

## MODIFIED CARBON PASTE ELECTRODE AS A TOOL FOR THE EVALUATION OF OXIDATIVE STABILITY OF RAPESEED OIL

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### ABSTRACT

Carbon paste electrode was used for evaluation of oxidative stability of rapeseed oil samples using cyclic voltammetry in 0.1 mol.L<sup>-1</sup> HCl as a supporting electrolyte. Rapeseed oil samples were exposed to daylight and oxygen in open glass baker at the laboratory condition in order to obtain oils with accelerated primary and secondary products of oxidation. The oxidation status was determined by peroxide value and p-anisidine value. Total oxidative stability was expressed as TOTOX index. The edible oils were used for preparation (modification) of the carbon paste composite material followed by the cyclic voltammetric measurement. Peroxide values significantly increased whereas p-anisidine value rather fluctuated during 40 days of storage in all the samples. Cyclic voltammograms showed anodic current peaks at 575 – 600 mV and cathodic current peaks at 400 – 425 mV. The oxidation and reduction waves diminished at pH ≥3.0 suggesting not only phenolic compounds contributed to the electrochemical characteristic of oil samples. The peroxide value or p-anisidine value did not correlate with oxidation or reduction peak currents at the potential 575 – 600 mV and 400 – 425 mV, respectively. Both cathodic and anodic currents increased with increasing TOTOX index exhibiting positive correlation with high Spearman correlation coefficient ( $r = 0.894$  and  $r = 0.914$  for anodic and cathodic current, respectively). Linear relationship was found for each sample individually. A caution has to be done when interpreting results since the correlation seems to be of oil sample specific. Nevertheless, the modified carbon paste electrode with rapeseed oil represents a suitable and alternative tool for determination of the oxidative state of edible oils without use of organic solvents.

**Keywords:** edible oil; peroxide value; cyclic voltammetry; carbon paste electrode

### INTRODUCTION

Plant seed oils represent the significant source of fatty acids, sterols and other biologically active substances for human nutrition (McKevith, 2005). Rapeseed oil belongs to the most frequently used vegetable oil in European Union countries. Global rapeseed production gradually increased over the past 20 years with the EU being the principal consumer (including biodiesel production) (Carré and Pouzet, 2014).

Rapeseed oil composition varies among the cultivars of *Brassica* sp. but it is well known that oleic, linoleic and  $\alpha$ -linolenic acids dominated together with other health-promoting compounds such as glucosinolates (progoitrin, gluconapin), tocoferols and phenolics (Pawłowicz et al., 2013; Zheng et al., 2014; Szterk et al., 2010). Phenolic substances also have protective effect against oxidative changes in the edible oils. The oxidation mechanism of the edible oils is very complex, and is influenced by the fatty acid composition, oil processing, energy of heat or light, the type and the concentration of oxygen and transition metals, pigments and antioxidants. The oxidation of the edible oils decreases their nutritional quality and sensory acceptance by the formation of aldehydes, carboxylic acids, alcohols and hydrocarbons as the secondary oxidation products (Choe and Min 2006). Therefore, the

knowledge of the oxidative status is crucial during manufacturing and storage of plant seed oils.

The analytical methods are divided in those determining the primary oxidation products (hydroperoxides) and the secondary ones. The versatile methods for determination of hydroperoxides and secondary oxidation products are peroxide value (PV) and p-anisidine value (p-AV), respectively. A comprehensive reviews upon the other methods for measurement of oxidation rancidity have been recently published (Gromadzka and Wardencki, 2011; Pignitter and Somoza, 2012) including UV/VIS, infrared and Raman spectroscopy, chromatography, nuclear magnetic resonance, scanning calorimetric techniques or luminescent method. Compared with those methods above, electrochemical techniques have some advantages such as high sensitivity, accuracy, simplicity, low expense and the possibility of miniaturization. The changes of electrical conductivity during the reaction of KI and hydroperoxides (Yang et al., 2014) or an optical transmission/capacitance bridge based on the reaction of moisture during heating of the edible oils (Shelke and More, 2013) represent interesting approaches to the evaluation of rancidity. Potentiometric determination of PV and p-AV in the edible oils with triiodide-selective membrane has been proposed for flow injection technique (Saad et al., 2006; Saad et

al., 2007) and Adhoumad and Monser (2008) described direct measurement of hydroperoxides using Prussian-blue modified glassy carbon electrode. Electrochemical characteristics of the edible oils were also used for the determination of the origin or adulteration of the seed oils using chemometric treatment of the data (Gambarra-Neto et al., 2009; Oliveri et al., 2009, Apetrei et al., 2005).

