Determination of Oxidative Stability in Mixtures of Edible Oil with Nonlipidic Substances

LUDMILA TROJÁKOVÁ, ZUZANA RÉBLOVÁ and JAN POKORNÝ

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Prague, Czech Republic

Abstract

TROJÁKOVÁ L., RÉBLOVÁ Z., POKORNÝ J. (2001): **Determination of oxidative stability in mixtures of edible oil with nonlipidic substances.** Czech J. Food Sci., **19**: 19–23.

The storage of lipid foods is mostly affected by the oxidation of lipid fraction. Dry foods are particularly sensitive because lipids are not protected by hydrated proteins against oxidation. A method suitable for testing dry foods was studied in model mixtures of rapeseed oil with albumin or cellulose. Oxipres apparatus was used, where the course of oxidation is monitored by changes of oxygen pressure. The end of induction period was more evident than in bulk oils as the contact of lipids with oxygen is better. The induction period was longer in mixtures of edible oil with albumin than in mixtures with cellulose. The induction period moderately decreased with increasing oxygen pressure, while the effect of sample weight was nearly negligible. The induction period length was a semilogarithmic function of reaction temperature. Variation coefficients and differences between the duplicates showed good reproducibility; they were lower in mixtures with albumin than in mixtures with cellulose, but both were of the same order as the respective values in bulk oils. At 120°C and 0.5 MPa oxygen, the induction periods could be usually measured within a working day.

Keywords: albumin; autoxidation; cellulose; edible oils; Oxipres apparatus; stability against oxidation

Lipid foods can be stored for only limited time periods because of their insufficient resistance to oxidation by air oxygen. In water containing foods, lipid droplets or membranes are usually protected against oxygen access by a layer of hydrated proteins or carbohydrates. In dry foods, oxygen can easily enter the lipid phase and the stability of such foods is substantially lower than in the presence of water. Therefore, the stability of dry foods should be tested, and if necessary, the lipid fraction should be protected by addition of antioxidants.

Storage tests take too a long time, so that accelerated tests are regularly used, where the rate of oxidation is enhanced either by using higher temperature or by addition of prooxidants. The Schaal Oven Test (carried out at 40–60°C) gives results close to storage conditions, but the test takes several weeks or even months (POKORNÝ *et al.* 1985). The most widely used method is the Oxygen Stability Index (OSI), using the Rancimat apparatus (HILL & PERKINS 1995), which has been standardized (AOCS 1996). Its use was criticized as the procedure conditions are too different from storage conditions so that the interpretation is difficult, often with unreliable conclusions (FRANKEL 1993).

The ASTM Oxygen Bomb method (POHLE *et al.* 1963) has the advantage that the sample is not aerated, and that the oxygen consumption is measured, which corresponds to the primary oxidation reactions. On the contrary, the formation of volatile acidic products, measured by the OSI method, corresponds to secondary reactions of lipid hydroperoxides. The Oxipres apparatus is based on a similar principle like the ASTM Oxygen Bomb Method; the sample is stored in oxygen atmosphere at a high temperature without stirring. The method was recently tested in our laboratory for the determination of oxidative stability of edible oils, and the conditions were optimized (TROJÁKOVÁ *et al.* 1999). The pressure showed hardly any changes during the induction period, and started to decrease rapidly after its end. The repeatability was completely satisfactory, and the analysis could be finished within a working day.

Supported by Grant No. OC918.20 of the Ministry of Education, Youth and Sports of the Czech Republic.

The same procedure was tested for the analysis of lipid foods. The optimization of the procedure and the repeatability were measured in model foods, consisting of mixtures of rapeseed oil with albumin or cellulose as typical representatives of proteins and carbohydrates, respectively, which are the most common food components.

MATERIAL AND METHODS

Material: Rapeseed oil Vegetol was produced from low-glucosinolate zero-erucic rapeseed of Czech origin by combined expeller pressing and solvent extraction, followed by superdegumming of crude oil, and physical refining. The oil was used within the recommended period for consumption. Crude egg albumin was produced by Sigma, microcrystalline cellulose by Lachema, Brno.

Model mixtures were prepared by thoroughly mixing one part of rapeseed oil with three parts of either egg albumin or microcrystalline cellulose. Both nonlipidic constituents were air dry preparations.

