PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

CAPACIDADE DEGRADATIVA DE OLEOS VEGETAIS POR *BACILLUS SUBTILIS* E CARACTERIZAÇÃO FÍSICO-QUÍMICA DE ÓLEOS RESIDUAIS DE FRITURA

VEGETABLE OILS DEGRADING CAPACITY BY *BACILLUS SUBTILIS* AND PHYSICO-CHEMICAL CHARACTERIZATION OF WASTE FRYING OILS

DE SOUZA, Eliane Ferreira[1], SANTOS, Maria do Socorro Mascarenhas[1], SILVA, Cinthia Aparecida de Andrade[1], FIORUCCI, Antonio Rogério[1], BATISTOTE, Margareth[1]

[1] Universidade Estadual de Mato Grosso do Sul, Programa de Pós-Graduação em Recursos Naturais

** Correspondence author*
e-mail: mariseliana@hotmail.it

Received 21 March 2019; received in revised form 22 April 2019; accepted 12 May 2019

RESUMO

Os óleos vegetais são considerados importantes fontes energéticas que fornecem ácidos graxos essenciais e vitaminas indispensáveis à manutenção do equilíbrio fisiológico do organismo humano, além de ressaltar características sensoriais dos alimentos, como a textura, o aroma e o sabor. O processo de oxidação que ocorre em óleos, gorduras e alimentos gordurosos constitui uma das principais causas da deterioração de alimentos, ocasionando a perda da qualidade organoléptica e a diminuição de suas propriedades nutricionais. Esse processo pode estar associado ao aquecimento, como ocorre no processo de frituras, a presença de microrganismos, entre outros fatores. O presente trabalho teve como objetivo analisar duas amostras de óleo residual de frituras (ORF) pela determinação dos parâmetros físico-químicos e microbiológicos. Os métodos utilizados foram o teste de Rancimat, titulação potenciométrica, cromatografia e espectrofotometria UV-vis. Os resultados evidenciaram alto valor de índice de acidez (1,77 e 1,17 mg KOH g^{-1}) e baixa estabilidade oxidativa (OSI de 1,93 e 0,79 h). Para o índice de iodo, os valores encontrados (95,81 e 106,21 g $I_2$100 g^{-1}) estão abaixo do que requer a norma do Codex. A composição de ácidos graxos indicou que os ácidos em maior quantidade em ambas as amostras foram o ácido oleico C18:1 e linoleico C18:2. A amostra 1 apresentou em sua composição 23,64% e 76,58% de ácidos graxos saturados e insaturados, respectivamente. Enquanto a amostra 2 apresentou 18,38% para ácidos graxos saturados e 81,51% para insaturados. Foi identificada a presença da bactéria *Bacillus subtilis* nas amostras de ORF e a capacidade de degradação desta em óleos vegetais de soja e girassol.

Palavras-chave: *Aquecimento, Alimentos, Degradação.*

ABSTRACT

Vegetable oils are considered important energy sources that provide essential fatty acids and vitamins indispensable for maintaining the physiological balance of the human body, besides emphasizing sensorial characteristics of foods such as texture, aroma and taste. The oxidation process that occurs in oils, fats and fatty foods is one of the main causes of deterioration of food causing the loss of organoleptic quality and the reduction of the nutritional properties. This process may be associated with heating, as occurs in the frying process, the presence of microorganisms, among other factors. The study aimed to analyze two samples of waste frying oils (WFO) by determination of physico-chemical and microbiological parameters. The methods used were Rancimat test, potentiometric titration, chromatography and UV-vis spectrophotometry. The results showed high acid value (1.77 and 1.17 mg KOH g-1) and low oxidative stability (OSI of 1.93 and 0.79 h). The iodine value found (95.81 and 106.21 g I2100 g-1) is below the suggested by the Codex standard. The fatty acid composition indicated that the acids in greater amount in both samples were the oleic acid C18:1 and linoleic acid C18:2. Sample 1 showed 23.64% and 76.58% of saturated and unsaturated fatty acids, respectively, while sample 2 showed 18.38% of saturated and 81.51% of unsaturated fatty acids. *Bacillus subtilis* was isolated in samples of WFO, and its degrating capacity was proved for soybean and sunflower oils.

Keywords: *Heating, Food, Degradation.*

DOI: 10.52571/PTQ.v16.n32.2019.214_Periodico32_pgs_196_206.pdf

1. INTRODUCTION

Vegetable oils are considered important energy sources, composed especially by glycerides, and may also contain small amounts of phospholipids and free fatty acids (Codex, 2017). In addition to being a part of human food providing calories, the oils act as a vehicle for fat-soluble vitamins and are a source of essential fatty acids and contribute to the sensory attributes of food (Dorni, Sharma, Saikia & Longvah, 2018).

Foods processed by frying are enjoyed by all ages and social classes. During this process heat is provided continuously to maintain a high temperature of the vegetable oils. This procedure provides good taste for the food, crisp texture and golden color, however, exposes the vegetable oil to the air, water and other components (Nieva-Echevarría, Goicoechea, Manzanos & Guillén, 2016).