Gambarra-Neto et al., (2009) classified various kinds of edible oils and unsuccessfully attempted to distinguish expired and non-expired oil samples using square wave voltammetry and platinum- and gold-disk electrodes. Oliveri et al., (2009) used Pt-microelectrode in mixture of the edible oil with ionic liquid and evaluated the whole shape of cyclic voltammograms. Our research was inspired by Apetrei et al., (2005) who used different olive oils as a binder material for preparation of carbon paste electrode for their subsequent discrimination. Among all the carbon electrodes, carbon paste electrode (CPE) is an appealing and widely used electrode material in the fields of electrochemistry, electroanalysis, etc. due to its attractive advantages, such as simple preparation, low-cost implementation, renewability, low background current, and wide potential window (Švancara et al., 2012).

The aim of this study was to evaluate the rapeseed oils oxidation status measuring their electrochemical characteristics by cyclic voltammetry using the carbon paste electrode.

## MATERIAL AND METHODOLOGY

### Reagents and equipment

All the reagents were purchased in Sigma-Aldrich spol. s r. o. (Prague, Czech Republic). Deionized water was used in this study ( $G \leq 0.055 \mu\text{S}$ ).

A three-electrode system consisting of CPEs (working), Ag/AgCl/3.0 M KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Eindhoven, The Netherlands) was used for electrochemical measurement. The surfaces of CPE were regenerated by renewing and polishing them on wet filter paper before each measurement.

### Preparation of CPE

The carbon paste composite material was prepared by mixing of 0.5 g graphite powder  $5.5 \mu\text{m} - 7.0 \mu\text{m}$  (CR-5, Maziva Týn n. L. s. r. o., Czech Republic) with 140  $\mu\text{L}$  of rapeseed oil sample in a ceramic mortar. The resulting paste was packed into the teflon piston holder (3.0 mm inner diameter) (Švancara and Metelka, 2000). The resistance of the composite material was always  $\leq 15.0 \Omega$ .

The cyclic voltammetry (CV) was performed with CPE, where RO1-RO3 was used as a binder liquid in 0.1 mol.L<sup>-1</sup> HCl at a scan rate 100 mV.s<sup>-1</sup> (potential range from -200 to +1000 mV, potential step 25.0 mV) or in 0.4 mol.L<sup>-1</sup> Britton-Robinson buffer at the pH 3.0, 7.05 and 10.05 if necessary.

### Sample preparation

Two refined rapeseed (RO1, RO2) oils under private label were obtained from local market (Billa spol. s r. o., Prague and Lidl Holding s.r.o., Prague, Czech Republic), RO3 (*Brassica rapa*) sample was purchased in Sigma

Aldrich spol. s.r.o. (Prague, Czech Republic). The oil samples were stored in refrigerator until use.

After opening the original package of the oil, 75 mL of each sample was put into the 150 mL glass baker and was allowed to expose to daylight and oxygen at laboratory condition ( $23.0 \pm 2.0 \text{ }^\circ\text{C}$ ) for 40 days. The geometry of the liquid in baker was 65 mm  $\times$  35 mm (diameter  $\times$  height). A portion of each oil sample was taken off at 0, 7<sup>th</sup>, 14<sup>th</sup>, 28<sup>th</sup> and 40<sup>th</sup> day of storage for determination of PV (AOAC 2007; Method 965.33), p-AV (IUPAC 1992; Method 2.504) and CPE preparation. Total oxidation value (TOTOX) was calculated as follows (Cao et al., 2014):

$$\text{TOTOX} = 2 \times \text{PV} + \text{p-AV}$$

### Statistical analysis

Statistical analysis was performed by Origin Pro software (OriginLab Corp., MA, USA) on the probability level  $p < 0.05$ . Relationship among electrochemical characteristics of oil samples and oxidation values was evaluated using Spearman correlation coefficient ( $r$ ). Comparison of the dataset was performed using analysis of variance. The chemical and electrochemical analysis was performed at six replicates.

