

USE OF NMR H^1 FOR PHYSICO-CHEMICAL PROPERTIES OF VEGETABLE OILS

Ester Carvalho de Souza, esterfisica@yahoo.com.br

Éder Cícero Adão Simêncio, ecasime@yahoo.com.br

Rosa Lúcia Simencio Otero, rosa_simencio@yahoo.com.br

Sylvana Cardoso Miguel Agostinho, sylvana@iqsc.usp.br

Lauralice de Campos Franceschini Canale, lfcanale@sc.usp.br

Universidade de São Paulo, Escola de Engenharia de São Carlos, São Carlos, SP. Brasil.

George Edward Totten, GETotten@aol.com

Portland State University, Department of Mechanical and Materials Engineering, Portland, OR, USA.

Abstract. *Much of all energy consumed worldwide comes from petroleum, coal and natural gas. These sources are limited and estimated to be exhausted in the near future. For this reason, the search for alternative energy sources is extremely important. In this context, the vegetable oils are presented as an alternative study. Analysis of vegetable oils by traditional physical-chemical methods can introduce errors which are often due to deficiencies inherent in the methodology. The fatty acid composition as well as their derivatives, is clearly an issue paper on the physico-chemical properties of vegetable oils and their various applications. The gas chromatography (GC) is the analytical method commonly used in determining the composition of fatty vegetable oils. The Hydrogen Nuclear Magnetic Resonance (NMR H^1) has been very effective in a variety of quantitative analytical purposes related to the chemistry of vegetable oils. In this study, it was proposed an alternative method for determining the average molecular weight, iodine value, saponification number and acid value, and to determine the fatty acids linolenic, linoleic, oleic and saturated (palmitic and stearic acid) of the studied oils (cotton, canola, sunflower, corn and soybeans). The method was created from the equations that consider the spectrum of NMR H^1 . This method uses the area of the protons (for integration) and provides equations for quantitative determination of unsaturated fatty acids present in the oils studied. The results showed that it is possible to use the NMR H^1 with good correlation compared with the official method.*

Keywords: *Vegetable oils, NMR H^1*

1. INTRODUCTION

The steels and other metallic alloys, depending on the phase present and their composition can exhibit a wide variety of properties. Its microstructure and properties can be modified by heat treatment. The quenching heat treatment consisting of hardening steel by quenching of austenite to its transformation into a structure very hard and brittle, the martensite. The cooling rate is specific for each steel depends on its chemical composition and also the ability to extract heat from the quenchant used. The cooling rate can be varied depending on the quenchant. Among the most commonly used quenchants, mineral oil is one of the most favorable characteristics of heat extraction necessary to quench. However, import dependence, vulnerability to price, the large potential for environmental contamination and the fact that inexhaustible source of energy, has attracted the interest of manufacturers and suppliers of oil to quench the research of new fluids and renewable sources in the area quenching heat treatment, Souza (2007).

Vegetable oils have been studied recently for applications quench, especially in the formulation of the basic fluids of cooling, due to the advantages of being inherently biodegradable and renewable source. However, its instability of oxidation and its narrow range of viscosity are the main obstacles in the use of vegetable oils as a quenchant, Canale (2005).

Vegetable oils are of particular interest in Brazil, because Brazil is one of the largest producers of such oil, mainly soybean oil. Several other oils are studied as a basis for formulation of quenching fluids. As an example, Brennan et al (1996 and 1997) studied the canola oil, Honary (1996) studied soybean oil, Lazzeri et al (1999) crambe oil, Prabhu and Fernandes (2007a, 2007b), Prabhu and Prasad (2003), Fernandes and Prabhu (2008) studied the oil palm, coconut, sunflower, peanut and castor.

This work, characterized some vegetable oils through some physico-chemical properties, beyond this analysis be done using traditional methods, is proposed here only the use of Nuclear Magnetic Resonance (NMR) to calculate all the physico-chemical properties of vegetable oils.

