

Estabilidade oxidativa de óleo de soja contendo concentrações contrastantes para ácido linolênico durante o processamento
Oxidative stability of soybean oil contrasting for linolenic acid content during processing

Eleonice Moreira Santos¹; Solange Aparecida Paula²; Everaldo Gonçalves de Barros¹; Marco Túlio Coelho Silva¹; Luiz Antonio dos Santos Dias¹

¹Universidade Federal de Viçosa – Av. PH Holfs, S/N – Campus Universitário – Viçosa/MG

²Departamento de Química e Exatas, Universidade Estadual do Sudoeste da Bahia, Av. José Moreira Sobrinho, S/N, 45206-191, Jequié - BA, Brasil

Resumo

O conteúdo de ácidos graxos poliinsaturados está diretamente relacionado com a estabilidade oxidativa do óleo. Este trabalho avalia a estabilidade oxidativa de amostras de óleo, obtidas a partir de isolinhas de soja contendo diferentes concentrações de ácido linolênico em alguns estágios de refino, por meio de testes acelerados com aumento de temperatura e de luz. As isolinhas usadas continham em média 4,63 (LLA) e 11,73% (NLA) de ácido linolênico. Ao final do processo de extração do óleo a isolinha LLA mostrou um rendimento de óleo 6% maior que a isolinha NLA. No teste oxidação acelerada pelo aumento de temperatura os óleos da isolinha LLA nos vários estágios de refino, foram significativamente mais estáveis que os da isolinha NLA. No teste de oxidação acelerada pelo aumento da exposição a luz, ambas isolinhas mostraram uma diminuição da estabilidade oxidativa, independentemente do conteúdo de ácido linolênico.

Palavras-chave: oxidação lipídica, processo de refino, oxidação termal e fotoxidação

Abstract

The content of polyunsaturated fatty acids is directly linked to the oil oxidative stability. This work evaluated the oxidative stability of oil samples obtained from soybean isolines contrasting for linolenic acid content in the refining stages, through oxidative accelerated tests by increased temperature and light. The isolines used have, on average, 4.63 (LLA) and 11.73% (NLA) of linolenic acid. At the end of the extraction process the isoline LLA showed an oil yield of 6% higher than NLA. In the accelerated oxidation test by using increased temperature the oils from LLA isoline, from the various refining steps, were significantly more stable than those of NLA isoline. The accelerated oxidation test by using increased light exposure showed a decrease of the oil oxidative stability from both isolines, independent of the linolenic acid content.

Keywords: lipid oxidation, refining process, thermal oxidation and photooxidation

Introduction

Oil is the most common of several products from soybeans. It is widely consumed for domestic and industrial use, due to its versatility and its market value [1]. The soybean oil is refined and the components that affect its stability and final quality are removed [2].

Each refining stage is responsible for removing one or more substances. In the degumming stage the phospholipids are removed (1st stage); in the neutralization stage the content of free fatty acids is reduced (2nd stage); in the bleaching stage the pigments present in the oil are reduced (3rd stage); and

in the last stage of refining, the oxidized compounds and part of the residual compounds from previous treatments are removed [3,4].

Soybean oil, like most edible oils, is composed of triglycerides, and contain on average 11% palmitic acid, 4% stearic acid, 24% oleic acid, 54% linoleic and 7% linolenic acid [5]. This relatively high linolenic acid content makes the soybean oil more susceptible to oxidation [6]. The distribution of fatty acids in the triacylglycerol molecules, especially the proportion of unsaturated fatty acids determines the oil quality, nutritional value, flavor and physical properties, such as oxidative stability and melting point [7,8].

The polyunsaturated fat acid content is a critical parameter for the oil industry, due to their low oxidative stability. Oils with high concentration of polyunsaturated fatty acids must be chemically hydrogenated to increase their oxidative stability [9,10]. However, the use of chemical hydrogenation may produce significant amounts of trans fat acids [11,7]. Thus, breeding soybean cultivars for low linolenic acid content is assumed to increase the oil oxidative stability [12-15].