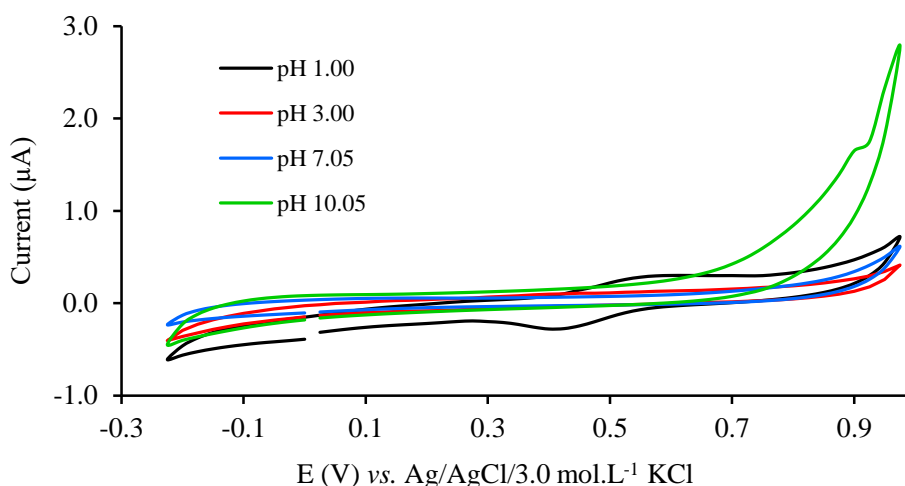
## RESULTS AND DISCUSSION

Peroxide value and p-anisidine value were determined for three rapeseed oil samples during storage in open baker at ambient temperature exposing to daylight for 40 days. As can be seen from Table 1, the initial PVs for RO1 and RO2 samples are below the required level ( $< 10.0 \text{ mmol.kg}^{-1}$ ) for refined vegetable oils recommended in International Food Standard by Codex Alimentarius (IFS 1999).

The sample RO3 is not primarily designed for human consumption but for analytical purposes. The exposition of rapeseed oil samples to daylight and oxygen resulted in increase of PV during 40 days of storage. It was previously published that the light is more important in <sup>1</sup>O<sub>2</sub> oxidation than temperature (Choe and Min, 2006) forming the hydroperoxides, which are slowly decomposed to the secondary oxidation products. It is in agreement with our results where PVs gradually increased until the end of experiments whereas p-AV showed fluctuations in all the samples.

A typical cyclic voltammogram of the CPE modified with RO1 sample is depicted in Figure 1. An anodic wave appeared at the potential range from +575 to +600 mV followed by the cathodic wave from +400 to +425 mV at the pH 1.0 in the reverse scan. Apetrei et al., (2005) stated that some phenolic compounds were oxidized and reduced when they examined olive oils modified CPE. However, electrochemical studies of various phenolic compounds using CPEs described the shifting of the redox potentials to negative region with increasing pH (Švancara et al., 2012).

In our study, the redox waves disappeared even at pH 3.0. We may imply that phenolics naturally occurred in rapeseed oils did not act electrochemically at the experimental conditions described above.



**Figure 1** Cyclic voltammogram of carbon paste electrode modified with rapeseed oil (RO1) at different pH. Scan rate  $100 \text{ mV}\cdot\text{s}^{-1}$  and potential step  $25.0 \text{ mV}$ .