The vegetable oil used in frying undergoes changes caused by physico-chemical processes with visible changes, among them the darkening, increased viscosity, foaming and smoke formation (Laranjeira, Ventura, Bermejo, Ribeiro & Henriques, 2013). During the heating process, the deterioration of vegetable oil increases the greater its degree of unsaturation and according to the period of use (Osawa, Gautam & Mendes, 2010), which may result in formation of harmful substances to human health, as the 4-hydroxy-2-nonenal (HNE), an unsaturated aldehyde extremely reactive, cytotoxic, mutagenic and carcinogenic (Sahin & Barutcu, 2012).

Lipidic residues from frying processes are distinguished by a significant variation in the amount of solid materials, water, polar compounds and free fatty acids, according to the origin of the raw material and operating conditions to which they were subjected (Serna-Saldivar & Rooney, 2015).

The degrading process that occurs in waste frying oils-WFO generates hydroperoxides and peroxides, which originate volatile compounds, such as aldehydes, ketones and carboxylic acids (Waghmare, Patil, LeBlanc, Sonawane & Arya, 2018), products from reactions of hydrolysis, oxidation and triglycerides polymerization. Due to its structure, water as one of the components of this type of oil provides greater number of free oxygen atoms that favors occurrence of hydrogen bonds, resulting in an increase in availability and enabling the development of microorganisms (Freitas & Figueiredo, 2000).

The presence of microorganisms can also be one of the causes that accelerate WFO degradation interfering significantly in processes that allow its reuse and compromising its quality (Laranjeira, Ventura, Bermejo, Ribeiro & Henriques, 2013). Many microorganisms present in these oils are lipolytic, producers of enzymes called lipases. These belong to a rather heterogeneous group and produce extracellular enzymes that catalyze the hydrolysis process of lipids (Laachari *et al*., 2014).

The action of lipases can provide desirable or undesirable effects to some food products, and may lead to the appearance of compounds and unpleasant odors (acids, aldehydes, ketones) or even food deterioration (Uppada, Akula, Bhattacharya & Dutta, 2017). According to Prasad and Manjajunath (2011), these enzymes produced by bacteria such as *Bacillus subtilis*, *Bacillus licheniformis, Bacillus amyloliquefaciens, Serratia marcescens, Pseudomonas aeruginosa* and *Staphylococcus aureus* are considered potent agents for lipid degradation.

Therefore, the degradative process of vegetable oils is influenced by physical and chemical factors, such as heat, humidity and presence of oxygen, in addition, microorganisms present in this raw material can contribute to this process, generating a need to assess the resistance degree of this energy source, especially regarding the stability during storage and thermal stress (Waghmare, Patil, LeBlanc, Sonawane & Arya, 2018; Laranjeira, Ventura, Bermejo, Ribeiro & Henriques, 2013). Thus, this study aims to assess the degradation of two samples of waste frying oil-WFO for determination of physico-chemical parameters, as well as isolate and characterize microorganisms with possible potential for vegetable oils degrading.

2. MATERIALS AND METHODS

2.1. Characterization of samples of waste frying oil-WFO

The two samples of WFO were collected in snack bars and restaurants in the city of Dourados/MS and stored in polyethylene bottles

wrapped in tinfoil, refrigerated at 10°C. The analyses were performed in triplicate using reagents of analytical grade and without prior purification, acquired from Sigma (USA) and VETEC (Brazil).

2.1.1. Physico-chemical analyses

For determination of acid value-AV, acidity in oleic acid (expressed as mg KOH/g and %, respectively) and iodine value-IV (Wijs) a Potentiometric Titler Titrino Plus 848 (Metrohm, Switzerland) was used, in accordance with the recommendations Ca 5a-40 and Cd 1-25 of the American Oil Chemists' Society - AOCS, respectively.

The oxidation stability analysis for determination of oil/oxidative stability index (OSI) was performed using 3 ± 0.01 g of sample in reaction tubes that were placed in the heating block of the equipment for analysis of Oxidative Stability (Metrohm, Switzerland), model PROFESSIONAL BIODIESEL RANCIMAT 893. Samples were analyzed under constant air flow of 15 L h^{-1}, at 120°C, and their respective volatile products of oxidation were collected in a container containing 50 mL distilled and deionized water (Farhoosh, Niazmand, Rezaei & Sarabi, 2008). The volatile products of oxidation were absorbed by the water, resulting in increased conductivity. Water conductivity was monitored in order to determine the OSI, acquired by the software StabNet (Metrohm) at the time resulting in the maximum value in the 2nd derivative curve of conductivity in function of time.

The fatty acid profile was determined according to Wychen, Ramirez & Laurens (2015), by transesterification of 10 mg of biomass with 0.2 mL of chloroform: methanol (2:1 v/v) and 0.3 mL hydrochloric acid 0.6 M in methanol heated at 85°C using a dri-block heater for 1 hour and following extraction of fatty acid methyl esters in 1 mL hexane and assessed by gas chromatography. Methyl esters were separated by gas chromatography using a gas chromatograph Agilent (Agilent Technologies, California, EUA), coupled to flame ionization detector (FID) and a fused silica capillary column (100 m x 250 µm x 0.2 µm, Supelco SP). The operating parameters were optimized as follows: injector and detector temperature of 260°C, oven temperature of 140°C for 5 minutes, reaching 240°C at a rate of 4°C/min, with final time of 48 minutes, using helium as carrier gas at flow rate of 1.2 mL min^{-1} and injection volume of 10 µL of sample. For identification, the retention times of fatty acids were compared to those of standard methyl esters (Sigma-Aldrich, St. Louis, MO, EUA). The retention times and peak area percentage were automatically calculated by the Software ChemStation.

