

Chapter 4

Hydroxychalcones: Synthetic Alternatives to Enhance Oxidative Stability of Biodiesel

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Abstract In this chapter, the main aspects about biodiesel synthesis and established quality parameters are reviewed and discussed in the perspective of oxidative stability. First, the characteristics, which define biodiesel as a promising, renewable green fuel, are listed. On the other hand, the propensity of biodiesel to oxidation is explained as one of the limitations to its application. Along this line of thought, the majority of natural and synthetic antioxidants usually applied in the industry are discussed, in which it can be noted that phenolic compounds are very representative. Chalcones containing hydroxyl groups in its structure are phenolic compounds, and so, their origins, sources, and synthetic approaches to obtain these types of molecules are presented. Antioxidant effects of hydroxychalcones already reported in the literature are pointed, and then an experimental topic shows the influence of two synthetic hydroxychalcones, namely (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one and (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one, on the oxidative stability of biodiesel obtained from frying soybean oil. The effects assessed by calorimetric analysis revealed interesting effects on the enhancement of biodiesel oxidative stability.

Keywords Biodiesel · Oxidative stability · Antioxidant · Hydroxychalcone
Synthetic chalcones

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4.1 Biodiesel

Biodiesel is a combustible chemically constituted of a mixture of several alkyl esters of fatty acids. This product can be obtained from vegetables, animals, microorganisms, and waste materials sources (Rockembach et al. 2014; Vieira et al. 2016; Marcon et al. 2017). These biological matrices make biodiesel a green alternative to fossil fuels since it is less aggressive to the environment, contributing to the recycling of resources and generating less pollution (Sajjadi et al. 2016). Moreover, it is known that the reserves of fossil fuels will be depleted in the near future although the energy demand continues to increase. In this way, the development of new sources of power, such as biodiesel, is considerably important for the sustainability of future generations (De Oliveira et al. 2013; Knothe and Razon 2017).

Although biodiesel is considered a green fuel for having several advantages, such as being biodegradable, non-toxic, environmental-friendly, having low sulfur content and being renewable, it faces numerous limitations in order to be fully implemented (Mekhilef et al. 2011). Major limitations are the higher cost of production compared to conventional fuels and the possible deviation of food resources to produce energy (Singh et al. 2011). Besides, due to its chemical constitution, biodiesel is susceptible to oxidation and has some other properties drawbacks involving cold flow and viscosity (Hoekman et al. 2012).

Generally, biodiesel is synthesized throughout a transesterification reaction, which converts triglycerides from the fatty matrix to alkyl esters of fatty acids. This process is accomplished employing methanol or another short-chain alcohol as a reactant and alkali or acid as catalysts (Pereira et al. 2012). The final product of this reaction—biodiesel—is more suitable to be used as a combustible than the crude oil since its viscosity, density, and volatility are more adequate for combustion in engines. Moreover, prejudicial factors that could happen when a crude oil is combusted, such as gumming and formation of carbon deposits, are avoided in this conversion procedure (Singh and Singh 2010).

In order to be used, the characteristics of the biodiesel produced must agree with the minimum standards set by Europe (EN 14214) or by the USA (ASTM D6751) (Knothe 2006). Both these requirements serve as models worldwide and are related to several properties of the fuel, such as kinematic viscosity, iodine value, cetane number, acid value, mineral content, and oxidation stability. Ideally, a certain synthesized biodiesel should be in line with the values established for these parameters. In most of the cases, however, it fails in one or more of the parameters, mainly due to its fatty acid composition (Pinzi et al. 2009).

Nowadays, edible oils such as soybean, rapeseed, palm, and sunflower oil are the main sources for the production of biodiesel. However, alternative feedstock like non-edible and waste oils are gradually growing in the market, since 75% of biodiesel production costs are related to its source (Gui et al. 2008). These alternative oils also have other advantages compared to conventional feedstock, since

they are not used in the food market and may occur abundantly from region to region (Sajjadi et al. 2016; Knothe and Razon 2017).

In many countries, the production of biodiesel has been promoted or facilitated through regulatory efforts, in which the top producer is the USA (4.7 billion liters). Overall, other countries with a considerable production of biodiesel include Brazil, Germany, Argentina, Indonesia, and France (Knothe and Razon 2017). In Brazil, the synthesis of this fuel was approximately 3.87 billion liters in 2016, with the majority of its feedstock being from soybean oil (68%). Figure 4.1 shows other sources that include animal tallow (14%) and waste cooking oil (2.5%).