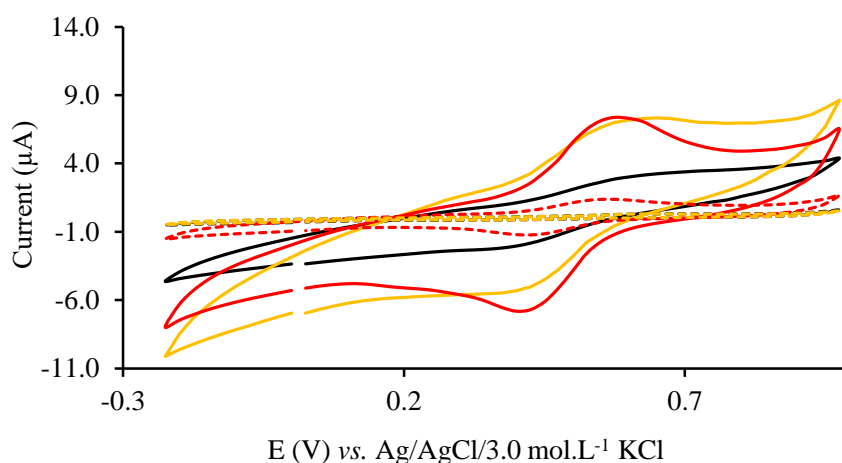
**Table 1** Changes in peroxide value and p-anisidine value during the autooxidation of rapeseed oils (RO1-RO3)\*\* at  $23 \text{ }^\circ\text{C}$  exposed to sunlight ( $n = 6$ ).

| Storage time<br>Days | Peroxide value<br>$\text{mmol}\cdot\text{kg}^{-1} \pm \text{SD}^*$ |                               |                                | p-Anisidine value<br>$\pm \text{SD}^*$ |                               |                              |
|----------------------|--|-------------------------------|--------------------------------|--|-------------------------------|------------------------------|
|                      | RO1  | RO2                           | RO3                            | RO1                                    | RO2                           | RO3                          |
| 0                    | <sup>a</sup> $4.35 \pm 0.72$                                       | <sup>a</sup> $1.82 \pm 0.23$  | <sup>a</sup> $25.50 \pm 1.31$  | <sup>b</sup> $2.41 \pm 0.43$           | <sup>cd</sup> $3.11 \pm 0.12$ | <sup>c</sup> $6.33 \pm 0.25$ |
| 7                    | <sup>a</sup> $5.42 \pm 0.63$                                       | <sup>b</sup> $9.21 \pm 0.20$  | <sup>b</sup> $35.62 \pm 1.45$  | <sup>a</sup> $1.30 \pm 0.21$           | <sup>ad</sup> $2.70 \pm 0.11$ | <sup>b</sup> $5.81 \pm 0.36$ |
| 14                   | <sup>b</sup> $9.53 \pm 0.54$                                       | <sup>c</sup> $11.55 \pm 0.19$ | <sup>b</sup> $42.11 \pm 1.26$  | <sup>c</sup> $3.51 \pm 0.09$           | <sup>bc</sup> $3.34 \pm 0.16$ | <sup>b</sup> $5.23 \pm 0.21$ |
| 28                   | <sup>c</sup> $15.91 \pm 1.00$                                      | <sup>d</sup> $16.50 \pm 0.91$ | <sup>d</sup> $68.90 \pm 0.36$  | <sup>e</sup> $5.34 \pm 0.15$           | <sup>b</sup> $3.62 \pm 0.23$  | <sup>a</sup> $2.64 \pm 0.30$ |
| 40                   | <sup>d</sup> $41.90 \pm 1.75$                                      | <sup>f</sup> $47.22 \pm 0.16$ | <sup>f</sup> $105.71 \pm 0.45$ | <sup>c</sup> $3.85 \pm 0.35$           | <sup>b</sup> $3.71 \pm 0.30$  | <sup>c</sup> $6.84 \pm 0.42$ |