The oxidative stability is a measure of resistance to oxidation and indicates the oils and fats qualities. It can be evaluated through accelerated tests that monitor the resistance to oxidation. The chemical changes suffered by oils during oxidation can be evaluated by measuring the primary oxidation products (hydroperoxides) and/or the secondary compounds resulting from the peroxides decomposition (aldehydes, ketones, alcohols, esters and others) [16].

The aim of this work was to characterize the soybean oil derived from isolines containing contrasting concentrations of linolenic acid, and the oxidative stability during some stages of refining, through accelerated tests with temperature and light increased.

Materials and Methods

Genotypes characterization

For selecting the soybean isolines used in the experiment, 42 lipoxygenases free genotypes obtained from the soybean breeding program for quality Improvement being carried out at the Federal University of Viçosa, MG, Brazil, were evaluated. The evaluation consisted in quantifying the seed fatty acids (palmitic, stearic, oleic, linoleic and linolenic) by gas chromatography using the method described by Bubeck and others [17]. We used a gas chromatograph model Shimadzu GC-17A equipped with FID detector and a DB-Wax column - J&W Scientific (30 m x 0.25 mm) with the following chromatographic conditions: injector 245 °C, detector 280 °C and the column with programmed temperature of 200 °C with an increase of 3 °C / min until the final temperature of 225 °C. The carrier gas used was nitrogen at a rate of 1.3 mL. min⁻¹ and the results were expressed as percentage of relative area of the compounds.

Crude oil

Crude oil was obtained from about 35 kg of each soybean isolate, were submitted to pressing. Subsequently, the residual oil from the derived cake was extracted, using petroleum ether as solvent.

The extraction process quantification

Samples of soybean cake were quantified in relation to the percentage of residual oil by the method described by IAL 2005 [18] to verify the effectiveness of the procedures used in the oil extraction.

Refining Process

Degummed oil

Degummed oil was obtained by the method described by Erickson and Diez [19,20]. To the crude oil was added 2% distilled water, heated to 65 °C and simultaneously stirred at 230 rpm for 30 min. After that, the mixture was centrifuged for 25 min at 25 °C and 6.000 x g. The supernatant was used after drying.

Neutralized oil

A solution of NaOH 8% was added to degummed oil and it was stirred for 20 min, at 60 °C. Then the temperature was increased to 80 °C for 5 min, and after that the mixture was centrifuged. The supernatant was washed with distilled water, citric acid 0.5% and then dried [21].

Bleached oil

To obtain the bleached oil, 2% of activated clay was added to neutralized oil. The process was carried out using a rotary evaporator under vacuum of 200 mm Hg, at 80 °C, and agitation for 20 min [22].

Temperature test: Schaal oven test

The stability of oils was determined by modification of Schaal Oven test. About 20 grams of oil in each refining stage, corresponding to the treatments crude, degummed, neutralized and bleached oil, were placed in beakers with a capacity of 100 mL and sealed with film paper, without the addition of antioxidants, and placed in oven at 60 ± 1 °C for 144 h. Periodically (0, 24, 48, 72, 96, 120, 144 h), a sample of each treatment was collected and submitted to measurements of peroxide value, free acidity, quantification of fatty acids and volatile compounds (hexanal) [23].

Light test

Light chamber

A light chamber adapted from Siqueira and Moser [24,25], was built for packaging of samples for the accelerated photooxidation test. The chamber consisted of a masonry rectangular container, with dimensions 80 x 35 x 60 cm. Inside it 6 fluorescent lamps were placed with power of 20 W - 3 on the upper part and 3 arranged on the bottom of the chamber - the light intensity was 8370 lux.

Photooxidation test

About 20 grams of oil of each refining stage were placed in beakers with a capacity of 50 mL and sealed with film paper without addition of antioxidants, and they kept in light chamber, with an average temperature of 39 ± 1 °C for 144 h. Periodically (0, 24, 48, 72, 96, 120, 144 h), a sample of each treatment was collected and submitted to measurements of peroxide value, free acidity, quantification of fatty acids and volatile compounds (hexanal).