As mentioned above, the use of waste oils is promising starting materials for the production of biodiesel. These types of oils can be reutilized, thus returning to the energetic chain and minimizing possible environmental impacts (Kulkarni and Dalai 2006). On the other hand, some barriers prevent the use of these oils, mainly due to lack of infrastructure and logistics for their efficient harvest. Moreover, most waste oils need to overcome a previous cleaning process in order to be transesterified, which could increase its production cost, making it difficult for an extensive implementation (Wang et al. 2007; Lapuerta et al. 2008).

Generally, the fatty acid profile of most vegetable oils is constituted of common biomolecules such as palmitic, stearic, oleic, linoleic, and linolenic acid with concentrations that vary from matrix to matrix (Atabani et al. 2013). These variations are linked directly to the properties of biodiesel including oxidative stability. A higher amount of unsaturated fatty acids leads to an oxidative instability in the fuel as the double bonds present in the fatty acids are reactive toward oxidation reactions (Knothe 2005).

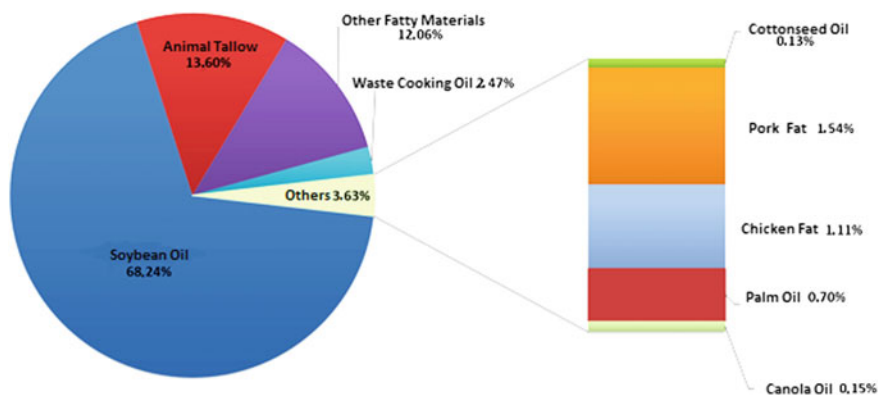


Fig. 4.1 Brazilian feedstock matrix of biodiesel (Pinto 2017)

4.1.1 Oxidative Stability

Oxidative stability can be defined as the amount of time required to oxidize a certain portion of biodiesel. This subject is extensively researched since it affects the fuel in early stages of use, during transportation, and storage (Knothe 2007). The presence of unsaturation is related to autoxidation, which is influenced by the presence of air, heat, metals, peroxides, and light, among other factors. This important parameter is evaluated by ASTM D6751 and EN 14214 that indicate a minimum induction time of 3 and 8 h, respectively (Dantas et al. 2011).

The oxidation of biodiesel influences greatly in the quality of the fuel. Its degradation is linked to variations in others physicochemical properties such as acid number, kinematic viscosity, polymer content, iodine value, and peroxide value. In this context, the oxidized biodiesel can lead to several problems in the operational system of a vehicle, causing corrosion, obstruction, and formation of deposits in the interior of the engine (Kumar 2017).

The oxidation process in biodiesel that leads to its deterioration is very complex and involves the development of hydroperoxides. These reactive species initiate several secondary reactions, which result in the formation of carboxylic acids, ketones, aldehydes, polymers, and other undesired compounds. In general, the degradation is composed of a free radical process divided in stages, namely initiation, propagation, and termination, which are outlined in Fig. 4.2 (Fu et al. 2017).

As it can be seen in Fig. 4.2, the oxidation undergoes a primary reaction in which hydrogen is removed of a polyunsaturated fatty acid (1). This step is relatively slow and forms the first free radicals (2) that initiate the degradation of the biodiesel. The propagation stage is characterized by the reaction with oxygen, resulting in peroxide radicals (3) and hydroperoxides (4) that forms radicals that feed further reactions. At the termination of this process, the free radicals formed in the course of the oxidation combine and form stable products (Yaakob et al. 2014).