2.2. Isolation and identification of microorganisms

The isolation of the bacteria was performed using two samples of WFO. 1.0 mL of sample was collected and added to 9.0 mL of sterile saline solution (NaCl 0.85%) and homogenized for 30 minutes. Then, a serial dilution from $1x10^{-1}$ to $1x10^{-4}$ was performed and 100 µL of the dilutions were inoculated in Petri dish containing Tryptone Soy Broth medium, plus 1.0% of olive oil. Samples were scraped with a Drigalski handle and incubated at 37°C for 24 to 72 hours at 250 rpm. The growth was analyzed by colony forming unit (CFU). In order to obtain pure sample the stretch marking technique was used. The identification of the microorganism was performed at the food laboratory of National Service of Industrial Learning (SENAI) in Chapecó, Santa Catarina, using the BD Phoenix Automated Microbiology System (BD Diagnostic Systems) equipment.

2.3. Rhodamine B test

To detect the activity of the extracellular lipolytic enzyme, the adapted rhodamine B test were used (Laachari *et al*., 2014). The pre-inoculum was prepared in 20 mL of cerébrle-BHI infusion medium in Erlenmeyer of 125 mL and incubated at 37ºC for 24 hours at 250 rpm. The sample was centrifuged (800x *g*), re-suspended in saline solution (NaCl 0.85%) and washed three consecutive times. 1.0 mL of sample was inoculated in the center of the Petri dish in solid minimum medium containing 2% agar (w/v), 1% Tween 80 (w/v), 0.01% rhodamine B (w/v), in the presence of 1% soybean or sunflower oil (v/v) and incubated at 32°C for 5 days, the tests were performed in triplicate. The plates were visualized in dark chamber under ultraviolet light at 365 nm, and samples that displayed fluorescent orange halos were considered positive.

2.4. Degradation analysis of samples of vegetable oil

For the analysis of degrading capacity,

sterile sunflower or soybean oil and 2.0 mL of bacteria were placed in bottles of 20 mL and incubated at 37°C at 250 rpm. Aliquots were collected at the times of 0, 24, 48, 96 and 120 hours for analysis. Samples were diluted in isopropanol at the ratio of 0.1:1000 (v/v). Samples were analyzed in quartz cuvette with 1 mm optical path using a spectrophotometer (Hitachi, model U-3000) in the UV-Vis absorbance spectra between 200 and 800 nm. The determination of the iodine value-IV of the oils was performed after different periods of degradation according to the AOCS (Cd-25) recommendation.

3. RESULTS AND DISCUSSION

3.1. Characterization of samples of waste frying oil-WFO

3.1.1. Physico-chemical analyses

Table 1 displays the physico-chemical analyses of the WFO samples. The values of acidity and acid value - AV determined for samples 1 and 2 were 0.89 and 0.61 g of oleic acid/100 g of sample or 1.77 and 1.17 mg KOH g^{-1}, respectively. Therefore, the studied samples exhibit values above the limit allowed by Brazilian (Anvisa, 2005) and international legislation (Codex, 2017), which are 0.3 g of oleic acid 100 g^{-1} of sample and 0.6 mg KOH g^{-1}.

The AV is a parameter to be monitored in quality assessment of waste oils, because the degrading process caused by hydrolysis, oxidation, or thermal degradation cause changes in acidity values (Sousa, Pereira Junior, Silva & Marques, 2014). Farhoosh, Einafshar & Sharayei (2009), studying soybean and canola oils, found values of AV (2.09 and 3.22 mg KOH g^{-1}) that reveal the high level of degradation of the conservation state of samples after the refining process, probably due to decomposition of glycerides accelerated by heating, steam and light.

Regarding the iodine value - IV, which relates to the percentage of unsaturated bonds present in the oil, the international standard (*Codex Alimentarius*) specifies a value that ranges from 120 to 143 g $I_2$100 g^{-1}. The IV values found in this study are below the required by Codex (95.81 and 106.21 g $I_2$100 g^{-1}), indicating oxidation of unsaturated fatty acids.

The accelerated oxidation test by the Rancimat method for samples 1 and 2 showed OSI values of 1.93 and 0.79 h, respectively. Farhoosh & Moosavi (2007) empirically correlated the maximum total polar compounds (TPC) established by the European legislation with the OSI of WFO samples and found that the OSI recommended values for this type of oil should be equal to or above 2.32 h. In this study, the samples analyzed did not respond to this recommendation, since the OSI values were far below 2.32 h. Sousa, Pereira Junior, Silva & Marques (2014) state that the oxidative stability of an oil decreases continuously throughout the frying process, the low values of OSI determined in this study suggest the presence of peroxides and hydroperoxides, oxidation products responsible for the deterioration of oils and fats (AOCS, 2004).

Jorge and Lunardi (2004) assessed changes in soybean oil used in potato discontinuous frying, at the temperature of 175°C, from samples collected at different time intervals. The results showed that acidity in oleic acid increases (0.10 to 0.31%) while the oxidative stability decreases (OSI ranging from 10.15 to 7.63 h) over frying time of 8.5 h.