Characterization of oils

Peroxide Value (PV)

PV of each oil fraction was determined according to AOCS Official Method Cd 8-53 [26].

Free Fatty acids (FFA)

The FFA content was determined by AOCS Official Method Ca 5a-40 [26]

Fatty acids methyl esters (FAME)

The FAME composition of each oil treatments was adapted of Bubeck and others [17], and performed by gas chromatograph, model GC-17A Shimadzu, equipped with FID detector using a DB-Wax column - J & W Scientific (30 m x 0.25 mm), with the following chromatographic conditions: injector temperature 245 °C, detector 280 °C and heating the column with programmed temperature of 200 °C with an increase of 3 °C / min until a final temperature of 225 °C. The carrier gas used was nitrogen with a flow of 1.3 mL. min⁻¹ and the results were expressed as percentage of relative area of the compound.

Volatile compounds (hexanal)

The analysis of volatiles were adapted from Snyder and others, Ulbert and Ulbert and others [27-29], using a gas chromatograph, model GC-17A Shimadzu, equipped with FID detector and headspace sampler. The initial set temperature was 30 °C with an increase of 5 °C / min to 80 °C and 15 °C / min until the final temperature of 220 °C. The carrier gas was nitrogen with a flow of 1 mL / min. Samples containing 1 g of soybean oil were placed in 20 mL vials. The vials were sealed and heated at 90 °C for 20 min for the thermostat time. After 1.5 mL the sample of the headspace gas was injected into the chromatograph and eluted onto a Carbowax-L & M (25 m x 0.32 mm) column. The quantification of hexanal (ppm) was obtained by making use a standard curve of pure hexanal obtained from Sigma.

Statistical Analysis

All data were analyzed by using analysis of variance (ANOVA) with the SAS - Statistical Analysis System.³⁰ For the qualitative factors, the means were compared using Tukey test, adopting the 5% level of probability. For the quantitative factors, the models were chosen based on the significance of regression coefficients using the t test, taking the 5% level of probability.

Results and Discussion

Extraction, refining and characterization of soybean oil

Two isolines were selected from the genotypes derived from Monarca cultivar to extraction of oil, due the present contrasting differences in linolenic acid content. Isoline 3033 TNCA CS-3 (LLA) containing 4.64% of linolenic acid and isoline 3033 TNKA CS-3512-34 (NLA) containing 11.7% of linolenic acid.

The oil content present in both isolines was about 18%, and after extractive processes, the LLA isoline showed yield 6% higher than the NLA. During the process of oil extraction there was a reduction in linolenic acid content of 20% and 33.6% for LLA and NLA respectively. Despite, this reduction was maintained a significant difference in linolenic acid content between the isolines, in different stages of oil refining (Table 1).

Table 1: Characterization of soybean oil

Parameter		P V (<i>meq/ kg oil</i>)	FFA (% <i>oleic acid</i>)	Fatty acids (area %)				
				16:00	18:00	18:01	18:02	18:03
LLA oils	Crude	0,18 ^b	0,97 ^b	11,0 ^a	3,5 ^a	18,9 ^a	62,7 ^a	3,7 ^b
	Degummed	0,70 ^b	0,87 ^b	11,1 ^a	3,6 ^a	18,6 ^a	62,5 ^a	3,6 ^b
	Neutralized	7,05 ^a	0,09 ^a	11,3 ^a	3,6 ^a	18,6 ^a	61,9 ^a	3,7 ^b
	Bleached	1,66 ^a	0,13 ^a	10,9 ^a	3,5 ^a	18,8 ^a	62,6 ^a	3,6 ^b
NLA oils	Crude	1,51 ^a	1,31 ^a	11,1 ^a	3,4 ^b	17,4 ^b	59,9 ^b	7,8 ^a
	Degummed	1,78 ^a	1,21 ^a	11,1 ^a	3,2 ^b	17,3 ^b	59,9 ^b	7,7 ^a
	Neutralized	7,10 ^a	0,13 ^a	11,1 ^a	3,3 ^a	17,8 ^a	60,0 ^b	7,8 ^a
	Bleached	0,93 ^a	0,12 ^a	11,0 ^a	3,46 ^b	17,3 ^b	59,9 ^b	7,7 ^a

Values in the same column and the same oils (Crude, Degummed, Neutralized and Bleached) with the same superscript letters are not significantly different ($P < 0,05$).