The degradation process has a correlation with the number of unsaturation, double bonds close to the ester group, higher chain length of the ester portion and *cis*-isomers. The acid number of a fuel is related to hydrolysis and the presence of oxygen. It can also occur polymerization between fatty acids when the biodiesel is exposed to high temperature conditions. The presence of trace metals can also contribute to the formation of radicals acting as catalysts to this process (Knothe and Razon 2017). Table 4.1 displays the oxidative stability of some biodiesel from distinct sources indicating that most of them do not reach accepted standards of stability.

In order to measure the progression of biodiesel decomposition, several techniques and methods were developed (Xin et al. 2009). One of the most used methods is the Rancimat, in which a stream of air is bubbled into the sample at elevated temperatures. Other methods can be cited, for example the iodine value, the active oxygen bomb test, and Schaal's oven test. It must be noted that thermal analysis is emerging in this field, mainly by differential scanning calorimetry (DSC) and differential thermal analysis (Herbinet et al. 2010; Saluja et al. 2016).

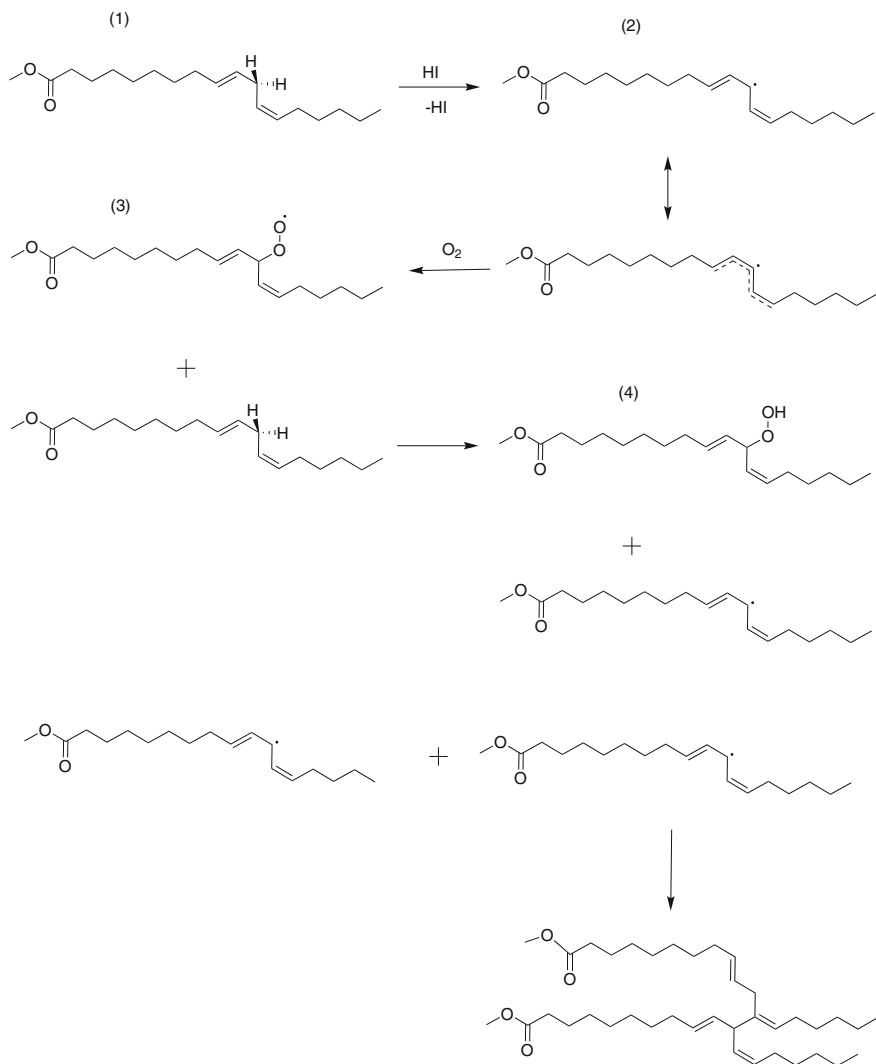


Fig. 4.2 Mechanism of linoleic acid methyl ester oxidation

DSC is a thermal technique that measures energetic differences between the sample and a reference material as a function of temperature. It has been extensively used to characterize several physical and chemical properties of combustibles, lubricant, oils, polymers, and other materials (Dunn 2006). In this technique, a small quantity of the sample is required and it is contained in a crucible. Then, the sample alongside a reference material is placed in a furnace in which the temperature and atmosphere can be controlled and measured, resulting in endothermic or exothermic events (Stuart 2012).

