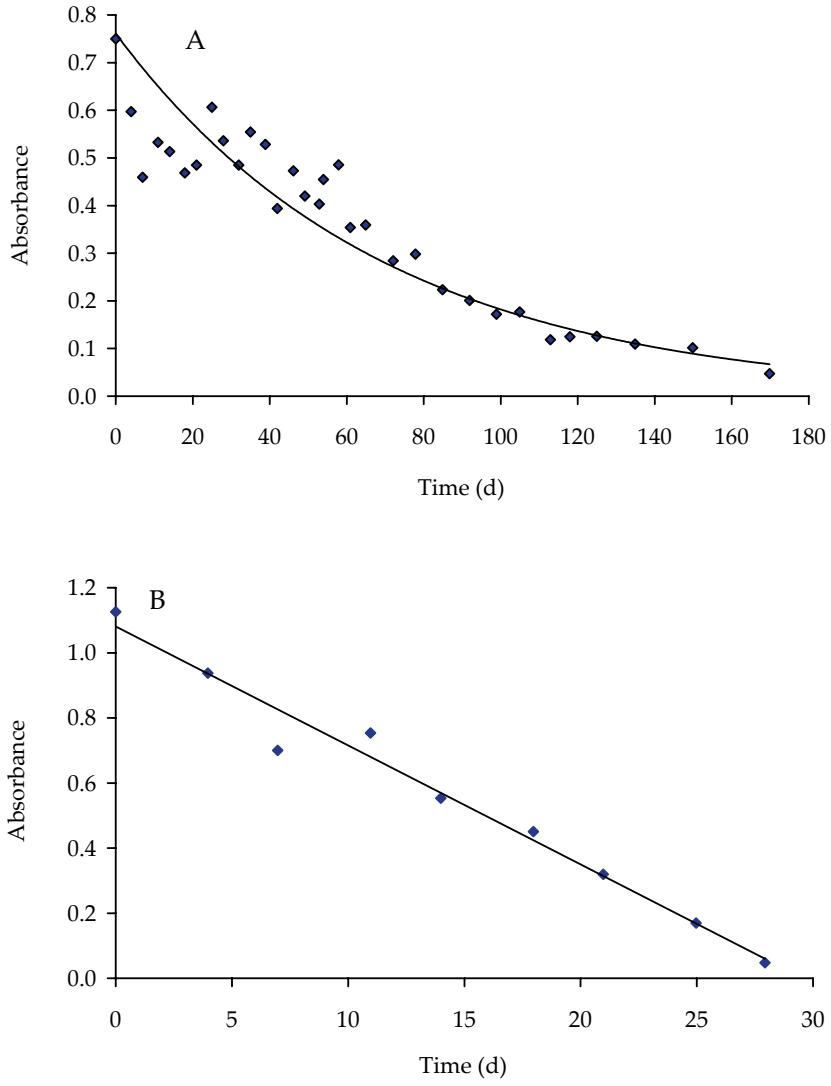

Figure 4. Degradation of β-carotene in peanut oil during storage at 60°C (absorbance at 452 nm; A = SunOleic oil, B = Virginia oil)

in Figure 4. The decomposition of carotene was much faster in Virginia peanut oil than in SunOleic peanut oil, most probably because of the higher initiation rate of linoleic acid oxidation in Virginia oil as compared to SunOleic oil. The decomposition followed an apparent zeroth order kinetics in the case of Virginia oil (the reaction rate constant k = 0.44 A_{452}). In Virginia oil, the content of linoleic acid remained nearly the same during the experiment because of its high initial content. On the contrary, the decomposition followed a first order kinetics in SunOleic peanut oil where changes of linoleic acid content during the β-carotene decomposition could not be neglected. They influenced the reaction rate because of its much lower initial value.

Table 7. Half-life times (d) of tocopherols and carotene in peanut oils at 60°C

Substance	Virginia peanut oil	SunOleic peanut oil
α-Tocopherol	15	87
γ-Tocopherol	22	96
β-Carotene	14	60

On the basis of our results, the SunOleic peanut oil is obviously more suitable as a raw material for the preparation of low-fat functional foods than are the conventional brands of peanut oil.

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Souhrn

Pokorný J., Parkányiová L., Réblová Z., Trojáková L., Sakurai H., Uematsu T., Miyahara M., Yano T. (2003): **Změny při skladování podzemnicového oleje s vysokým obsahem tokoferolů a β-karotenu**. Czech J. Food Sci., **21**: 19–27.

Srovnávali jsme změny tokoferolů a β-karotenu v tradičním podzemnicovém oleji (odrůda Virginia s 30,5 % linolové kyseliny) s modifikovaným podzemnicovým olejem o vysokém obsahu kyseliny olejové (odrůda SunOleic

s 2,7 % linolové kyseliny), vypěstovaným na Floridě v USA. Oba oleje měly srovnatelné výchozí obsahy tokoferolů a oxidačních produktů lipidů včetně hydroperoxidů. Stabilita proti oxidaci byla stanovena za podmínek Schaalova testu při 40 a 60 °C, metodou AOM, v přístrojích Rancimat a Oxipres při 100 °C. Tokoferoly byly stanoveny s použitím HPLC s elektrochemickou detekcí (bez předchozího zmýdelnění). Podzemnicový olej SunOleic s vysokým obsahem kyseliny olejové byl 4–8krát stabilnější proti oxidaci než tradiční podzemnicový olej Virginia. Obsah tokoferolů byl 303 mg/kg v oleji Virginia a 426 mg/kg v oleji SunOleic, což je relativně malý rozdíl, a poměr α- : γ- : δ-tokoferolů byl dosti podobný v obou olejích. Pozorované rozdíly oxidační stability tedy nemohly být způsobeny jen tokoferoly. Rozklad tokoferolů v podzemnicových olejích obsahujících přídavek 500 mg/kg γ-tokoferolu byl během skladovací zkoušky podstatně pomalejší u podzemnicového oleje SunOleic než u oleje Virginia. Obdobné rozdíly byly zjištěny u podzemnicových olejů s přídavkem 50 mg/kg β-karotenu. Vitaminová hodnota se tedy lépe uchovávala v oleji s vysokým obsahem kyseliny olejové než v tradičním podzemnicovém oleji.

Klíčová slova: antioxidanty; autooxidace; karoten; podzemnicový olej; skladování; tokoferoly

Corresponding author:

Prof. Ing. Jan Pokorný, DrSc., Vysoká škola chemicko-technologická, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 224 353 264, fax: + 420 233 339 990, e-mail: jan.pokorny@vscht.cz