According to Gerd and others [14] the reduction of linolenic acid content, the main fatty acid involved in the oxidation of vegetable oils, during the oil processing, provides a better quality of the final product. However, in this case, the reduction was not necessary because the difference in the linolenic acid content between NLA and LLA was important for the evaluations performed in this study.

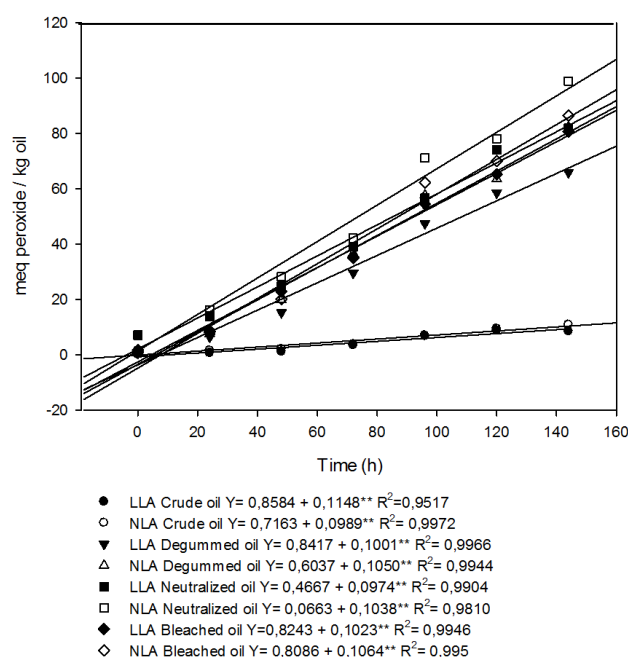
The initial characterization of isoline oils in each stage of refining, crude, degummed, neutralized and bleached before the temperature accelerated test were similar (Table 1). The crude and degummed NLA oils showed higher values for the PV and FFA, while the neutralized and bleached oils showed no significant difference. The considerable increase in PV was found in neutralized oils from the NLA and LLA. However in the subsequent step, the bleached oil, the values for peroxide again were reduced. The FFA content of NLA and LLA oils reduced significantly after the neutralization step, ensuring the reduction expected with the procedure used. This reduction is expected since in the neutralizing step the free fatty acids neutralized by the reaction with an alkaline solution are eliminated as soaps [3,21].

Oxidative stability

Peroxide value: Temperature test

The NLA and LLA crude oils for most of the period showed similar rate of oxidation. For the NLA degummed and neutralized oil, the PV during the time interval measured was higher. The same behavior was observed in the NLA bleached oil in the final period (96, 120 and 144 h). The profile of PV of all evaluated oils was obtained by linear regression (Fig.1), and they all maintained a gradual increase of PV until the end of 144 hours, showing no tendency of stabilization.

Vegetable oils with no antioxidants in temperature test, as in this case, has no tendency to stability [31]. The significant difference for most oxidation of NLA refining oils is mainly due to its high content of linolenic acid, which is the most important precursor in the deterioration of oils. This is attributed to its number of double bonds [32,33].