Table 4.1 Biodiesel and its respective oxidative stability

Biodiesel source	Oxidative stability (h)	References
Corn oil	6.5	Serqueira et al. (2014)
Sunflower oil	1.73	Sarin et al. (2007)
Soybean oil	3.80	Sarin et al. (2007)
Waste cooking oil	2.6	Serqueira et al. (2014)
Colza oil	11.2	Serqueira et al. (2014)
Karanja oil	2.35	Sarin et al. (2007)
Jatropha oil	3.23	Sarin et al. (2007)
Palm oil	13.37	Sarin et al. (2007)
Beef tallow	1.2	Mittelbach and Schober (2003)
Rapeseed oil	9.1	Mittelbach and Schober (2003)
Safflower oil	0.86	Xin et al. (2009)
Cottonseed oil	3.6	Serqueira et al. (2014)

4.1.2 Antioxidants in Biodiesel

It has been established that susceptibility to oxidation is one of the major drawbacks of biodiesel usage. In this way, it is very important to combine biodiesel with antioxidants that are effective for the minimization of oxidation stability issues (Yaakob et al. 2014). Antioxidants may be isolated from natural sources, serving as examples α -tocopherol (vitamin E), citric acid, and caffeic acid (Fig. 4.3).

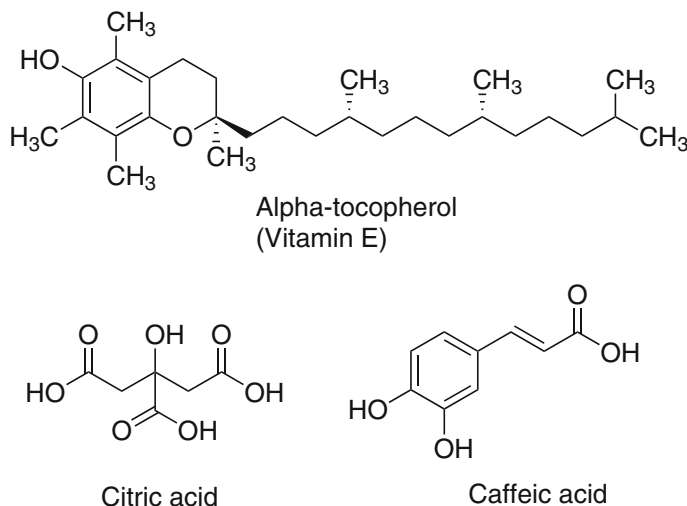


Fig. 4.3 Structure of some naturally derived biodiesel antioxidants: vitamin E, citric acid, and caffeic acid (Yaakob et al. 2014)

Vegetable oils may have considerable quantities of natural antioxidants, but they are reduced during oil refining processes.

Antioxidants derived from synthetic sources are generally preferable and more effective, in which the major examples include, but are not limited to: butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylated hydroxyquinone (TBHQ), propyl 3,4,5-trihydroxybenzoate (PG), 1,2,3-trihydroxybenzene (pyrogallol—PY), and gallic acid (GA) (Fig. 4.4). Depending on their mode of action, they can be classified as primary antioxidants that are free radical terminators, metal ion chelators, or oxygen scavengers. Secondary antioxidants are preventive and can act as chain breakers (peroxide radical quenchers), or reducing agents (hydroperoxide decomposers), retarding the chain initiation reaction (Shahidi and Wanasundara 1992; Yaakob et al. 2014).

These antioxidants or potentially new ones are blended with biodiesel in relatively low concentrations ranging from 200 to 2000 ppm (1 ppm: parts per million = 1 $\mu\text{g}/\text{mL}$ = 1 mg/L) to minimize added costs (Dunn 2008; Pullen and Saeed 2012). Effectiveness of antioxidants will vary depending on the composition of fatty acid methyl esters, feedstock, and oxidative stability of the biodiesel (Rizwanul Fattah et al. 2014b; Yaakob et al. 2014).