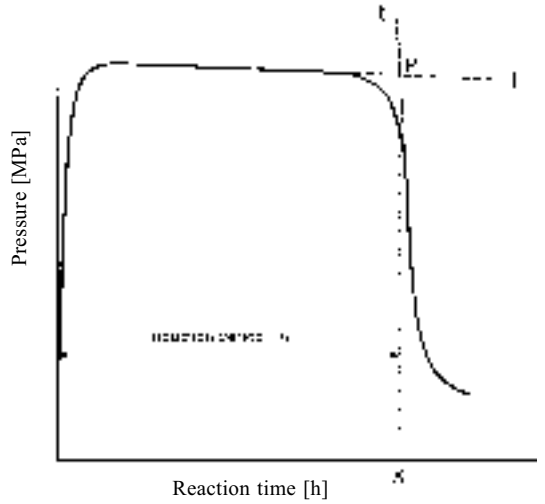
Analytical Methods: The stability against oxidation was determined as described in our previous paper (TROJÁKOVÁ et al. 1999). The apparatus ML OXIPRES (manufactured by Mikrolab Aarhus A/S, Hojbjerg, Denmark) was provided with two measuring cells. The sample was weighed to the nearest 0.01 g into a reactor tube, the reactor was connected to a pressure bottle of oxygen, washed with oxygen, and then filled with oxygen to the defined initial pressure. The tube was then placed into a thermostat, and pressure changes were recorded. The induction period was calculated as the time after which the pressure began to decrease abruptly (its end was measured from the cross-section point of tangents of the first part and the subsequent part of the curve recording the pressure changes, as evident from Fig. 1). For the analysis of edible oils, the optimum conditions are as follows: 5.0 g samples; initial pressure 0.5 MPa; temperature 100°C.

The software Microsoft STATISTICA 6.0 was used for the calculation of variation coefficients, using a one-way ANOVA procedure. The average difference between duplicates was calculated according to Gini (WEBER 1957).

RESULTS AND DISCUSSION

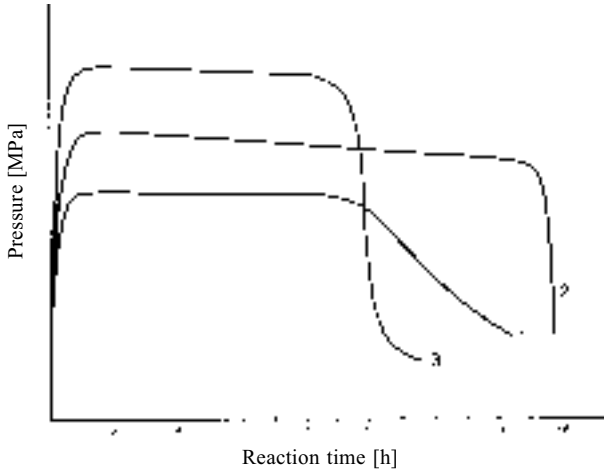
Typical courses of oxidation are evident from Fig. 2. The change of pressure after the end of induction period is more pronounced in the case of model mixtures of oil with albumin or cellulose than in the case of bulk oil because the pressure decrease becomes steeper after the end of induction period. The reason is that the lipid layer on protein or carbohydrate particles is thin and the oil surface large so that oxygen may easily penetrate through the whole volume of lipid phase, while in the case of bulk oil, the layer is much thicker and the oil/air interface smaller, which prevents rapid access of oxygen to lower layers of the analyzed sample.

The effect of the sample weight is evident from Table 1. No effect of the amount of sample was observed, with the exception of lowest weight in the mixture with egg albumin. It could be expected as oxygen can easily enter into channels between particles of albumin or cellulose covered with a film of rapeseed oil. The contact is thus almost independent of the sample volume which determines the pathway of oxygen into the sample. In the case of



P = oxygen pressure [MPa]; t = reaction time [h]; t_1 = tangent to the branch of the time-pressure curve corresponding to the induction period; t_2 = tangent to the branch of the time-pressure curve corresponding to the subsequent stage of rapid oxidation; P = cross section of the two tangents; X = end of the induction period

Fig. 1. Evaluation of the end of induction period



1 = rapeseed oil; 2 = mixture of rapeseed oil with albumin; 3 = mixture of rapeseed oil with cellulose

Fig. 2. Reaction course in the Oxipres apparatus

Table 1. Effect of sample weight [g] on the induction period [h] in mixtures of rapeseed oil with non-lipidic substrates