Storage studies performed by Thode Filho, Cabral, Maranhão, Sena & Silva (2014) in soybean oil samples exposed or not to the ambient luminosity for two months, revealed increase in acidity value (0.2 to 0.6% and 0.2 to 0.4%, respectively) over time of analysis.

The assessment of fatty acid composition of WFO samples, regarding saturated fatty acids showed that sample 1 had 23.64% of its composition represented by the following fatty acids myristic, pentadecilic, palmitic, margaric, stearic, arachidic and behenic, while sample 2 presented 18.38%, being this composition distributed among the fatty acids palmitic and stearic (Table 2).

Regarding unsaturated fatty acids, the palmitoleic, oleic, linoleic and linolenic acid represent 76.58% of the WFO-1 sample, and oleic, vacenic, linoleic and linolenic, 81.51% of WFO-2.

For both samples the fatty acids present in greater amount were the oleic and linoleic acids, both unsaturated. Our data corroborate Bautista, Vicente, Rodrígues & Pacheco (2009) evaluating frying oil collected in the kitchen of the

restaurant of University Rey Juan Carlos (Mostoles, Madrid, Spain), which found oleic and linoleic acid in greater amount with values of 45.15% and 39.74%, respectively.

Comparing the content of polyunsaturated fatty acids (PUFA) of sample 1 and 2 of the WFO studied, 40.83% and 49.67%, with the values of OSI, 1.93 h and 0.87 h respectively, it is possible to observe that the lower the PUFA content the greater the value of OSI, i.e., a lower content of PUFAs results in greater oxidative stability and increased shelf life of the oil (Bodoira, Penci, Ribotta & Martínez, 2017). This is because the higher the amount of unsaturations, the more susceptible to degradation the oil will be (Farhoosh, Einafshar & Sharayei, 2009; Bodoira, Penci, Ribotta & Martínez, 2017).

3.2. Isolation and identification of microorganism

The bacterium *Bacillus subtilis* was identified in the samples of WFO. This microorganism is a Gram-positive bacilli that develops at relatively high temperatures ranging from 45°C to 122°C (Sakthipriya, Doble & Sangwai, 2015).

Bacillus subtilis is a thermophilic bacterium, not pathogenic, that has protective structures resistant to physical and chemical agents (Vlamakis, Chai, Beauregard, Losick & Kolter, 2013). The endospore of *Bacillus subtilis* provides the bacteria with resistance and survival in adverse environments such as shortage of nutrients, temperature changes among other adverse conditions (Alves *et al*., 2018; Wang, Wang & Yang, 2017). It is likely that the presence of the endospore in the *Bacillus subtilis* isolated in this study allowed the bacteria to remain and survive under adverse conditions such as those of frying oils.

3.3. Rhodamine B test

The bacterium *Bacillus subtilis* produced extracellular lipase enzyme in the presence of the sunflower and soybean oils, with formation of a fluorescent orange halo under UV light.

Studies have reported the production of lipase using several bacteria and rhodamine B method (Table 3). Carvalho-Gonçalves and Gorlach-Lira (2018) using the Rhodamine B method to isolate bacteria present in contaminated soil with vegetable oil isolated 38 strains capable of producing lipase. This method highlights the halo formation when the dye reacts with the detached fatty acids, suggesting the excretion of lipase (Peil, Kuss, Rave, Villarreal, Hernandes & Nascente, 2016).

Microorganisms are a potential source for enzymes production. Although they are found in plants and animals, microbial enzymes are more advantageous in relation to the others, due to the lower cost of production and large-scale achievement in fermenters, and by having an extensive spectrum and physicochemical particularities (Roveda, Hemkemeier & Colla, 2010). It is estimated that more than 4000 enzymes are known and approximately 200 are employed commercially with the vast majority being microbial strains (Cognette & Pereira, 2017).

The lipase from microorganisms shows numerous biotechnological applications, especially due to its properties and versatility regarding the substrate, and for being relatively easy to produce, it is one of the most employed enzymes in industry (Feitosa, Barbosa, Orellana, Lima & Soares, 2010). Several species of bacteria are important from the industrial point of view, such as the genera Arthrobacter, Bacillus, and Pseudomonas (Gutiérrez & Acevedo, 2016).

3.4. Assessment of biodegrading capacity of *Bacillus subtilis* in vegetable oils

In the assessment of biodegrading capacity, *Bacillus subtilis* showed degrading potential in sunflower and soybean oils, indicated by the increase of absorbance intensity at UV-Vis of compounds resulting from degradation over time of analysis, with greater absorbance intensity at 225 nm corresponding to conjugated dienes, and at 260, 270 and 280 nm related to conjugated trienes, regarding the analyzed oils (Figures 1 A and B).

Studies on edible oils have detected changes in UV-Vis spectra caused by formation of conjugated dienes and trienes at similar spectrum ranges to those obtained in this study. Changes in UV-Vis spectra and transmission density occur due to absorption by primary (230-235 nm) or secondary products from oxidation (260, 270 and 280 nm), and are oil change indices (Laranjeira, Ventura, Bermejo, Ribeiro & Henriques, 2013; Raza, Rashid, Qureshi, Asim & William, 2009). The bacterium isolated in this study, present in waste frying oils, may have survived the adversities of the medium due to its

endospore and degraded the sunflower and soybean oils using them as a carbon source.