It can be seen that antioxidant agents are diverse in molecular structure, but most of them are phenolic substances. Phenolic compounds tend to have a proximate relationship with antioxidant activity, because the hydrogen from hydroxyl groups can be easily donated, forming relatively stable radical intermediates (Cao et al. 1997; Heim et al. 2002; Senevirathne et al. 2006; Detsi et al. 2009). The $-\text{OH}$ groups scavenge reactive radicals, such as peroxy radicals, and can be stabilized by

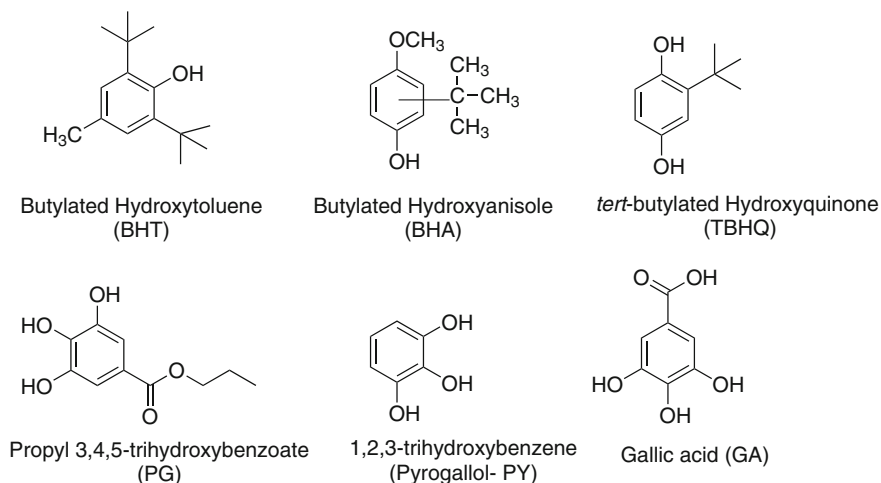
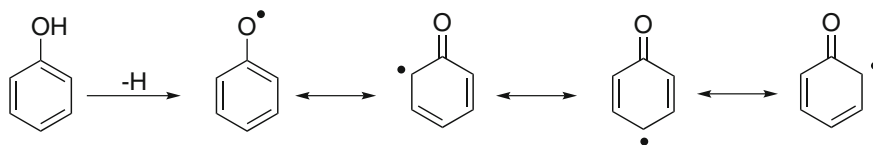


Fig. 4.4 Structure of some synthetic antioxidants used for biodiesel storage (Rizwanul Fattah et al. 2014b; Yaakob et al. 2014)



Scheme 4.1 Stabilization of phenoxy radical by delocalization of unpaired electrons around the aromatic ring (Rizwanul Fattah et al. 2014b)

resonance delocalization and lack of apt sites to be attacked by oxygen (Scheme 4.1) (Rizwanul Fattah et al. 2014a, b).

Phenolic groups are frequently present in chalcones, which are molecules naturally abundant in edible plants or obtained synthetically. Chalcones are very versatile molecules and have been associated with many biological activities, in which antioxidant activity can be highlighted (Detsi et al. 2009; Righi et al. 2011; Serifi et al. 2013; Días-Tielas et al. 2016).

4.2 Chalcones and Antioxidant Activity

Chalcones (1, 3-diaryl-2-propen-1-ones) are a class of molecules within the family of flavonoids, which present two aromatic rings connected by a three-carbon linker of an α - β -unsaturated system. As it can be seen from Fig. 4.5, one of the aromatic rings (Ring “A”) is connected to a carbonyl group, while the other aromatic ring (Ring “B”) is connected to the α - β -unsaturation (Dawane et al. 2009).

Chalcones are a category of open-chain flavonoids, and in fact, some of them are immediate precursors of other classes of flavonoids, such as aurones. It can be seen from Fig. 4.6 that chalcones have a close structural and biogenetic relationship with flavonoids, including aurones, and so they can co-occur in many natural products (Veith and Grayer 2006).