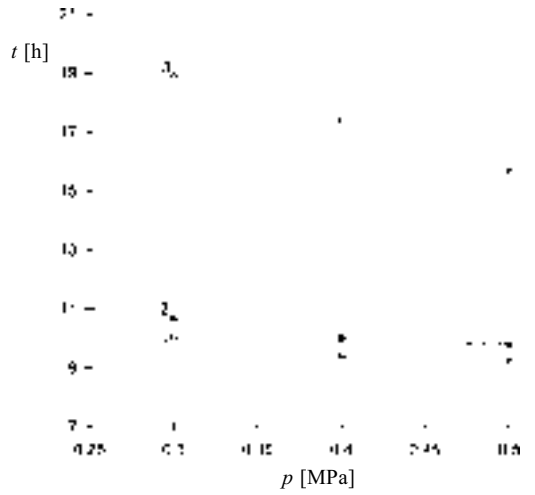
Sample weight	Mixture with	
	egg albumin	cellulose
4	15.23	9.70
8	15.50	9.83
12	15.53	10.05
16	15.45	9.68
20	15.68	9.78
24	15.55	9.63

bulk oil, the effect of sample weight is much more pronounced (TROJÁKOVÁ *et al.* 1999) as oxygen has to penetrate into oil only by diffusion, and the amount of oxygen present in oil depends on the path from the interphase, which is given by thickness of the oil layer. For practical reasons, it is better to weigh between 10–20 g of the sample, especially if the oxidized sample has to be further analyzed, and more sample is thus necessary. The latter value corresponds to 5 g lipids, which was found as the optimum in the case of fats and oils.

The effect of oxygen pressure is shown in Fig. 3. Naturally, higher oxygen pressure results in higher concentration of dissolved oxygen in the lipid phase. The higher concentration of reactant causes higher oxidation rate, and thus, more rapid consumption of present antioxidants. The length of the induction period is nearly proportional to the oxygen pressure, and decreases proportionally to the absolute value of the induction period. As the experiment should be sufficiently short, the initial oxygen pressure of about 0.5 MPa is to be recommended. At a very high oxygen pressure, the higher initiation rate would decrease the antioxidant activity.

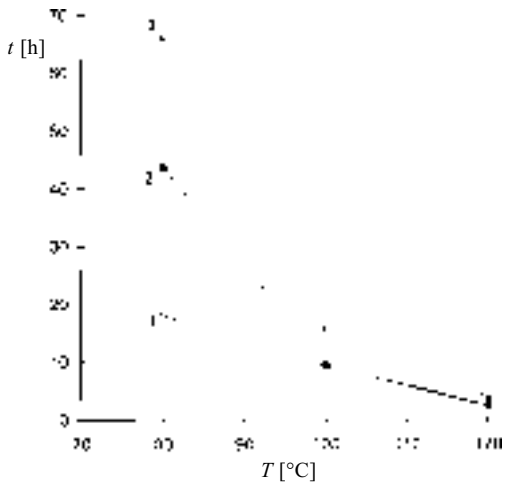
Another variable is the reaction temperature. An example is shown in Fig. 4. The influence of reaction temperature is very pronounced. A semilogarithmic plot yields a nearly linear relation, which is in agreement with the Arrhenius law. The choice of the optimum temperature is difficult, as higher temperatures correspond to shorter reaction times. At 120°C, the reaction is nearly always shorter than a working day, but the results are less easily interpreted as the stability under storage conditions. The antioxidant activity decreases with increasing temperature (MARINOVA & YANISHLIEVA 1992) so that misleading results may be obtained at high temperatures. Depending on the system studied, temperatures of 100°C or slightly higher should be used.