2. EXPERIMENTAL

It was used for this study, samples of different vegetable oils, cotton, canola, sunflower, corn and soybeans, edible and available in supermarkets of Brazil. Characterized these oils and their physicochemical properties using the following techniques:

- Acidity Index: ABNT NBR 14248 – 05/04, Petroleum products - Determination of the number of acidity and basicity - Method indicator.
- Iodine Index: According to the Farmacopéia Brasileira (1977)
- Saponification Index: ASTM D 94 – 02, Standard Test Methods for Saponification Number of Petroleum Products.

Also performed the following tests:

2.1. Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance of hydrogen-1 was done in oils using about 10 to 20 mg of sample are dissolved in 0.7 mL of CDCl_3 and their NMR spectra were recorded on Bruker spectrometer, model AC-200, operating in the FT at room temperature.

For the H^1 NMR (nuclei of hydrogen-1) used the following acquisition parameters: pulse: 45° ; relaxation time: 3.0 s, acquisition time: 3.14 s, scanning width: 2,600 Hz, width Line: 0.1 Hz. 16 repetitions were accumulated for each free induced decay (FID).

The analysis of NMR spectra is based on Adhvaryu et al (2000), Reda and Carneiro (2006) and Guillén and Ruiz (2003). Figure 1 shows an example of analysis by Reda.

CAPTION:

- a = methyl protons;
- b = methyl protons of lonilenic acid;
- c = methylene protons of fatty acids of triacylglycerol;
- d = β -carboxylic protons;
- e = allylic protons outside;
- f = α -carboxylic protons;
- g = internal allylic protons;
- h + i = methylenic protons of the glycerol;
- j = H-2 proton of glycerol methylenic;
- k = olefinic protons.