Chalcones and aurones are major contributors to the yellow to orange pigments seen in flowering plants, mainly isolated from Leguminosae, Asteraceae, and Moraceae families (Rozmer and Perjési 2016). These phytochemicals are widely distributed in seeds, leaves, and flowers of plants and are associated to protection against photosynthetic stress, reactive oxygen species (ROS), and pathogenic microorganisms (Serifi et al. 2013). The key enzyme to chalcone synthesis in the flavonoid biosynthesis pathway, chalcone synthase, is also known to be induced in plants under stressful conditions, such as high irradiation of ultraviolet light, and bacterial, or fungal infections, which corroborates the protective effective demonstrated by chalcones (Dao et al. 2011). The distribution of these compounds is not limited to plants, as they are also present in fruits (apples, citrus), vegetables (tomatoes, potatoes), beer, wine, and other dietary components (Tomás-Barberán and Clifford 2000; Orlikova et al. 2011).

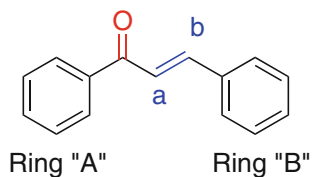


Fig. 4.5 General structure of a chalcone (Detsi et al. 2009)

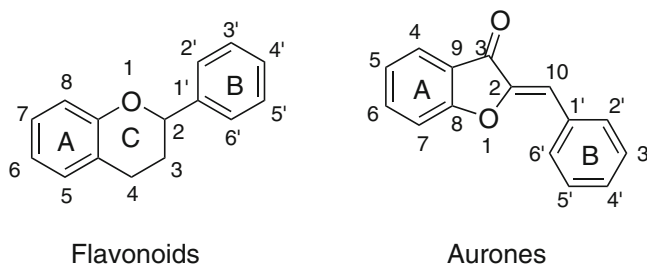


Fig. 4.6 General structure of flavonoids (Heim et al. 2002) and aurones (Detsi et al. 2009)

Apart from the α - β -unsaturation, the general structure of chalcones is highly versatile because the aromatic rings can present a wide range of substituents in any position. Thus, many molecules of natural or synthetic chalcones can be isolated or obtained, which will differ structurally depending on the substitution pattern of the parent chalcone (Orlikova et al. 2011; Ritter et al. 2015). Possibilities include, but are not limited to, methoxylated, hydroxylated, halogenated, and *N*-containing chalcones (Padhye et al. 2010). Simple examples of this classification can be seen in Fig. 4.7.

Chalcones can be multifunctional molecules since one particular compound may exhibit more than one biological activity, such as the antioxidant, anti-inflammatory, and chemopreventive activities associated with licochalcone (Fig. 4.8), which is present in licorice (Lee et al. 2008; Franceschelli et al. 2011).

Many studies have reported an antioxidant activity exhibited by chalcones and chalcone derivatives, especially chalcones containing hydroxyl groups (hydroxy-chalcones) (Mathiesena et al. 1996; Heim et al. 2002; Rezk et al. 2002; Sikander et al. 2011). The radical-scavenging activity of chalcones is influenced by the aryl groups and their substituents (rings "A" and "B" in Fig. 4.5). It is believed that the presence of one or more hydroxyl groups can be key contributors to the antioxidant effect associated with chalcones (Mathew et al. 2017).

Sikander et al. (2011) showed that trans-chalcone (labeled as compound 1 in Fig. 4.9) inhibited reactive oxygen-triggered lesions induced by hydrogen peroxide (H_2O_2), resulting in the protection of hepatocellular carcinoma cells.

Rezk et al. (2002) showed the potent antioxidant activity of a dihydrochalcone, phloretin (2) and of its respective glucoside, phloridzin (phloretin 2- β -D-glucose)