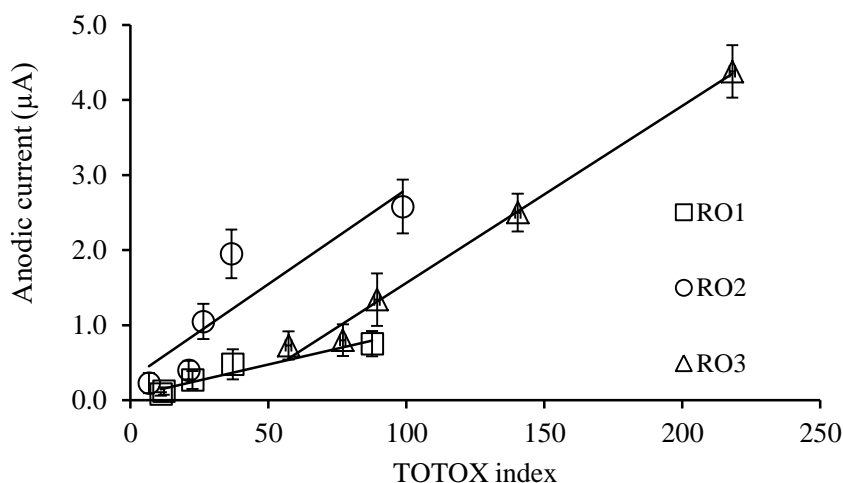
Different small letters (a-f) in superscript indicate statistical differences in columns ( $p < 0.05$ ).

\* Values are presented as mean  $\pm \text{SD}$  of duplicate samples obtained from three individual trials.

\*\* RO1 (Rapeseedoil 1, Billa spol. s r. o., Prague, Czech Republic), RO2 (Rapeseed oil 2, Lidl Holding s.r.o., Prague, Czech Republic), RO3 (Sigma Aldrich spol. s r. o., Prague, Czech Republic).



**Figure 2** Cyclic voltammogram of carbon paste electrode modified with rapeseed oil samples RO1 (black line), RO2 (yellow line) and RO3 (red line) in initial oxidation state (dotted lines) and after 40 days (solid lines) of storage exposed to daylight and oxygen at  $23 \text{ }^\circ\text{C}$ . Scan rate  $100 \text{ mV}\cdot\text{s}^{-1}$ , potential step  $25.0 \text{ mV}$ , pH 1.0.



**Figure 3** Linear regression of oxidation current of CPE modified with rapeseed oil samples upon TOTOX index. Mean  $\pm$ SD ( $n=6$ ). RO1 (Rapeseed oil 1, Billa spol. s r. o., Prague, Czech Republic), RO2 (Rapeseed oil 2, Lidl Holding s.r.o., Prague, Czech Republic), RO3 (Sigma Aldrich spol. s r. o., Prague, Czech Republic).

The same type of working electrode may give different results at similar conditions. For instance, **Oliveri et al., (2009)** analyzed the whole CV curves using Pt-microelectrode for discrimination of edible oils and found no specific oxidation and reduction wave. On the other hand, **Garbarra-Neto et al., (2009)** used Pt-discelectrode for the same purpose, and used currents appearing in cathodic potential region specific for fatty acids detection for chemometric analysis. When performing cyclic voltammetry with CPEs prepared with rapeseed oils in different oxidation state, an increase of the both oxidation and reduction currents appeared (Figure 2). An attempt to find correlation between peroxide value and oxidation ( $I_a$ ) and reduction ( $I_c$ ) current was performed resulting in Spearman correlation coefficients  $r = 0.321$  ( $p = 0.508$ ) and  $r = 0.412$  ( $p = 0.516$ ) for  $I_a$  and  $I_c$ , respectively. Correlations of some edible oil quality parameters have been studied in some research papers.

**Zheng et al., (2014)** found negative linear correlation between the canolol content in rapeseed oils and PV and p-anisidine values. The concentration of nonpolar carbonyl compounds correlated with the TOTOX index in vegetable oils (**Cao et al., 2014**). Statistically significant positive correlation of the current and TOTOX index for all the samples was found in our study giving  $r = 0.894$  ( $p = 7.125 \cdot 10^{-6}$ ) and  $r = 0.914$  ( $p = 1.841 \cdot 10^{-6}$ ) for  $I_a$  and  $I_c$ , respectively. No correlation was found between the current and p-AV in this study. The TOTOX index is used to estimate total oxidative deterioration of oils combining the amounts of primary oxidation products (hydroperoxides) with secondary oxidation products (principally alkenals and alkadienals) (**Cao et al., 2014**). In addition, **Apetrei et al., (2005)** found strong correlation between anodic potential of the virgin olive oil and specific absorbance at 232 nm and 270 nm (showing the amount of polar compounds with conjugated multiply bonds), free fatty acid content, and peroxide value. In our study, the shift of both positive and negative potential values was observed but correlation was not confirmed.

