Original Article

EVALUATION OF THE TEMPERATURE EFFECT ON VEGETABLE OILS BY CHEMICAL ANALYSIS AND ULTRAVIOLET–VISIBLE SPECTROSCOPY

AVALIAÇÃO DO EFEITO DA TEMPERATURE EM ÓLEOS VEGETAIS POR ANALYSES QUÍMICAS E ESPECTROSCOPIA ULTRAVIOLETA VISÍVEL

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ABSTRACT: Several methodologies could be used to characterize vegetable oil besides estimating thermal modification provided by high temperatures. These techniques are used as a proper tool to determine compositional and functional analysis of food ingredients and finished products. In general, vegetable oils are extracted from seeds like the rapeseed (canola oil) soybean (soybean oil), among others. In the present study vegetable oils such as canola and soybean were heated at a temperature of 100°C from 1h up to 28 h and the degraded products were measured to assess the oil stability at temperature. The determination of acid number, peroxide value and iodine number by chemical analysis was carried out for the estimation of the oxidative heat stability of these oils. Ultraviolet–visible spectroscopy strategy was also used to better comprehend this phenomenon, since it greatly improve the performance of measurements, in order to step up sensitivity. The findings demonstrated that vegetable oils thermal deteriorated as seen in the batocromic displacement of the samples heated and increasing the specific absorption in 350 nm. These data were capable to highlight the differences observed in the degree of unsaturation of oils.

KEYWORDS: Soybean. Canola. Acid number. Peroxide value. Iodine number. Ultraviolet–visible spectroscopy.

INTRODUCTION

Vegetable oils recovered by solvent extraction or mechanical pressure contain diverse kinds of lipids. In general, it was found many molecules such as triacylglycerols, free fatty acids and minor compounds like phospholipids, sterol, tocols, carotenoids, etc. Special attention can be paid to the fatty acids, molecules made of carbon, hydrogen and oxygen and potentially responsible for oxidative instability of oil. In fatty acids it is possible to observe the carbon atoms connected by one or two bonds. Molecules of oil with single bonds between carbons atoms are said to be saturated, on the other hand, oil with two or more double bonds are referred to as unsaturated. It is generally known that oil molecules could be degraded as follows: (i) oxidation or autoxidation in contact with ambient air; (ii) thermal or thermal-oxidative decomposition from excess heat; (iii) hydrolysis from contact with water or moisture in tanks and fuel lines; or (iv) microbial contamination from migration of dust particles or water droplets containing bacteria or fungi into the oil.

Canola and soybean oils present significant differences in terms of degree of unsaturation. As discussed by Wanasundara et al. (1995), the content of saturated fatty acids in canola oil is the lowest among vegetable oils and it contains 94% unsaturated fatty acids. Canola oil has a high content (8-12%) of α-linolenic acid as compared to other vegetable oils such as soybean (8%). However, the content of linoleic acid is reasonably lower in canola (22-25%) than soybean (57-60%), as discussed by Lin et al. (2011) and Park 2012. This project reported the thermal oxidation of these vegetable oils and the influence of this parameter on the saturation condition. Monitoring the thermal oxidation on vegetable oils quality during heat presents a major concern for oil producers, suppliers, and consumers. Current literature discussed that iodine number (IV) is the method often used to determine the amount of unsaturation in fatty acids (Henna Lu and Tan, 2009). This unsaturation is in the form of double bonds, which react with iodine compounds. In addition, peroxide and acid numbers are variables that can provide relevant information about the quality of oil after heating. Peroxides produced by lipid oxidation decompose into various smaller molecules such as aldehydes, ketones, alcohols, and carboxylic acids. Some of these volatile products influence flavor, even at very low concentrations, in which both the oil and the food prepared from it become

unpalatable. Therefore, the evaluation of oxidative stability is a key factor to develop new oils for food applications.