In studies with *B. subtilis*, it was observed growth and increase of the degrading process in fatty acids which were used as carbon source (Gudinã, Pereira, Costa, Coutinho, Teixeira & Rodrigues, 2013). Sakthipriya, Doble & Sangwai (2015) assessed the capacity of *Bacillus subtilis* as biodegrading agent of crude oil, and the results showed that more than 95% of the degradation was observed within 15 days for crude oil samples at 50°C.

The iodine value is related to the total number of unsaturations present in the fatty acid chains that compose the vegetable oils. The greater the number of unsaturations, the more susceptible to oxidative process is the oil. There is a decrease in IV values of the sunflower and soybean oils (39.85 to 24.50; 48.30 to 30.83 g I_2 100 g^{-1}), respectively (Figures 2 A and B), biodegraded by *Bacillus subtilis* during 120 hours.

Juarez, Osawa, Acunã, Sammán & Gonçalves (2011), in studies of degradation by immersion of breaded products and churros in soybean and sunflower oils at 180°C, found a decrease in values of IV, 5.3 and 3.0% during heating for up to 42 hours, due to breaking of the double bonds by reactions of polymerization, ciclyzation and oxidation. In the present study the same behavior was observed, since there was a decrease in IV values of soybean (36.2%) and sunflower (38.5%) oils analyzed for up to 120 hours of biodegradation by *Bacillus subtilis*.

The oxidation of sunflower or soybean oils consists of a complex series of chemical reactions characterized by a decrease in the total content of unsaturated fatty acids due to the elimination of hydrogen adjacent to double bonds and formation of free radicals (Bouaid, Martinez & Aracil, 2007), which consequently lead to an increase in the amplitude of the absorbance at 235, 260, 270 and 280 nm and decrease in values of IV as shown in Figure 2. The results showed an inverse correlation between the values of absorbance at the wavelengths cited and IV, as a result of the decrease in the oil degree of unsaturation and the increase in the absorbance amplitude at ranges corresponding to conjugated dienes and trienes, products of the degrading process (Laranjeira, Ribeiro, Lima, Henriques & Bermejo, 2014; Raza, Rashid, Qureshi, Asim & William, 2009).

4. CONCLUSIONS

The analyzed samples of WFO had acidity and iodine value above the suggested by ANVISA and Codex Alimentarius and both showed low oxidative stability determined by the Rancimat method. The fatty acid composition indicated that the fatty acids in greater amount in both samples were the oleic and linoleic acids. Comparing the PUFA content of samples with values of OSI, it was possible to conclude that the higher the content of PUFAs, the lower the value of OSI and consequently the lower the oxidative stability and the quality of the sample. The bacterium *Bacillus subtilis* was isolated in the samples of WFO and showed lipase production in the presence of rhodamine B and degrading capacity of edible soybean and sunflower oils under the conditions tested. It was possible to prove that besides the physical and chemical factors, the bacteria are associated to the degradation of vegetal oils.

5. ACKNOWLEDGMENTS

The authors would like to thank FUNDECT, Brazil, CNPq, Brazil, and CAPES, Brazil (Edital Nº 11/2009 - Pró-Equipamentos Institucional) for financial support and scholarships.

6. REFERENCES

1. Alves, K.C.; Almeida, M.E.M.; Glória, J.C.; Santos, F.A.; Pereira, K.D.; Castro, D.P. & Mariúba, L.A.M. Bacillus subtilis: uma versátil ferramenta biotecnológica. *Scientia Amazoni,* **2018**, *7*(2): 15-23.

2. AMERICAN OIL CHEMISYS SOCIETY – AOCS - Official Methods and Recommended Practices of the American Oil Chemists Society. Washington. Oficial methods and recommended practices of the American oil Chemists Society. 27th ed. Arlington: A.O.C.S. Official Method Ce; 1993. *American Oil Chemists Society;* 1–62. **2004.**

3. ANVISA - Agência Nacional da Vigilância Sanitária. Technical Regulation for vegetable oils, fats vegetables and vegetable cream. Brazil. RDC/ANVISA/MS Nº 270, **2005**.

4. Bautista, L. F.; Vicente, G.; Rodríguez, R. & Pacheco, M. Optimisation of FAME

production from waste cooking oil for biodiesel use. *Biomass and bioenergy*, **2009**, *33*(5): 862 – 872.