The non-lipidic matrix is another important factor as its physical structure and chemical properties affect the reaction course. Mixtures with cellulose had lower resistance to oxidation than mixtures with albumin, which is in agreement with our former results obtained at atmospheric oxygen pressure (POKORNÝ 1981). The lipidic fraction is stabilized in the presence of protein by reactions of lipid hydroperoxides with amine or sulphur groups of protein (ZAMORA *et al.* 1987, 1989; YEN & FOK 1988; KOUŘIMSKÁ *et al.* 1993). Substantial losses of the respective basic and sulphuric amino acids were observed (NIELSEN *et al.* 1985). The inhibition was observed at higher water activities, too (JANITZ 1987; WANG *et al.* 1991). Fluorescent products, formed during the production, may be used as a measure of reaction products be-



t = induction period [h]; *p* = initial oxygen pressure [MPa]
1 = rapeseed oil; 2 = mixture of raapeseed oil with cellulose; 3 = mixture of rapeseed oil with albumin

Fig. 3. Effect of the initial oxygen pressure on the induction period



t = induction period [h]; *T* = reaction temperature [°C]
1 = rapeseed oil; 2 = mixture of rapeseed oil with cellulose; 3 = mixture of rapeseed oil with albumin

Fig. 4. Effect of the reaction temperature on the induction period

tween proteins and oxidizing lipids (IIO & YODEN 1988). The amphoteric character of proteins contributes to the interaction (SCHWARZ *et al.* 1999), which could decrease the antioxidant activity in the presence of proteins. The results obtained with the edible oil are not necessarily proportional to those obtained in mixtures with non-lipidic materials. The results obtained in carbohydrate-lipid mixtures need not be necessarily correlated with those obtained in protein-lipid mixtures.

The repeatability of analysis was tested under the optimum conditions: 20 g of the reaction mixture (corresponding to 5 g of the lipid phase), 0.5 MPa oxygen pressure, the temperature of 100°C. The results for the mixtures of rapeseed oil with albumin and with cellulose are given in Table 2, and compared with that of rapeseed oil. The difference between duplicates (N = 28), and the standard deviation of 7 determinations was lower in the case of mixtures with albumin than in the case of mixtures with cellulose. Shorter reaction time could contribute to the higher value of oil-cellulose mixtures. The values for pure oils were of the same order, in agreement with our previous results (TROJÁKOVÁ *et al.* 1999).

Table 2. Repeatability of the induction period of rapeseed oil in mixture with a nonlipidic substrate (N = 7)

Statistical parameter	Mixture with	
	albumin	cellulose
Mean [h]	15.68	8.78
Maximum [h]	15.85	10.15
Minimum [h]	15.50	9.55
Standard deviation [h]	0.10	0.23
Variation coefficient [%]	0.66	2.35
Mean difference between duplicates [h]	0.10	0.24

Our results give sufficient evidence that the OXIPRES method may be used for the determination of oxidative stability of dry foods.

References

FRANKEL E.N. (1993): In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends Food Sci. Technol., **4**: 220–225.

IIO T., YODEN K. (1988): Formation of fluorescent substances from degradation products of methyl linoleate hydroperoxides with amino compounds. Lipids, **23**: 1069–1072.

JANITZ W. (1987): Interaktionen der Fette und Eiweiße des Fleisches, I. Dynamik der Fettoxidation und quantitative Bestimmung der Eiweiß-Fett-Komplexe. Z. Ernährungs-Wiss., **26**: 62–72.

HILL S.E., PERKINS E.G. (1995): Determination of oxidation stability of soybean oil with the oxidative stability instrument: Operation parameter effects. J. Am. Oil Chem. Soc., **72**: 741–743.

KOUŘIMSKÁ L., POKORNÝ J., JASCHKE A., RÉBLOVÁ Z. (1993): Reaction of linoleic acid hydroperoxides with muscle proteins under storage conditions. In: SCHWENKE K.D., MOTHES R. (Eds.): Food Proteins: Structure and Functionality. VCH, Weinheim: 236–238.

MARINOVA E.M., YANISHLIEVA N.V. (1992): Effect of temperature on the antioxidative action of inhibitors in lipid autoxidation. J. Sci. Food Agr., **60**: 313–318.

NIELSEN H.K., LÖLIGER J., HURRELL R.F. (1985): Reactions of proteins with oxidizing lipids. 1. Analytical measurements of lipid oxidation of minor acid losses in a whey protein-methyl linoleate model system. Brit. J. Nutr., **53**: 61–73.

POHLE W.D., GREGORY R.L., VAN GIESSEN B. (1963): A rapid oxygen-bomb method for evaluating the stability of fats and shortenings. J. Amer. Oil Chem. Soc., **40**: 603–605.

POKORNÝ J. (1981): Influence des substance non lipidiques sur l'oxydation des corps gras. Rev. Fr. Corps Gras, **28**: 151–160.