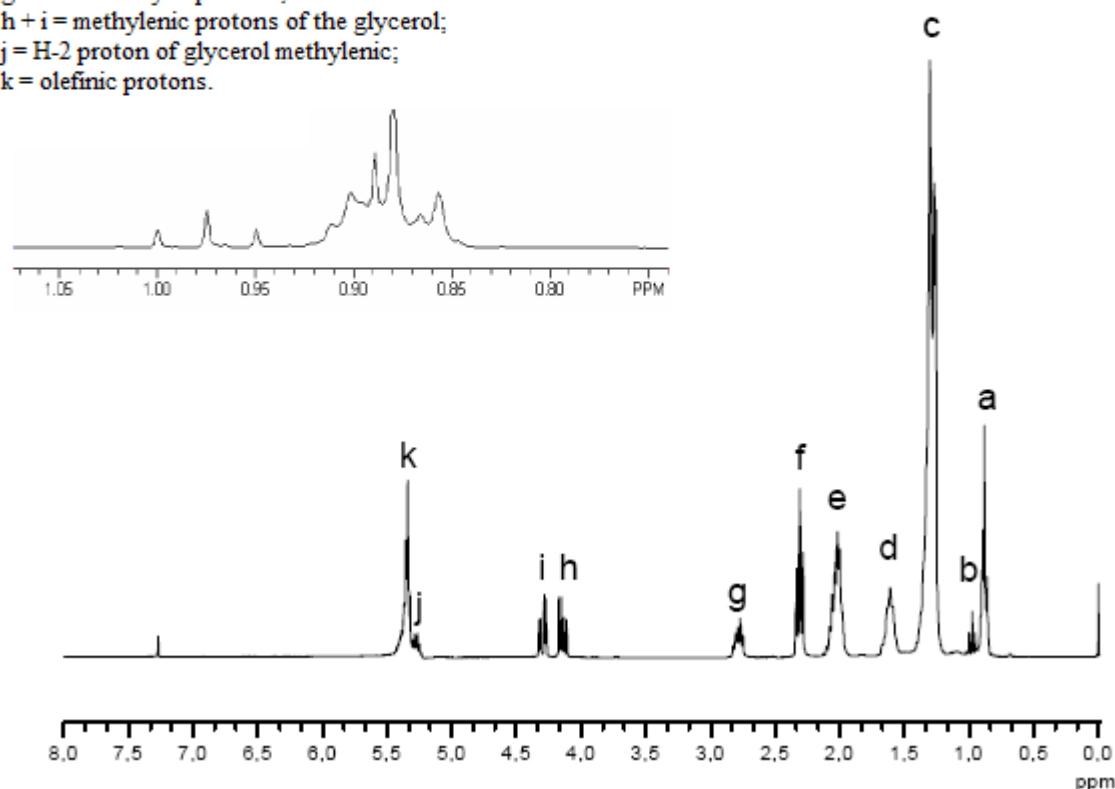


Figure 1. H^1 NMR spectrum of a generic vegetable oil, Reda (2006).

Analyzing an NMR spectrum of H^1 as shown in Fig. 1, the calculated iodine and saponification index was made, according to Reda and Carneiro (2006), with the following equations:

Calculating the area of a proton (A_p):

$$A_p = \frac{i + h}{4} \quad (1)$$

Calculus of olefinic protons:

$$V = \frac{(k + j) - A_p}{A_p} \quad (2)$$

where:

(k + j) = represents the area corresponding to vinyl protons, obtained by direct reading of the integrated spectrum;
 (i + h) = protons of two methylene groups of glycerol. The methine hydrogen (H-2) glycerol appears at 5.26 ppm overlapped to vinyl protons in the curve of integration. Therefore, the area will be on a proton (i + h)/4.

Calculation of the total protons (T):

$$T = \frac{(k + j + i + h + f + e + d + c + b + a)}{A_p} \quad (3)$$

Calculation of average molecular weight of triacylglycerol (MW):

$$MW = 119.7 + 7.036T + 5.983V \quad (4)$$

Calculation of iodine index by H¹ NMR:

$$I.I = \frac{126.91 \times 100V}{MW} \quad (5)$$

The calculation of the saponification index (SI) can be obtained by the following equation:

$$S.I = (MW \times -0.2358) + 398.42 \quad (6)$$

For the linear correlation between the ratio olefinic protons/aliphatic (Ro, a), according to Reda et al (2007):

$$Ro, a = \frac{V}{a + b} \quad (7)$$

The acidity index can be calculated by the following equation:

$$A.I = -0.0298(Ro, a)^2 + 0.1265(Ro, a) + 0.683 \quad (8)$$

The determination of the proportion of oleic (O), linoleic (L), linolenic (Ln) and saturated (S) can be performed using the following equations, according to Guillen and Ruiz (2003):

$$Ln(\%) = 100[B/(A + B)] \quad (9)$$

$$L(\%) = 100\{(E/D) - 2[B/(A + B)]\} \quad (10)$$

$$O(\%) = 100\{(C/2D) - (E/D) + [B/(A + B)]\} \quad (11)$$

$$S(\%) = 100[1 - (C/2D)] \quad (12)$$

2.2. Gas Chromatography (GC)

The samples were brought to the testing of gas chromatography to analyze the composition of fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids, main components of vegetable oils.

The chromatographic analysis of samples were performed in a gas chromatograph (GC-17-A) from Shimadzu, equipped with a flame ionization detector (FID) set to 300 °C and a "split" injection system ratio of 1:30 at 280 °C for the injection volume of 1 µL. N₂ was used as an auxiliary gas and average linear H₂ gas flow rate was 35 cm/s. The

column used was LM-100 (100% polyethylene glycol) with dimensions 25 m x 0.25 mm id. x 0.25 μ m. The temperature of the column was maintained isothermally at 190 °C for 25 minutes. These tests were performed according to an adaptation of the method described by Christie (1989) and Neto (2000).

3. RESULTS AND DISCUSSION

3.1. Nuclear Magnetic Resonance

After completion of the NMR at 200 and 400 MHz, and obtained the spectra of each oils studied, calculated the areas under each as shown in Fig. 1 and these values are shown in Tab. 1 (200 MHz) and Tab. 2 (400 MHz).