5. Bodoira, R. M.; Penci, M. C.; Ribotta, P. D. & Martínez, M. L. Chia (Salvia hispanica L.) oil stability: Study of the effect of natural antioxidants. *LWT - Food Science and Technology,* **2017**, *75*(107).
6. Bouaid, A.; Martinez, M. & Aracil, J. Long storage stability of biodiesel from vegetable and used frying oils. *Fuel,* **2007**, *86*(16): 2596-2602.
7. Carvalho-Gonçalves, L. C. T. & Gorlach-Lira, K. Lipases and biosurfactants production by the newly isolated Burkholderia sp. *Brazilian Journal of Biological Sciences,* **2018**, *5*(9): 57-68.
8. CODEX ALIMENTARIUS- CODEX STAN 210-1999- *Normas Internacionales de los Alimentos.* Roma, **2017**.
9. Cognette, R.C. & Pereira, E.B. Otimização e caracterização de micropartículas de quitosana imobilizada em lipase pancreática comercial. *Revista da Universidade Vale do Rio Verde, Três Corações,* **2017**, *15*(1): 03-13.
10. Dorni, C., Sharma, P., Saikia, G. & Longvah, T. Fatty acid profile of edible oils and fats consumed in India. *Food Chemistry*, **2018**, *238*: 9-15.
11. Feitosa, I.C.; Barbosa, J.M.P.; Orellana, S.C.; Lima, A.S. & Soares, C.M.F. Produção de lipase por meio de microrganismos isolados de solos com histórico de contato com petróleo. *Acta Scientiarum Technology,* **2010**, *32*(1): 27-31.
12. Farhoosh, R.; Einafshar, S. & Sharayei, P. The effect of commercial refining steps on the rancidity measures of soybean and canola oils. *Food Chemistry,* **2009**, *115*(3): 933-938.
13. Farhoosh, R. & Moosavi, S.M.R. Rancimat test for the assessment of used frying oils quality. *Journal of Food Lipids*, **2007**, *14*(3): 263-271.
14. Freitas, A.C. & Figueiredo, P. Conservação de alimentos. *Lisboa: Acribia, SA,* (Chapter 10) **2000**.
15. Gudiña, E.J., Pereira, J.F.B., Costa, R., Coutinho, J.A.P., Teixeira, J.A. & Rodrigues, L.R. Biosurfactant-producing and oil-degrading *Bacillus subtilis* strains enhance oil recovery in laboratory sand-pack columns. *Journal of Hazardous Materials,* **2013**, *261*: 106-113.
16. Gutiérrez, L.K.B. & Acevedo, M.V.H. Isolation of biosurfactant producing microorganisms and lipases from wastewaters from slaughterhouses and soils contaminated with hydrocarbons. *Scientia Agropecuaria,* **2016**, *7*(1): 23-31.
17. Hasan, F.; Shah, A.A. & Hameed, A. Industrial applications of microbial lipases. *Enzyme Microbial Technology,* **2006**, *39*(2): 235-251.
18. Jorge N. & Lunardi V.M. Comportamento de óleos poliinsaturados em frituras descontínuas de batatas. *Alimentos e Nutrição,* **2004**, *15*(2): 119-24.
19. Juárez, M.D., Osawa, C. C., Acuña , M.E., Sammán, N. & Gonçalves, L.A.G. Degradation in soybean oil, sunflower oil and partially hydrogenated fats after food frying, monitored by conventional and unconventional methods. *Food Control,* **2011**, *22*: 1920-1927.
20. Laachari, F., El Bergadi, F., Bahafid, W., Sayari, A., Elabed, S., Mohammed, I. & Ibnsouda, S. K. Biochemical study of lipases from Bacillus subtilis. *Moroccan Journal of Biology,* **2014**, *11*: 1-9.
21. Laranjeira, C.; Ventura, C.; Bermejo, S.; Ribeiro, M. & Henriques, M. Óleos alimentares usados: caracterização físico-química para selecção de indicadores de degradação de qualidade e de melhores práticas laboratoriais disponíveis. *Revista da UIIPS*, **2013**, *2*: 256-286.
22. Laranjeira, C.; Ventura, C.; Bermejo, S.; Faro, M.; Torgal, I; Lima, M.; Oliveira, M.; Henriques, M. & Ribeiro, M. Óleos alimentares usados: Indicadores de degradação de qualidade. Definição de critérios e de melhores práticas laboratoriais disponíveis, no âmbito da prevenção, requalificação e valorização de OAU's. *Revista da 180 UIIPS: Número especial do Congresso UIPPS "Investigação, Inovação e Tecnologia: Novos Desafios*", **2014**, *1*: 44.
23. Nieva-Echevarría, B., Goicoechea, E.,

Manzanos, M.J., & Guillén, M.D. The influence of frying technique, cooking oil and fish species on the changes occurring in fish lipids and oil during shallow-frying, studied by 1H NMR. *Food Research International,* **2016**, *84*: 150-159.