Nowadays, several investigations are conducted using spectroscopy techniques such as nuclear magnetic resonance, infrared, UV–Vis, fluorescence, mid-infrared, among others, to determine authenticity or adulteration in food, including vegetable oils. As showed by Nawrocka and Lamorska (2013) spectroscopy methods are highly desirable for analysis of food components because they often require minimal sample preparation, provide rapid analysis, and have the potential to run multiple tests on a single sample. It is important to note that UV/Vis spectroscopy is routinely used as a quality assurance tool to determine compositional and functional analysis of food ingredients, process intermediates, and finished products such as refined vegetable oils.

Absorption spectroscopy in the UV/Vis region is based on the Lambert-Beer's law, expressed by the following Equations (1) and (2):

$$I = I_0 \, 10^{\varepsilon c L} \qquad (1)$$

$$\log\left(\frac{I}{I_0}\right) = \varepsilon c L = A \qquad (2)$$

where I_0, I – intensity of light coming in and out of the sample, respectively; ε – extinction molar coefficient; c– molar concentration of substance; L– thickness of the sample (cm). Plotting the absorption A against a series of oil concentrations (in this case from 10 up to 45%), a linear graph is obtained with slope of $\varepsilon\,(\lambda) * L$.
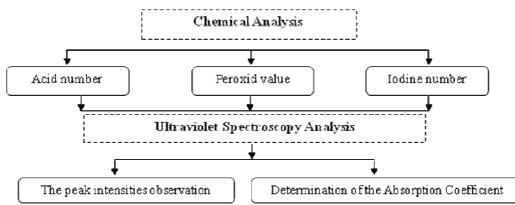
This method could be also attractive to observe changing in vegetable oil compositions after heating. Souza *et al.* (2011) evaluated the 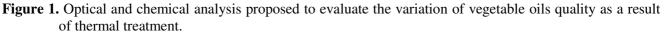oxidative degradation of sunflower biodiesel by UV-visible spectroscopy to monitor the changes using peroxide. The authors observed the peroxide value showed that sunflower biodiesel possesses susceptibility for oxidation, under extreme conditions (120°C and air atmosphere). In addition, this work reports information about the solvent effect on the photophysical properties of vegetable oils exposed to high temperature, and thermal degradation could be provided. It is known that the photophysical behavior of dissolved oil depends on the absorption wavelength of oil in solutions. Besides, maximum absorption also depends on the solvent-solute interactions and solvent nature. It is important to note the spectrophotometric analysis is accurate, low cost and highly reproducible; in addition the coefficient of absorption in the visible range can be a high-quality parameter to indicate the degradation process of such oils.

In the present study vegetable oils, canola and soybean oils, were heated at a temperature of 100°C from 1h up to 28 h and the degraded products were measured to assess the oil stability at temperature.

MATERIAL AND METHODS

Figure 1 present a schematic drawing of the methodologies used in this paper to estimate the thermal decomposition products in vegetable oils (canola and soybean). At least, three chemical-physics parameters (acid number, peroxide value and iodine number) were used to determine the quality of oils. Furthermore, optical analyses were done. UV/vis can be considered as a forward spectroscopy methodology that is sensitive to the electronic transitions in several molecular species. It has been used by many authors helping to note the degradation products of heated oil.

Figure 1. Optical and chemical analysis proposed to evaluate the variation of vegetable oils quality as a result of thermal treatment.

Heating procedure

Refined vegetable oils (canola and soybean) obtained from the local market in Uberlandia (Brazil) were placed in a glass and heated at 100°C for 1, 2, 4, 12 and 28 hours using a oil heater (TECNAL, TE-044-05/50). After each heating period, oil samples were analyzed. The temperature of 100°C was used as a rapid method for simulation of heating in real conditions.