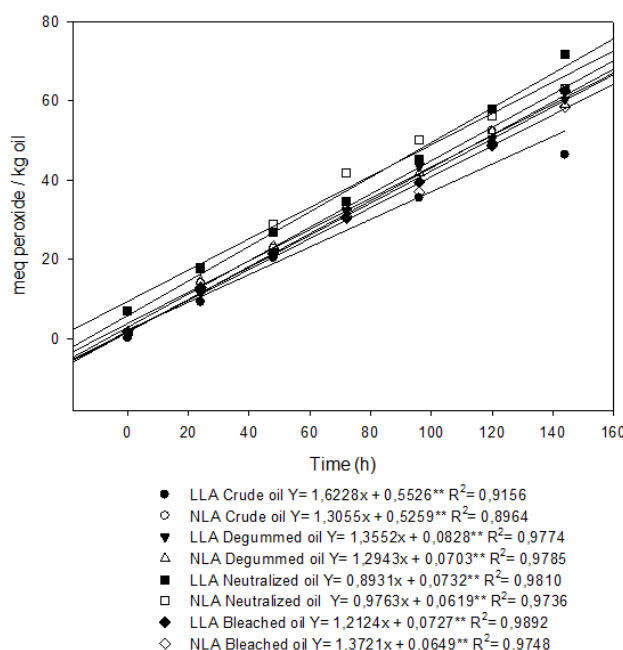
** Linear regression plot PV against time: significantly at 1% level of probability by F test.

Figure 1: PV evolution in crude, degummed, neutralized and bleached oils, submitted a temperature test at 60 ± 1 °C for 144 hours.

Peroxide value: Photooxidation test

During the evaluation of oils in accelerated photo-oxidation chamber, the PV increased linearly along the 144 hours for all oils with unstable oscillations among them in some time intervals. The profiles obtained by linear regression are shown in Fig. 2. The NLA neutralized oil had the highest rate of oxidation and LLA crude oil the lowest. The light effect on oxidative stability measured by hydroperoxides formation was very similar for the oils from both isolines in the refining stages, despite the significant difference in the linolenic acid content.

The similarity of behavior among the stages of refining is due to the time of light exposure. The longer the time, the greater is the oil oxidation. This is perceived in a few hours of light exposure, with significant increase in PV. In photochemical oxidation, the amount of hydroperoxide formed is proportional to total amount of light absorbed [34-36,25].



** Linear regression plot PV against time: significantly at 1% level of probability by F test.

Figure 2: PV evolution in crude, degummed, neutralized and bleached oils, submitted a photooxidation test at 39 ± 1 °C for 144 hours

Free Fatty Acids contents

The FFA values showed similar profiles for temperature and photooxidation tests (Fig. 3). Higher values of FFA were found for NLA crude and degummed oils, followed by LLA crude and degummed oils during the 144 hours. For the remaining oils the FFA values were lower and they did not show significant difference along the period analyzed. The high FFA values for crude and degummed oils are due to their refining stage, in which the free fatty acids have not yet been removed. The little variability observed in the FFA during the time evaluated among the treatments is mainly due to low humidity present in the oils (data not shown), once the increase in free acidity may be caused by hydrolytic rancidity which is influenced by the presence of humidity [37].

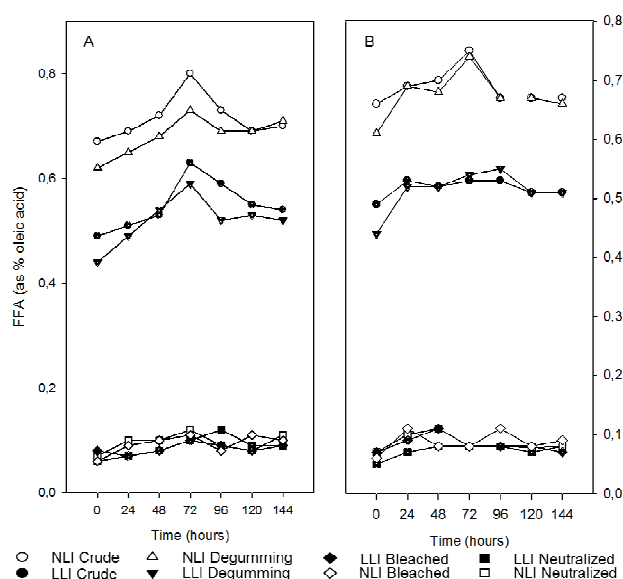


Figure 3: FFA % the crude, degummed, neutralized and bleached oils submitted a temperature test at 60 $\pm$ 1 °C (A) and stored in light chamber (B), for 144 hours.