Table 1. Value of the area of H¹ NMR spectrum (200 MHz).

Vegetable Oils	1	2	3	4	5	6	7	8	9	10
	A	B			C	D	E			F
	a	b	c	d	e	f	g	i + h	j	k
Canola	7.6	1.1	53.0	7.0	10.8	6.0	2.5	3.8	1.5	6.7
Sunflower	7.8	1.0	51.5	7.3	10.9	5.9	2.9	3.8	1.6	7.3
Cottonseed	8.5	0	52.7	8.2	9.0	6.1	3.2	4.0	1.5	6.8
Corn	8.3	0.8	52.7	7.1	10.1	5.9	2.8	3.8	1.6	6.9
Soybean	7.8	1.2	50.5	7.2	10.3	6.1	3.8	3.9	1.6	7.7

Table 2. Value of the area of H¹ NMR spectrum (400 MHz).

Vegetable Oils	1	2	3	4	5	6	7	8	9	10
	A	B			C	D	E			F
	a	b	c	d	e	f	g	i + h	j	k
Canola	7.9	0.3	66.2	7.6	12.5	6.9	1.9	4	1.1	7.7
Sunflower	6.3	0.5	55.2	5.7	10.2	5.3	2.4	4	1.4	7.5
Cottonseed	7.1	0	55.4	6	8.2	5.6	2.8	4	1.4	7
Corn	6.4	0	55	6.2	9.1	5.6	2.4	4	1.4	7.4
Soybean	5.3	0.2	52.7	6.4	8.9	6.4	3.5	4	1.4	8.2

Using these values, calculated the iodine index, saponification index and index acidity for each of the vegetable oils studied according to Eq. 5, 6 and 8, respectively. These values are shown in Tab. 3.

Table 3. Physico-chemical characteristics of the oils studied.

Vegetable Oils		Canola	Sunflower	Cottonseed	Corn	Soybean
Iodine Index (CgI ₂ /g)	Conventional method	109	125	110	115	130
	NMR (200MHz)	110	118	110	113	125
	NMR (400MHz)	102	119	113	118	132
Acidity Index (mgKOH/g)	Conventional method	0	0	0	0	0.2
	NMR (200MHz)	0.77	0.78	0.77	0.77	0.78
	NMR (400MHz)	0.78	0.79	0.78	0.79	0.81
Saponification Index (mgKOH/g)	Conventional method	-	-	-	-	188
	NMR (200MHz)	191	189	199	189	193
	NMR (400MHz)	170	200	203	201	203

For the iodine index presents three results, the conventional method, by NMR at 200 MHz and NMR at 400 MHz. By the conventional method of iodine index ranged from 109 to 130, cited by NMR at 200 MHz from 110 to 125 and NMR at 400 MHz from 102 to 132. As always the lowest value for the canola oil and higher value for soybean oil. It is this small variation, and this method can be applied to get an idea of the values of iodine index without having to perform the conventional method.

As for the acidity index ranged from 0.77 to 0.78 for NMR at 200 MHz and 0.78 to 0.81 for the NMR at 400 MHz, results similar to those found by Reda et al (2007). For the conventional method was obtained 0 and 0.2 for soybean oil. The results were quite different for the two methods, but both pointed soybean oil with higher acidity.

The saponification index was measured by NMR at 200 MHz (189 to 199) and by NMR at 400 MHz (170 to 203), the conventional method this result was only measured for soybean oil which made the value of 188. For all oils, the NMR at 400 MHz shows better results obtained by NMR at 200 MHz, with the exception of canola oil that had a

reduction. The iodine and saponification index found by NMR for the corn oil are similar to those reported by Reda and Carneiro (2006).

Through the data presented in Tab. 1 and 2, it is also possible to calculate the percentages of fatty acids present in vegetable oils. Were calculated the oleic, linoleic, linolenic and saturated by Eq. 9 to 12 respectively. The values obtained are presented in Tab. 4.