Determination of the acid number (AN)

The acid number could be defined as the number of milligram of KOH required to neutralize one gram of oil. This simple verification is observed when 5 g of oil sample in 25 mL of ether-alcohol (2:1) is placed. Thereon, a few drops of phenolphthalein into it were added and the solution was titrated with NAOH until pink color persists. The AN determination is verified by Equation 3, where A is the volume, in milliliters, of NAOH solution used to sample titration. C is the NAOH solution concentration (0.1 mol L^{-1}), f is a correlation factor of the NAOH solution (0.1 N) and M is the weight (g) of sample.

$$Acid\ number\ (\%) = \left(\frac{A*C*f*100}{M}\right) \qquad (3)$$

Determination of peroxide value (PV)

As International Fragrance Association, the peroxide value is a parameter specifying the content of oxygen as peroxide, especially hydroperoxides in a substance. The peroxide value is an indirect measure of the oxidation presence. To determine PV, 5 g of the vegetable oil was placed into a 250 mL Erlenmeyer flask. After that, 30 mL acetic acid-chloroform (3:2) was added to dissolve oil. Thus, 0.5 mL KI saturated solution was added and the erlen was maintained for 1 minute in a dark place. Thereafter, 30 mL of distilled cold water and 0.5 mL starch indicator were supplemented. Solution titrate with sodium thiosulfate (0.1 mol L^{-1}) was used until blue color disappears. It is important to note a blank determination - 30 mL acetic acid-chloroform + 0.5 mL KI + 30 mL cold water. Finally, starch indicator (0.5 mL) before titrating should be also used. The PV of a pure compound can be computed by Equation 4 where, A and B are the volume, in milliliters, of $Na_2S_2O_3$ solution used to the sample and the blank titration, respectively. C is the sodium thiosulphate ($Na_2S_2O_3$) solution concentration (0.1 mol L^{-1}), f is a correlation factor of the $Na_2S_2O_3$ solution (0.1 N) and M is the weight (g) of the sample.

$$Peroxide\ value\ \left(\frac{meq}{Kg}\right) = \left(\frac{(A-B)*C*f*1000}{M}\right) \qquad (4)$$

Determination of Iodine number (IN)

In the presence of other chemicals, such as iodine, one of the multiple bonds breaks and the iodine atoms attach to the carbon atoms until four atoms are bonded to each carbon atom. In this experiment, iodine is responsible for the amber color of the tincture of iodine solution. In fact, oils combine with iodine in proportion to the number of double bonds the oils contain. The IN of a pure compound can be computed by Equation 5, where, B and A are the volume, in milliliters, of $Na_2S_2O_3$ solution (0.1 N) used to blank and sample titration, respectively. C is the sodium thiosulphate ($Na_2S_2O_3$) solution concentration (mol L^{-1}), f is a correlation factor of the $Na_2S_2O_3$ solution, 12.69 = factor proceeding from the reason between the number of transferred electrons and molar mass of iodine numbers and M is the weight (g) of sample.

$$Iodine\ number\ \left(\frac{gI_2}{100g}\right) = \left(\frac{(B-A)*C*f*12.69}{M}\right) \qquad (5)$$

The procedure involves dissolving the sample (0.1 g) in ethylic alcohol (15 mL) under vigorous stirring and heating (50 °C) for 2 minutes. After that, add 20 mL iodine ethanol solution (0.1 mol L^{-1}) keeping the sample in the dark. Add cold deionized water (200 mL) and titrate excess iodine with sodium thiosulphate. AN, PV and IN were measured by protocols from LUTZ (1985).

UV/Vis spectroscopy oil evaluation

UV/Vis absorption spectra were recorded with USB-650 UV/VIS Spectrometer from Ocean Optics using deuterium tungsten halogen light source (350-1000 nm). Quartz cuvettes with 1 cm optical path length were used for measurements in solution. To absorbance coefficient determination, several dilution ratios (from 10 up to 45 %) of vegetable oil in hexane were employed. Reagents and solvents of analytical grade were used, and the findings were collected in the range of 200-400 nm.