Linolenic acid and hexanal contents

Linolenic acid content of NLA isolate oils remained statistically superior to the oils obtained from LLA during 144 h in the temperature and photooxidation test (Fig. 4 and 5). Among the oils (crude, degummed, neutralized and bleached), the neutralized oils of isolines NLA and LLA showed a significant decrease in linolenic acid content over the 144 h period, showing its greatest potential for oxidation, which could be confirmed by their high PV (Fig. 1 and 2). The reduction in linolenic acid content, especially in situations in which the oil is subjected to heating, is due to oil degradation, as reported by other authors [14,38].

The hexanal concentration (data not shown) did not show a stable tendency of increasing for the oils from NLA and LLA during tests. Despite the instable tendency of hexanal concentration, in the temperature test, highest values of hexanal were found for NLA oils, and in the photooxidation test in the final periods (144 h), the hexanal concentration was higher for NLA oils. The gradual increase of volatile compounds is due to time exposure of packed oils in an oven at temperatures between 50 and 60 °C or time of light exposure - reported by several authors [35,36, 39-41].

Conclusions

The LLA soybean isolate showed considerable oxidative stability during the extractive and refining processes, as well as higher yield of final oil. The oils in different refining stages of this isolate showed the best results in the temperature test. Thus, the replacement of partially hydrogenated oils, which require chemical processing and cause negative impacts on health, for oils with low linolenic acid content, which may not require this treatment, is a good alternative. Industrially, the advantage for processing and production of oil from soybeans with low linolenic acid content is the yield improvement and the quality of the final product.

Acknowledgments

This work was carried out by the financial support of CAPES, Coordination for the Improvement of Higher Education.