Despite the strong correlation between TOTOX index and anodic or cathodic current, it was unable to mathematically describe such relationship for prediction purposes for all the samples. Therefore, we tried to evaluate each sample independently. The results presented in Figure 3 show the linear relationship between anodic peak current and TOTOX value for each of the rapeseed oil sample with  $r^2$  to be 0.915 ( $p = 0.007$ ), 0.730 ( $p = 0.041$ ) and 0.989 ( $p = 3.394 \cdot 10^{-4}$ ) for RO1, RO2 and RO3, respectively. The same linear correlation was found between TOTOX value and cathodic current with the calculated values  $r^2$  to be 0.971 ( $p = 0.014$ ), 0.819 ( $p = 0.022$ ) and 0.979 ( $p = 8.510 \cdot 10^{-4}$ ) for RO1, RO2 and RO3, respectively.

## CONCLUSION

Rapeseed oil used as a binder liquid instead of mineral oils for the preparation of CPE is an alternative tool for determination of its oxidative stability. Despite the positive correlation between electrochemical characteristics of the binder liquid and the TOTOX index, one should be cautious interpreting results. We proved that each oil sample has different linear relationship of both anodic or cathodic currents, and oxidation quality parameters (TOTOX, p-AV). This behavior is probably rapeseed oil specific and should not be generalized. Nevertheless, the results of our research introduced a novel and a different approach for determination of oxidation stability of edible oils.

## REFERENCES

- Adhoum, N., Monser, L. 2008. Electrochemical sensor for hydroperoxides determination based on Prussian blue film modified electrode. *Sensor. Actuator. B*, vol. 133, p. 588-592. <http://dx.doi.org/10.1016/j.snb.2008.03.039>
- AOAC, 2005. *Official Methods of Analysis of AOAC International*. 18<sup>th</sup> ed. GAITHERSBURG, MD, USA: AOAC International, Official Method 965.33. ISBN: 0-935584-78-1.
- Apetrei, C., Rodríguez-Méndez, M. L., de Saja, J. A. 2005. Modified carbon paste electrodes for discrimination of