Table 4. Composition of fatty acids in vegetable oils ¹.

Vegetable Oils		Canola	Sunflower	Cottonseed	Corn	Soybean
Oleic (%)	GC	65.4	19.4	17.0	35.0	20.8
	NMR (200MHz)	61.9	53.3	20.9	47.5	35.1
	NMR (400MHz)	66.7	58.3	23.2	38.4	18.5
Linoleic (%)	GC	20.2	58.6	56.7	48.0	58.6
	NMR (200MHz)	15.2	27.3	53.1	29.3	36.6
	NMR (400MHz)	20.2	30.6	50.0	42.9	47.4
Linolenic (%)	GC	5.2	0.5	0.4	0.8	5.0
	NMR (200MHz)	13.0	11.2	0	9.2	13.1
	NMR (400MHz)	3.7	7.4	0	0	3.6
Saturated (%)	GC	6.6	10.2	24.0	14.0	13.2
	NMR (200MHz)	9.9	8.2	26.0	14.0	15.2
	NMR (400MHz)	9.4	3.8	26.8	18.8	30.5

1 - Percentages may not add up to 100% due to presence of other minor constituents not listed.

The method has different results for the oils studied. For the amount of oleic acid have values very close to 65.4, 61.9 and 66.7 (conventional method, NMR at 200 MHz and NMR 400 MHz, respectively), what is considered as good results, but for the same acid, we found a wide variation when it comes to sunflower oil, this shows the value of 19.4 for the conventional method and 53.3 and 58.3 for the NMR methods at 200 and 400 MHz, far from the values found by many conventional method.

3.2. Gas Chromatography

The composition of the different vegetable oils used for the work were quantified by gas chromatographic analysis and the results obtained are shown in Tab. 5. Substantial variation in the compositions of these vegetable oils is evident.

Table 5. Composition percentage of fatty acids present in samples of vegetable oils. ¹

Vegetable Oils	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Relative Reactivity to Oxidative Degradation ²
Corn	12.0	2.0	35.0	48.0	0.8	1.43
Soybean	10.5	2.7	20.8	58.6	5.0	1.90
Canola	4.5	2.1	65.4	20.2	5.2	1
Cottonseed	22.0	2.0	17.0	56.7	0.4	1.60
Sunflower	7.0	3.2	19.4	66.0	0.5	1.85

1. Percentages may not add up to 100% due to presence of other minor constituents not listed.

2. This is a value calculated from the stearic acid, oleic acid, linoleic acid and linolenic acids using the reported relative reactivity to oxidation of: 1/10/100/200 respectively. Place the lower value to 1 and other figures with him.

The stability of vegetable oils to oxidation depends not only on the degree of unsaturation (double bond content) but also on the degree of double bond conjugation. For example, linoleic acid is especially reactive relative to oleic acid and linolenic acid is more reactive than linoleic acid. The most stable form of the fatty acid components is when there are no double bonds present (completely saturated) such as stearic acid and palmitic acid. The following approximate relative oxidation rates were reported: stearic (1), oleic (10), linoleic (100), linolenic (200), according Kodali (2002) and Schneider (2002).

It is known that the overall reaction rate of a process is the sum of the various individual reaction rates of a process. Since vegetable oils possess different fatty acid components, each with a particular and different oxidation rate, the overall oxidation rate is approximately equivalent to the sum of the oxidation rates of the fractional composition of stearic (and palmitic), oleic, linoleic and linolenic acids. This is only approximate since there are other unidentified

unsaturated fatty acid components in very low concentrations. This is the reason why different vegetable oils exhibit different properties.

The data in Tab. 5 show that vegetable oils have relative stability to oxidation of:

$$\text{Canola} < \text{Corn} < \text{Cottonseed} < \text{Sunflower} < \text{Soybean}$$

The sequence presented is the same obtained by Chasan (1994), where the canola oil has a lower tendency to oxidize. While soybean oil is one of the vegetable oils of greatest interest for quenchant formulation, it is almost twice the potential for oxidation than canola oil, the most stable oil in this study.