RESULTS

Chemical analysis to determination of the temperature effect in canola and soybean oils

Table I and II summarize the acid and iodine numbers together with peroxide values for canola and soybean oils submitted at different

heating times. It is important to note that fatty acids percentage was reduced with the increases in the period of time thermal exposition. In addition, peroxide value increments were verified. Probably it resulted from the fatty acids oxidation. In general, the primary products of lipid oxidation are hydroperoxides which are commonly referred to as peroxide. As commented by Cao *et al.* (2008), the average for refined soybean oil to acid value is < 0.05 %. On the other hand, Warner and Mounts (1993) reported acid number to canola samples of 0.15 %. Both values are lower than those observed in this data probably due to commercial oil quality. Low peroxide value was also observed (Talpur *et al.*, 2010) to canola samples, 0.17 meq 1000g^{-1},

when compared with this data (from 7 up to 11 meq 1000g^{-1}). Although the peroxide value (PV) is a common measurement of lipid oxidation, its use is limited to the initial stages of oxidation. Iodine numbers, for both oils, continuously decreased with the increments of the heating times at 100°C, since the total unsaturation is also reduced. It is notorious that with high temperature levels carbon double bonds could be broken, and saturated molecules became majority compounds of vegetable oils. Moser (2011) observed iodine number, at room temperature, of 106-109 gI$_2$ 100g^{-1} and 129-132 gI$_2$ 100g^{-1} for canola and soybean oil samples, respectively, corroborating our determinations.

Table 1. Chemical analysis – Canola oil samples heated at 100°C

Sample Conditions	AN (%)	PV (meq 1000g^{-1})	IN (gI$_2$ 100g^{-1})
Control (Not heated)	0.57± 0.06	7.08± 0.01	119.92 ± 2.22
Heated for 1h	0.40 ± 0.08	4.41 ± 1.33	97.81 ± 11.05
Heated for 2h	0.42 ± 0.01	4.98 ± 0.29	82.55± 7.63
Heated for 4h	0.38 ± 0.02	6.83 ± 0.93	75.65 ± 3.45
Heated for 12h	0.21 ± 0.09	11.08 ± 2.13	59.55 ± 8.05
Heated for 28h	0.13 ± 0.10	11.15 ± 1.45	57.67 ± 6.48

Table 2. Chemical analysis – Soybean oil samples heated at 100°C

Sample Conditions	AN (%)	PV (meq 1000g^{-1})	IN (gI$_2$ 100g^{-1})
Control (Not heated)	0.54 ± 0.16	4.66 ± 0.85	139.91 ± 1.97
Heated for 1h	0.83 ± 0.14	7.01 ± 1.18	113.58 ± 13.16
Heated for 2h	0.65 ± 0.09	7.84 ± 0.42	110.81 ± 1.39
Heated for 4h	0.47 ± 0.09	6.76 ± 0.54	95.40 ± 7.71
Heated for 12h	0.12 ± 0.17	9.94 ± 1.60	81.72 ± 6.84
Heated for 28h	0.10 ± 0.10	10.11 ± 0.66	62.48 ± 6.58

UV/Vis spectroscopy as a technique to oil quality evaluation

Figure 2 shows the UV/Vis spectrum for samples after heating at 100°C. This technique is quite useful since it shows the presence of instauration in the organic compounds. It is well known that several products from thermal oxidation

processes show maximum absorption at specific wavelengths, as discussed by Vieira and Regitano-d'Arce (2001). The oxidation of polyunsaturated fatty acids is accompanied by enhanced UV absorption. Fatty acids with conjugated unsaturations strongly absorb in the 230 to 375 nm region (GRAY, 1978; ROVELLINI *et al.*, 1997).
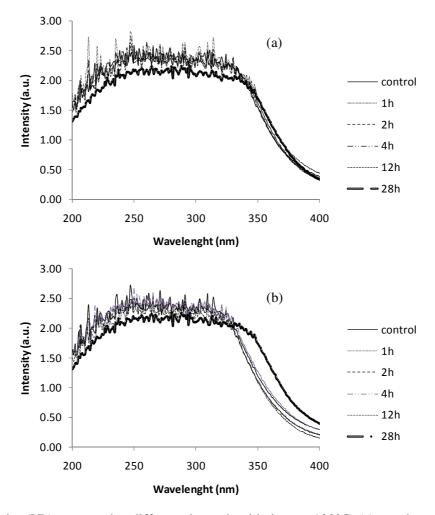
Figure 2. OA intensity (I/I_0) measured at different thermal oxidations at 100°C. (a) canola and (b) soybean.