POKORNÝ J., DOBIÁŠOVÁ S., DAVÍDEK J. (1985): Repeatability of the determination of oxidative stability of vegetable oils using the Schaal test. Sbor. VŠCHT Praze, E **58**: 163–173.

SCHWARZ K., STÖCKMANN H., HUYNH-BA T. (1999): Physicochemical parameters influencing antioxidant activity in lipid-containing systems. 23rd World Congr. and Exhibition Int. Soc. Fat Research ISF, Brighton; Proc. Abstr.: 60.

TROJÁKOVÁ L., RÉBLOVÁ Z., POKORNÝ J. (1999): Determination of the oxidative stability of fats and oils using the Oxipres apparatus. Czech. J. Food Sci., **17**: 68–72.

WANG J.Y., MIYAZAWA T., FUJIMOTO K. (1991): Inhibition of methyl linoleate peroxidation by maize zeine in powder model system at high water activity. Agr. Biol. Chem., **55**: 1531–1536.

WEBER E. (1957): Grundriß der biologischen Statistik für Naturwissenschaftler, Landwirte und Mediziner. 3. Aufl. G. Fischer, Jena: 66 & 400.

YEN G.C., FOK C.L. (1988): Changes in functional properties following reaction with oxidizing methyl linoleate. J. Chinese Chem. Soc., **26**: 234–244.

ZAMORA R., MILLÁN F., HIDALGO F.J., ALAIZ M., MAZA M.P., OLÍAS J.M., VIOQUE E. (1989): Interaction between the peptide glutathione and linoleic acid hydroperoxide. Nahrung, **33**: 283–288.

ZAMORA R., HIDALGO F.J., ALAIZ M., MILLÁN F., MAZA M.P., VIOQUE E. (1987): Interacción entre el glutation y diferentes oxoácidos grasos. Grasas Aceites, **38**: 318–322.

AOCS Standard Methods and Recommended Practices (1996). AOCS Press, Champaign, IL.

Received for publication January 20, 2000
Accepted for publication March 8, 2000

Souhrn

TROJÁKOVÁ L.,RÉBLOVÁ Z., POKORNÝ J. (2001): **Stanovení oxidační stability směsí jedlého oleje s nelipidovými látkami.** Czech. J. Food Sci., **19**: 19–23.

Skladovatelnost potravin obsahujících tuk je nejčastěji omezena vlivem oxidačních produktů v lipidovém podílu. Suché potravinové přípravky jsou zvláště citlivé k oxidačnímu žluknutí, protože lipidy v nich přítomné nejsou účinně chráněny před přístupem kyslíku vrstvou hydratovaných bílkovin. Pro zkoumání stability suchých potravinových výrobků byl zvolen postup využívající přístroj Oxipres, který se osvědčil již při sledování stability tuků a olejů. Jeho principem je skladování vzorku za zvýšené teploty a zvýšeného tlaku kyslíku; průběh reakce se sleduje ze změny tlaku. Jako modelové potraviny byly zvoleny směsi řepkového oleje s vaječným albuminem nebo s mikrokrystalickou celulosou. Konec indukční periody byl charakterizován zrychleným úbytkem tlaku a byl nápadnější u směsí než u samotných tuků a olejů, protože u směsí je tuk vystaven na větším povrchu působení kyslíku. Indukční perioda byla u směsí oleje s albuminem delší než u směsí oleje s celulosou. Navážka vzorku měla jen nepatrný vliv na indukční periodu. Logaritmus její délky byl nepřímo úměrný teplotě reakce. Variační koeficienty a rozdíly mezi paralelními analýzami prokazovaly dobrou opakovatelnost. Tyto parametry byly nižší u směsí s albuminem (u nichž absolutní hodnoty byly vyšší) než u směsí s celulosou, ale hodnoty zjištěné pro oba typy směsí se podstatně nelišily od hodnot pro čisté rostlinné oleje. Při teplotě 120 °C a tlaku 0,5 MPa trvalo stanovení indukční periody obvykle jeden pracovní den.

Klíčová slova: albumin; autooxidace; celulosa; oleje jedlé; přístroj Oxipres; stabilita proti oxidaci

Corresponding author:

Ing. LUDMILA TROJÁKOVÁ, Vysoká škola chemicko-technologická, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Czech Republic, tel.: + 420 2 24 18 32 64, fax: + 420 2 311 99 90, e-mail: ludmila.trojakova@vscht.cz