Doing the same analysis using the values of the acids obtained by NMR at 200 MHz and 400 MHz, the results are presented in Tab. 6 and Tab. 7.

Table 6. Composition percentage of fatty acids present in the sample of vegetable oils obtained by NMR 200 MHz

Vegetable Oils	Saturated	Oleic Acid	Linoleic Acid	Linolenic Acid	Relative Reactivity to Oxidative Degradation
Corn	14.0	47.5	29.3	9.2	1.11
Soybean	15.2	35.1	36.6	13.1	1.40
Canola	9.9	61.9	15.2	13.0	1
Cottonseed	26.0	20.9	53.1	0	1.17
Sunflower	8.2	53.3	27.3	11.2	1.16

Table 7. Composition percentage of fatty acids present in the sample of vegetable oils obtained by NMR 400 MHz

Vegetable Oils	Saturated	Oleic Acid	Linoleic Acid	Linolenic Acid	Relative Reactivity to Oxidative Degradation
Corn	18.8	38.4	42.9	0	1.37
Soybean	30.5	18.5	47.4	3.6	1.65
Canola	9.4	66.7	20.2	3.7	1
Cottonseed	26.8	23.2	50.0	0	1.53
Sunflower	3.8	58.3	30.6	7.4	1.49

The data in Tab. 6, which are related to the NMR 200 MHz, show that vegetable oils have relative stability to oxidation of:

$$\text{Canola} < \text{Corn} < \text{Sunflower} < \text{Cottonseed} < \text{Soybean}$$

Already looking at Tab. 7, which is related to the NMR 400 MHz, shows that vegetable oils have relative stability to oxidation of:

$$\text{Canola} < \text{Corn} < \text{Sunflower} < \text{Cottonseed} < \text{Soybean}$$

Both the results reported by NMR presents the same sequence of vegetable oils, and the only difference to the result by gas chromatography is that first comes the sunflower oil and cottonseed oil after. All results presented as canola oil as the more stable and soybean oil as the greatest potential to oxidize. Interestingly we have the same results by analyzing the oxidation stability of each oil, although the individual results for the values of the fatty acids are quite different in each analysis.

4. CONCLUSIONS

The use of H^1 NMR for determination of iodine index, acidity and saponification and determination of fatty acids have shown good results when compared to conventional methods. In all methods, soybean oil has the highest iodine index of canola oil and the lowest. Also, all results in soybean has the highest acidity.

Analysis of fatty acids from vegetable oils studied shows that canola oil is more stable and soybean oil is more favorable oxidation.

5. ACKNOWLEDGEMENTS

The authors acknowledge their appreciation to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support of this work and to MSc Renata Leal for performing the physical property analysis reported here.