On the other hand, isolated carbon double bonds have maximum absorption wavelengths nearly 250 nm with bathochromic displacement probably provided by chromophores linked in the π-electron system. This displacement is higher for soybean samples than canola oil due to different thermal degradation times. Figure 3 shows the comparison between soybean and canola oils after heating, when different thermal degradation times are evaluated. It is also important to note that Figure 3(f) confirmed the results presented in Tables 1 and 2, since after 28 h at 100 °C the iodine number is similar to canola and soybean oils, i.e., 57.67 ± 6.48 gI$_2$ 100g^{-1} and 62.48 ± 6.58 gI$_2$ 100g^{-1}, respectively.
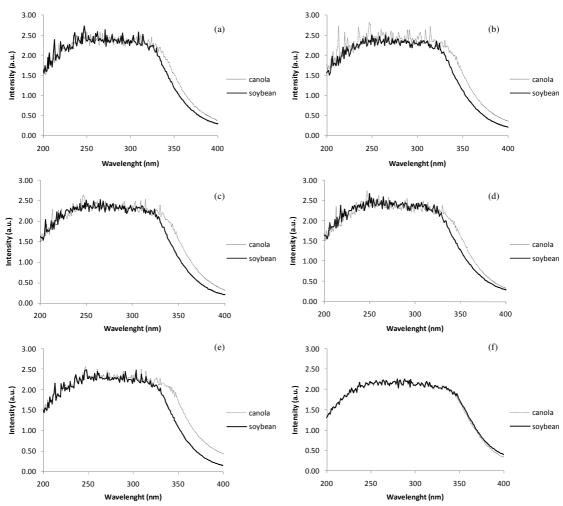
Figure 3. Comparison between the spectrum of canola and soybean oils at different thermal oxidation times at 100°C. (a) control sample not heated; (b) 1h; (c) 2h; (d) 4h; (e) 12h and (f) 28h.

According to the UV/VIS spectroscopy data, the specific absorbance ε is used as a parameter that can give indications about the quality of the vegetable oils and its thermal oxidation conditions. Figure 4 shows ε for both oils observed at 350 nm (on the base of the average of the set data) at the different heating times.
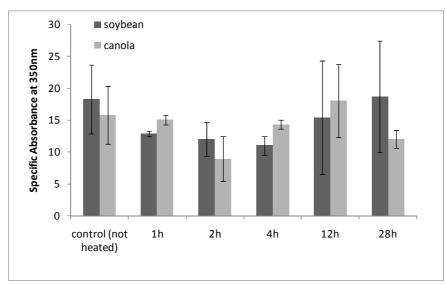

Figure 4. Specific absorbance at 350 nm of vegetable oils (soybean and canola) at different heating times.

DISCUSSION

Measuring vegetable oils thermal deterioration involves testing for the primary and secondary breakdown products. The most common test is peroxide value, although, other measurements of oxidation are the acid number (free fatty acids) and iodine number. Currently, the process of oil deterioration can be described by the peroxide value (PV). The deterioration takes place during lipids exposition to some external factor, including the temperature. It results in production of peroxides, which are regarded as products of fatty acids oxidation. Primary oxidation processes in oil mainly form hydroperoxides, which are measured by the PV. In general, reduced PV indicates a better quality of the vegetable oil. However PV decreases as secondary oxidation products appear. The highest value of PV for oil produced through cold extraction is 10 meq O_2 Kg^{-1}, while regarding refined oil it may reach the amount of 5 meq O_2 Kg^{-1} (NAWROCKA; LAMORSKA, 2013). Also, modern analytical methods are often used for determining the structure indices such as IV. It is important to note that this parameter, which indicates total unsaturation, has even been included in some standards for manufactured products such as vegetable oils.