24. Odeyemi A.T., Aderiye, B. & Bamidele, O.S. Atividade lipolítica de algumas cepas de Klebsiella, Pseudomonas e Staphylococcus spp. de águas residuais do restaurante e receber fluxo. Journal of Microbiology Research, **2013**, 3(1): 43-52.
25. Osawa, C.C.; Gonçalves, L.A.G. & Mendes, F.M. Avaliação dos Óleos e Gorduras de Fritura de Estabelecimentos Comerciais da Cidade de Campinas/SP. As Boas Práticas de Fritura Estão Sendo Atendidas? *Alimentos e Nutrição,* **2010**, *21*(1): 47– 55.
26. Peil, G.H.S., Kuss, A.V., Rave, A.F.G., Villarreal, J.P.V., Hernandes, Y.M.L. & Nascente, P.S. Bioprospecting of lipolytic microorganisms obtained from industrial effluents. *Anais da Academia Brasileira de Ciências,* **2016**, *88*: 1769-1779.
27. Prasad, M.P. & Manjunath, K. Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species. *Indian Journal of Biotechnology,* **2011**, *10*: 121-124.
28. Raza, S.A.; Rashid, A.; Qureshi, F.A.; Asim, M.F. & William, J. Analytical Investigation of oxidative deterioration of sunflower oil stored under different conditions. *Biharean Biologist*, **2009**, *3*(2): 93-97.
29. Roveda, M.; Hemkemeier, M. & Colla, L.M. Avaliação da produção de lipases por diferentes cepas de microrganismos isolados em efluentes de laticínios por fermentação submersa. *Ciência e Tecnologia de Alimentos,* **2010**, *30*(1): 126-131.
30. Sahin, S. & Barutcu, I. Handbook of Food Safety Engineering. In: Sun, D.W (Eds), *Frying of Foods* (pp. 412-443). **2012**, Oxford: Blackwell Publishing Ltd.
31. Sakthipriya, N., Doble, M. & Sangwai, J.S. Fast degradation and viscosity reduction of waxy crude oil and model waxy crude oil using *Bacillus subtilis. Journal of Petroleum Science and Engineering*, **2015**, *134*:158-166.
32. Serna-Saldivar, S.O. & Rooney, L. W. Tortillas: Wheat flour and corn products. (1th ed.). Paul, MN: AACC International, **2015**, (Chapter 13).
33. Sousa, E.R.; Junior, A.S.P.; Silva, G.S.; Marques, A.L.B. Avaliação da qualidade de óleos de origem vegetal oriundos de frituras. *Acta Tecnológica*, **2014**, *9*(2): 58-62.
34. Thode Filho, S.T.; Cabral, G.B.; Maranhão, F.S.; Sena, M.F.M. & Silva, E.R. Deterioração de óleos vegetais expostos a diferentes condições de armazenamento. *Revista Eletronica em Gestão, Educação e Tecnologia Ambiental,* **2014**, *18*: 07-13.
35. Uppada, S.R., Akula, M., Bhattacharya, A. & Dutta, J.R. Immobilized lipase from *Lactobacillus plantarum* in meat degradation and synthesis of flavor esters. *Journal of Genetic Engineering and Biotechnology,* **2017**, *15*(2): 331-334.
36. Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., & Kolter, R. Sticking together: building a biofilm the Bacillus subtilis way. *Nature Reviews Microbiology*, **2013**, *11*(3): 157–168.
37. Waghmare, A., Patila, A. S., LeBlancb, J. G., Sonawanea, S. & Aryaa, S. S. Comparative assessment of algal oil with other vegetable oils for deep frying. *Algal Research*, **2018**, *31*: 99–106.
38. Wang, H.; Wang, Y.; Yang, R. Recent progress in Bacillus subtilis spore-surface display: concept, progress, and future. *Applied Microbiology and Biotechnology*, **2017**, *101*: 933-949.
39. Wychen, S.V.; Ramirez, K. & Laurens, L.M.L. Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification laboratory analytical procedure (LAP). *National Renewable Energy Laboratory*, **2015**.
40. Zaki N.H. & Saeed, S.E. Production, purification and characterization of extra cellular lipase from Serratia marcescens and its potential activity for hydrolysis of edible. Jounal of Al-Nahrain University, **2012**, *15*(1): 94-102.

Table 1. *Analysis of acidity, acid value (AV), iodine value (IV) and oxidative stability index (OSI) of samples of waste frying oil (WFO).*

Parameters	Samples	
	WFO-1	WFO-2
AV (mg KOH g^{-1} sample)	1.77±0.00	1.17±0.01
*Acidity in oleic acid (%)	0.89±0.00	0.59±0.01
IV (g $I_2$100 g^{-1})	95.81±2.01	106.21±8.88
OSI (h)	1.93±0.02	0.79±0.01

**For conversion of AV to acidity in oleic acid the value of AV was divided by 1.99. Results expressed as mean ± S.D. of the three replicates.

Table 2. *Fatty acid composition (%) of samples of waste frying oil (WFO).*

Fatty Acids	**WFO-1 (%)**	**WFO-2 (%)**
Myristic acid **C14:0**	0.44±0.06	nd
Pentadecilic acid **C15:0**	0.10±0.01	nd
Palmitic acid **C16:0**	16.14±1.69	13.93±0.16
Palmitoleic acid **C16:1**	1.14±0.11	nd
Margaric acid **C17:0**	0.16±0.03	nd
Stearic acid **C18:0**	5.75±0.84	4.45±0.03
Oleic acid **C18:1n9**	35.19±1.93	30.35±0.08
Vacenic acid **C18:1n7**	Nd	1.49±0.01
Linoleic acid **C18:2**	34.97±1.69	45.89±0.12
Linolenic acid **C18:3**	5.28±0.77	3.78±0.01
Arachidic acid **C20:4**	0.58±0.09	nd
Behenic acid **C22:0**	0.47±0.07	nd
∑SFA	23.17±2.72	18.38±0.19
∑MUFA	36.33±2.04	31.84±0.09
∑PUFA	40.83±2.55	49.67±0.13
PUFA/SFA	1.76±0.01	2.70±0.02

nd: not detected; SFA: saturated fatty acids; Results expressed as mean ± standard deviation of the three replicates. MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids.