6. REFERENCES

- Adhvaryu, A., Erhan, S. Z., Liu, Z. S., Perez, J. M., 2000, "Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy", *Thermochimica Acta*, n.364, pp.87-97.
- Brennan, R. J., Faulkner, C. H., 1996, "A new quenching alternative", in Totten, G. E., Funatani, K., Howes, M. A. H., Sjoström, S. (Eds.), *Conf. Proceed. 2nd International Conference on Quenching and Control of Distortion*. ASM International, Materials OH, pp. 423-428.
- Brennan, R. J., Faulkner, C. H., Massuda, D., 1997, "A quenchant based on canola oil", *Advanced Materials & Processes*, Vol. 152, No. 2, pp. 32S – 32U.
- Canale, L. C. F., Fernandes, M. R., Agostinho, S. C. M., Totten, G. E., Farah, A. F., 2005, "Oxidation of vegetable oils and its impact on quenching performance", *Int. J. Materials and Product Technology*, Vol. 24, pp. 101-125.
- Chasan, D. E., 1994, "Oxidative stabilization of natural oils", *Society of Tribologists and Lubricants Engineers*, Ciba.
- Christie, W. W., 1989, "Gas Chromatography and Lipids: A Practical Guide", *The Oily Press, Bridgwater, United Kingdom*, pp.11-27, 69.
- Farmacopéia Brasileira, 1977, 3^a Edição, Atheneu Editora São Paulo Ltda.
- Fernandes, P., Prabhu, K. N., 2008, "Comparative study of heat transfer and wetting behaviour of conventional and bioquenchants for industrial heat treatment", *International Journal of Heat and Mass Transfer*, Vol. 51, No. 3-4, pp. 526-538.
- Guillén, M. D., Ruiz, A., 2003, "Rapid simultaneous determination by proton NMR of unsaturation and composition of acyl groups in vegetable oils", *Eur. J. Lipid Sci. Technol.*, 105, pp.688-696.
- Honary, L. A. T., 1996, "Performance of vegetable oils as a heat treat quenchant", in Totten, G.E., Funatani, K., Howes, M.A.H., Sjoström, S. (Eds.), 1996, *Conf. Proceed. 2nd International Conference on Quenching and Control of Distortion*, ASM International, Materials OH, pp. 595-605.
- Kodali, D. R. 2002, "High Performance Ester Lubricants from Natural Oils", *Industrial Lubrication and Tribology*, Vol.54, No.4, pp.165-170.
- Lazerri, L., De Mattei, F. Bucelli, F., Palmieri, S., 1999, "Crambe oil: A potentially new hydraulic oil and quenchant", *Ind. Lubr. Tribol.*, Vol. 49, No. 2, pp. 71-77.
- Neto, P. R., 2000, "Produção de biocombustível alternativo ao óleo diesel através da transesterificação de óleo de soja usado em frituras", *Química Nova*, São Paulo, Vol.23, No.4, pp.531-537.
- Prabhu, K. N., Prasad, A., 2003, "Metal/quenchant interfacial heat flux transients during quenching in conventional quench media and vegetable oils", *J. Mat. Eng. and Perf.* Vol. 12, No. 1, pp. 48-55.
- Prabhu, K. N., Fernandes, P., 2007a, "Effect of surface roughness on metal/quenchant interfacial heat transfer and evolution of microstructure", *Materials and Design*, Vol. 28, pp. 544-550.
- Prabhu, K. N., Fernandes, P., 2007b, "Determination of wetting behavior, spread activation energy, and quench severity of bioquenchants", *Metallurgical and Materials Transactions B*, Vol. 38, No. 4, pp. 631-640.
- Reda, S. Y., Carneiro, P. I. B., 2006, "Parâmetros físico-químicos do óleo de milho in natura e sob aquecimento calculado pelo programa proteus RMN H1", *Publ. UEPG Ci. Exatas Terra. Ci. Agr. Eng.*, Ponta Grossa, 12 (2): pp.31-36, ago.
- Reda, S. Y., Costa, B., Sossela R., 2007, "Determinação do índice de Acidez por RMN-H¹ do Biodiesel Etílico de Milho", 2^o Congresso da Rede Brasileira de Tecnologia de Biodiesel <http://www.biodiesel.gov.br/rede_arquivos/caracterizacaoII.htm>.
- Schneider, M., 2002, *Government Industry Forum on Non-food Uses of Crops (GIFNFG 7/7) - Case Study Plant Oil Based Lubricants in Total Los san Potential Loss Applications*, Final Report prepared for P. Smith Network Manager, University of York, England.
- Souza, E. C., 2007, "Estudo da oxidação do óleo de soja com diferentes concentrações de aditivos anti-oxidantes, para uso em tratamentos térmicos de têmpera", *Dissertação (Mestrado) – Interunidades em Ciência e Engenharia de Materiais*, Universidade de São Paulo, São Carlos.

7. RESPONSIBILITY NOTICE

The authors are the only responsible for the printed material included in this paper.