Complementarily, ultraviolet – visible (UV) spectroscopic analysis has become a versatile technique for providing analytical data on degradation of vegetable oils. Talpur *et al.* (2010) observed the UV method has a major advancement over titration method in terms of using less solvent and reagents. These authors reported that PV ranged between 0.15 and 11.6 meq of active oxygen per kilogram of oil as the canola oil was heated from 0 to 12 h at 180°C. For the temperature dependence, we also heated during different times (from 1h up to 28 h) the soybean and canola oils at 100 °C, and similar results were found for the canola oil, 10.11 meq Kg^{-1} after 28 h at 100 °C by titration methods. Confirming these findings, Figure 4 showed that ε, in general, during the heating dropped and returned to increase for both oils. This behavior could be attributed to the evaluation of the deteriorated compounds such as peroxides and hydroperoxides. In addition, the oxidation of polyunsaturated fatty acids is accompanied by the exchange of isolated double bounds into conjugated double bonds (SOUZA *et al.*, 2011) as observed in Figure 2.

It is important to note that the degradation of vegetable oils quality is caused by the unsaturated fatty acid oxidation that is a complex phenomenon once provides hydroperoxides by different stages. A comparison between soybean and canola oil, after heating up, showed that canola oil seems to be more unstable by thermal variation. As Souza *et al.* (2011), this kind of vegetable oil presenting double bonds are prone to losing hydrogen. In this case, the radical R* could rapidly reacts with oxygen to form a peroxide radical (**ROO***), via free radical chain reaction, and the **ROO*** can gain a hydrogen atom to form hydroperoxides.

Being a versatile technique for the analysis of degradation of vegetable oils, this UV-Vis absorption is also able to give an optical signature for fast and cheap measurement of the oil quality. Because the bathochromic shift can give very similar results compared to the chemical analysis about the oxidation of oils this could become a new tool for quality control with commercial purposes.

Kongbonga *et al.* (2011) discussed that numerous decomposition processes that lead to the formation of hydrocarbons, aldehydes, alcohols and volatile ketones by the formation of vinyl radical. At the same time, other non-volatile secondary compounds are also formed, such as aldehydes, oxidized triglycerides and their polymers. In conclusion, since the heating time is associated with absorptivity in the UV spectrum, this analysis can be used to compare the oxidative stability of oils under heating processes.

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RESUMO: Inúmeras metodologias podem ser utilizadas para caracterizar óleos vegetais, além de estimar modificações térmicas ocasionadas por altas temperaturas. Estas técnicas são usadas como ferramentas específicas para determinar a composição e função de ingredientes em alimentos e em produtos finais processados. Em geral, óleos vegetais são extraídos de sementes como a colza ou soja, entre outros. No presente trabalho, óleos vegetais como canola e soja foram aquecidos a temperatura de 100 °C entre 1 e 28 horas e os produtos de degradação foram medidos para avaliar a estabilidade do óleo na temperatura. As determinações da acidez, índice de peróxido e índice de iodo por análises químicas foram realizadas para estimar a estabilidade e a oxidação térmica dos óleos. A espectroscopia ultravioleta visível foi uma estratégia também utilizada para melhor compreender este fenômeno, uma vez que esta técnica aumenta o desempenho nas quantificações e quando se objetiva o incremento na sensibilidade. Os resultados demonstraram a

deterioração térmica dos óleos vegetais através do deslocamento batocrômico das amostras aquecidas e o aumento da absorção a 350 nm. Estes dados ressaltam as diferenças observadas no grau de insaturação dos óleos.

PALAVRAS CHAVES: Soja. Canola. Acidez. Índice de peróxido. Índice de iodo. Espectroscopia ultravioleta visível.

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