Table 3. *Detection of lipolytic activity by Bacillus subtilis in the presence of sunflower and soybean oils and lipase producing bacteria reported in the literature.*

Microorganism	Lipase producing			References
	Sunflower oil	Soybean oil	Olive oil	
Bacillus subtilis	+	+	np	This work
Serratia marcescens	np	np	+	Peil et al., 2016
Enterobacter aerogenes	np	np	+	Peil et al., 2016
Serratia sp	np	np	+	Odeyemi et al., 2013
Klebsiella sp	np	np	+	Odeyemi et al., 2013
Serratia marcescens	+	+	+	Zaki e Saeed, 2012

np: not performed by the author.

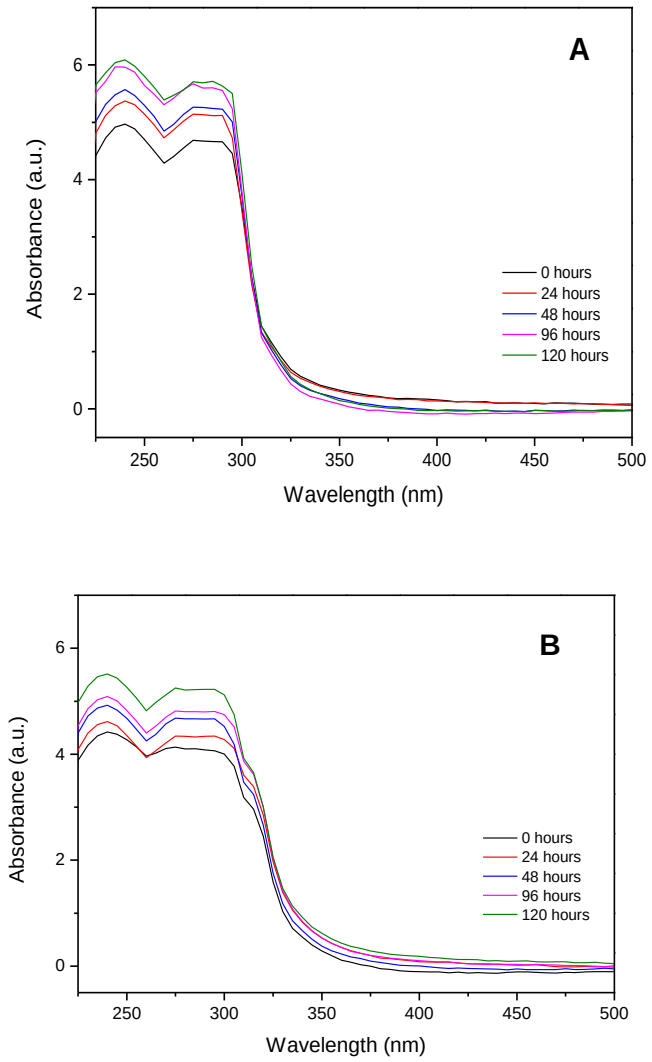

Figure 1. *UV-Vis spectra of samples of sunflower (A) and soybean (B) oils biodegraded at different times by Bacillus subtilis.*

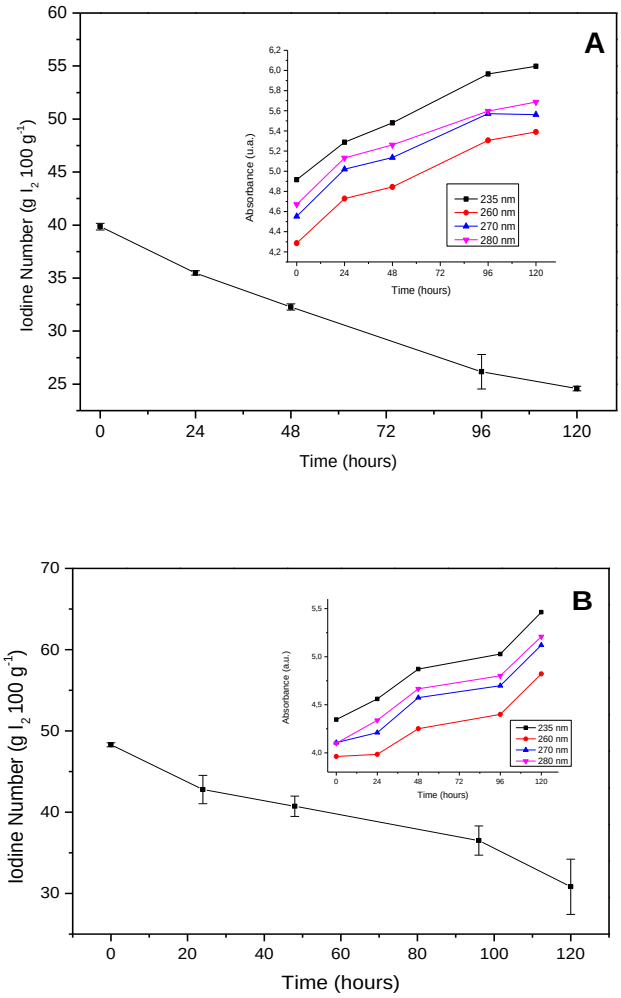

Figure 2. *Variation of iodine value and absorbances at UV-Vis of samples of sunflower (A) and soybean (B) oils biodegraded in different periods by Bacillus subtilis.*