- vegetable oils. *Sensor. Actuator. B*, vol. 111-112, p. 403-409. <http://dx.doi.org/10.1016/j.snb.2005.03.041>
- Cao, J., Deng, L., Zhu, X. M., Fan., Y., Hu, J. N., Li. J., Deng, Z. Y. 2014. Novel approach to evaluate the oxidation state of vegetable oils using characteristic oxidation indicators. *J. Agric. Food Chem.*, vol. 62, p. 12545-12552. <http://dx.doi.org/10.1021/jf5047656>
- Carré, P., Pouzet, A. 2014. Rapeseed market, worldwide and in Europe. *OCL*, vol. 21, no. 1, D102. <http://dx.doi.org/10.1051/ocl/2013054>
- Gambarra-Neto, F. F., Marino, G., Araújo, M. C. U., Galvão, R. K. H., Pontes, M. J. C., de Medeiros, E. P., Lima, R. S. 2009. Classification of edible vegetable oils using square wave voltammetry with multivariate data analysis. *Talanta*, vol. 77, p. 1660-1666. <http://dx.doi.org/10.1016/j.talanta.2008.10.003>
- Gromadzka, J., Wardencki, W. 2011. Trends in edible vegetable oils analysis. Part B. Application of different analytical techniques. *Pol. J. Food Nutr. Sci.*, vol. 61, no. 2, p. 89-99. <http://dx.doi.org/10.2478/v10222-011-0009-5>
- Choe, E., Min, D. B. 2006. Mechanisms and factors for edible oil oxidation. *Compr. Rev. Food Sci. F.*, vol. 5, no. 4, p. 169-186. <http://dx.doi.org/10.1111/j.1541-4337.2006.00009.x>
- IFS, 1999. Codex standarad for named vegetable oils. [online] Codex stan 210-1999 [cit. 2015-02-6]. Available at: [http://www.codexalimentarius.org/input/download/standards/336/CXS\\_210e.pdf](http://www.codexalimentarius.org/input/download/standards/336/CXS_210e.pdf)
- IUPAC, 1992. Standard methods for the analysis of oils, fats, and derivatives. 7<sup>th</sup> ed. OXFORD, GB: Blackwell Scientific, Official Method 2.504. ISBN:0-632-03337-1.
- McKeivith, B. 2005. Nutritional aspects of oilseeds. *Nutr. Bull.*, vol. 30, no. 1, p. 13-26. <http://onlinelibrary.wiley.com/doi/10.1111/j.1467-3010.2005.00472.x/abstract;jsessionid=11510D4615C35652C548858484C7C535.f01t04>
- Oliveri, P., Baldo, A., Daniele, S., Forina, M. 2009. Development of a voltammetric electronic tongue for discrimination of edible oils. *Anal. Bioanal. Chem.*, vol. 395, p. 1135-1143. <http://dx.doi.org/10.1007/s00216-009-3070-8>
- Pawłowicz, R., Gromadzka, J., Tynek, M., Tylingo, R., Wardencki, W., Karlovits, G. 2013. The influence of the UV irradiation on degradation of virgin rapeseed oils. *Eur. J. Lipid Sci. Technol.*, vol. 115, p. 648-658. <http://dx.doi.org/10.1002/ejlt.201200126>
- Pignitter, M., Somoza, V. 2012. Critical evaluation of methods for the measurement of oxidative rancidity in vegetable oils. *J. Food Drug. Anal.*, vol. 20, no. 4, p. 772-777. <http://dx.doi.org/10.6227/jfda.2012200305>
- Saad, B., Wai, W. T., Lim, B. P., Saleh, M. I. 2006. Flow injection determination of peroxide value in edible oils using triiodide detector. *Anal. Chim. Acta*, vol. 565, p. 261-270. <http://dx.doi.org/10.1016/j.aca.2006.02.039>
- Saad, B., Wai, W. T., Lim, B. P., Saleh, M. I. 2007. Flow injection determination of anisidine value in palm oil samples using a triiodide potentiometric detector. *Anal. Chim. Acta*, vol. 591, p. 248-254. <http://dx.doi.org/10.1016/j.aca.2007.03.067>
- Shelke, A. V., More, P. S. 2013. Synthesis and analysis of optical transmission/capacitance bridge system for oil deterioration identification. *Int. J. Instrument. Sci.*, vol. 2, no. 2, p. 41-45. <http://dx.doi.org/10.5923/j.instrument.20130202.04>
- Szterk, A., Roszko, M., Sosińska, E., Derewiaka D., Lewicki, P. P. 2010. Chemical composition and oxidative stability of selected plant oils. *J. Am. Oil Chem. Soc.*, vol. 87, p. 637-645. <http://dx.doi.org/10.1007/s11746-009-1539-4>
- Švancara, I., Kalcher, K., Walcarius, A., Vytras, K. 2012. *Electroanalysis with carbon paste electrodes*. BOCA RATON, FL: CRC Press. ISBN: 978-1-4398-3019-2.
- Švancara, I., Metelka, R. 2000. Piston-driven carbon paste holders for electrochemical measurements. In Vytřas, K., Kalcher, K. *Sensing in Electroanalysis*, vol. 1. Pardubice: University of Pardubice, p. 7 – 8. ISBN:80-7194-831-4.
- Yang, Y., Li, Q., Yu, X., Chen, X., Wang, Y. 2014. A novel method for determining peroxide value of edible oils using electrical conductivity. *Food Control*, vol. 39, p. 198-203. <http://dx.doi.org/10.1016/j.foodcont.2013.11.017>
- Zheng, C., Yang, M., Zhou, Q., Liu, C. S., Huang, F. H. 2014. Changes in the content of calanol and total phenolics, oxidative stability of rapeseed oil during accelerated storage. *Eur. J. Lipid Sci. Technol.*, vol. 116, p. 1675-1684. <http://dx.doi.org/10.1002/ejlt.20130229>

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