References

- [1] Amaral L.; Jaigobind S.J.; Jaigobind, A.G.A. Dossiê Técnico: Óleo de soja. Serviço Brasileiro de Respostas Técnicas – SBRT. 2006. Available from: http://pessoal.utfpr.edu.br/marlenesoares/arquivos/dossie_tecn_Tecpar.pdf, accessed Junho 2012.
- [2] Erickson D.R. In: Practical handbook of soybean processing and utilization. Erickson, D.R.; ed.; Illinois: AOAC Press, Champaign, (1995) 56-64p.
- [3] Gunstone, F.D.; Harwood, J. L.; Dijkstra, A. J. The lipid handbook, 3rd ed., CRC Press: New York, (2007) 177-204 p.
- [4] Moretto, E.; Fett, R. Tecnologia de óleos e gorduras vegetais na indústria de alimentos. 1nd ed., Livraria Varela: São Paulo, 1998.
- [5] Kinney, A.J.; J. Food Lipids, (1996) 3, 273.
- [6] Quinteiro, L.M.C.; Vianni, R.; Cienc. Tecnol. Aliment. (1995) 15, 29.
- [7] Yadav, N.S.; In: Soybean genetics, molecular biology and biotechnology; Verma, D.P.S.; Shoemaker, R.C., eds.; CAB International: U.S.A., (1996) 127-188p.
- [8] Somerville, C.; Browse, J.; Science. (1991) 252, 80.
- [9] McCormick, R. L.; Ratcliff, M.; Moens, L.; Lawrence, R.; Fuel Process Technol. (2007) 88, 651.
- [10] Erickson, D.R.; Erickson, M.D. In: Practical handbook of soybean processing and utilization. Erickson, D.R.; ed.; Illinois: AOAC Press, Champaign, (1995) 218-238p.
- [11] Simopoulos, A.P. In: Handbook of lipids in human nutrition. Spiller, G.A. ed.; Raton, Florida: CRC Press, Boca. (1996) 91-99p.
- [12] Clemente, T. E.; Cahoon, E. B.; Plant Physiol. (2009) 151, 1030.
- [13] Warner, K.; Fehr, W.; J. Am. Oil. Chem. Soc.(2008) 85, 945.
- [14] Gerd, J.; Hard, C.; Fehr, W.; White, J.P.; J. Am. Oil Chem. Soc. (2007) 84, 557.
- [15] Cherrak, M.; Pantalone, V.R.; Meyer, E.J.; Ellis, D.L.; Melton, S.L.; West, D.R.; Mount, J.R.; J. Am. Oil Chem. Soc. (2003) 80, 539.
- [16] Ross, C.F.; Smith, D.M.; Compr. Rev. Food Sci. Food Safety. (2006) 5, 18.
- [17] Bubeck, D.M.; Fehr, W.R.; Hammond, E.G.; Crop Sci. (1989) 29, 652.
- [18] Zenebon, O.; Pascuet, N.S.; Tiglea, P. (coord). Métodos físico-químicos para análise de alimentos. 4th ed., Instituto Adolfo Lutz: São Paulo, 2005.
- [19] Erickson, D.R. In: Practical handbook of soybean processing and utilization. Erickson, D.R.; ed.; Illinois: AOAC Press, Champaign, (1995) 174-183p.
- [20] Diez, R.; Óleos e Grãos. (1996) 28, 38.
- [21] Erickson, D.R. In: Practical handbook of soybean processing and utilization. Erickson, D.R.; ed.; Illinois: AOAC Press, Champaign, (1995) 184-202p.
- [22] Erickson, D.R. In: Practical handbook of soybean processing and utilization. Erickson, D.R.; ed.; Illinois: AOAC Press, Champaign, (1995) 203-217p.
- [23] Jham, G.N.; Teles, F.F.F.; Campos, L.G.; J. Am. Oil Chem. Soc. (1982) 59, 132.
- [24] Siqueira, F.M. Tese de Mestrado, Universidade Estadual de Campinas, Brasil, 1998.
- [25] Moser, H.A.; Evans, C.D.; Cowan, J.C.; Kwolek, W.F.; J. Am. Oil Chem. Soc. (1965) 42, 30.
- [26] AOCS - Official methods and recommended practices of the American Oil Chemists' Society. 4th ed.; IL, Champaign: AOCS Press, 1996.
- [27] Snyder, J.M.; Frankel, E.N.; Warner, K.; J. Am. Oil Chem. Soc. (1986) 63, 1055.
- [28] Ulbert, F.; In: Lipids analysis in oils and fats. Hamilton, R.J., ed.; Blackie Academic and Professional: London, Liverpool, (1998) 59-85p.
- [29] Ulbert, F.; Roubicek, D.; Food Chem. (1993) 46, 137.
- [30] SAS - Statistical Analysis System. Version 8.1. Cary, NC: SAS Institute Inc., 2001
- [31] Garcia, J.O.; Juárez, L.A.M.; Meza, N.G.; Rodriguez, J.A.N.; Food Sci. Technol. Int. (2005) 11, 443.
- [32] Dunn, R.O.; J. Am. Oil Chem. Soc. (2005) 82, 381.
- [33] Fatemi SH, Hammond EG. Analysis of oleate, linoleate, and linolenate hydroperoxides in oxidized ester mixtures. Lipids .(1980) 15: 379-385.
- [34] Coppin, E.A.; Pike, O.A.; J. Am. Oil Chem. Soc.(2001) 78, 13.

-
- [35] Mounts, T.L.; Warner, K.; List, G.R.; Neff, W.E.; Wilson, R.F.; J. Am. Oil Chem. Soc. (1994) 71, 495.
- [36] Sattar, A.; Deman, J.M.; J. Am. Oil Chem. Soc. (1976) 53, 473.
- [37] Barrera-Arellano, D.; Óleos e Grãos. (1993) 6, 10.
- [38] Onal-Ulusoy, B.; Hammond, E.; White, P.; J. Am. Oil Chem. Soc. (2005) 82, 433.
- [39] Neff, W.E.; Mounts, T.L.; Rinsch, W.M.; Konishi, H.; J. Am. Oil Chem. Soc. (1993) 70, 163.
- [40] Neff, W.E.; Selke, E.; Mounts, T.L.; Rinsch, W.; Frankel, E. N.; Zeitoun, M.A.M.; J. Am. Oil Chem. Soc. (1992) 69, 111.
- [41] Snyder, J.M.; Mounts, T.L.; J. Am. Oil Chem. Soc. (1990) 67, 800.