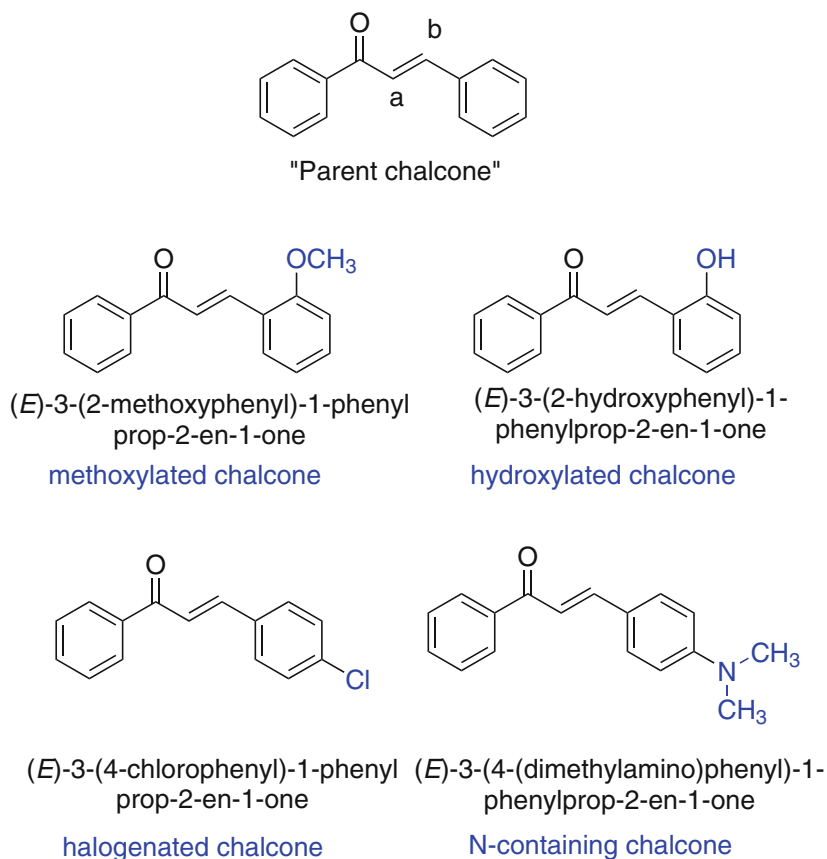


Fig. 4.7 Examples of different chalcones based on their substitution pattern

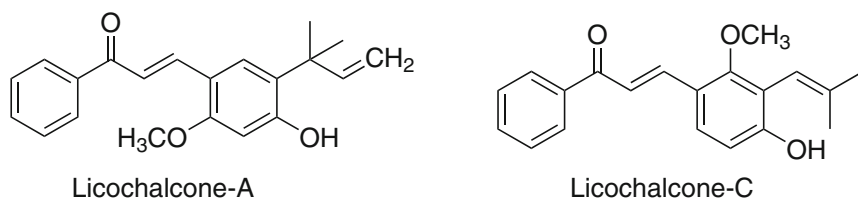
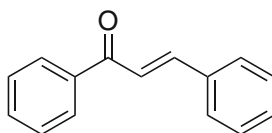


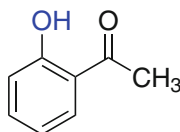
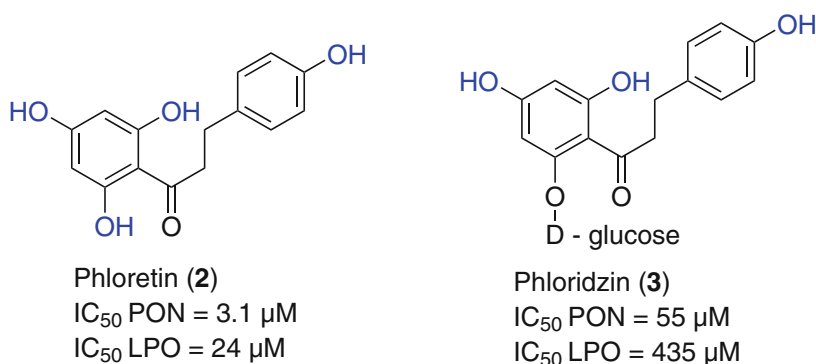
Fig. 4.8 Molecular structures of licochalcone-A and licochalcone-C associated with antioxidant, anti-inflammatory, and chemopreventive activities

(3), which are chalcones without α - β double bond containing four and three hydroxyl groups, respectively (Fig. 4.10). The concentration needed to scavenge 50% of peroxynitrite (IC_{50} PON) and the concentration needed to inhibit 50% of lipid peroxidation (IC_{50} LOP) were determined. For phloretin, IC_{50} PON was



Compound 1
Trans chalcone

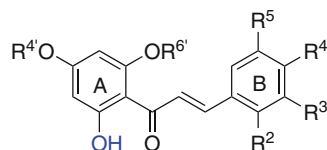
Fig. 4.9 Structure of trans-chalcone (compound 1) (Sikander et al. 2011)



2-hydroxyacetophenone
IC₅₀ PON >1000 μM
IC₅₀ LPO >1000 μM

Fig. 4.10 Structures of phloretin, phloridzin, 2-hydroxyacetophenone, and concentrations needed to scavenge 50% of peroxynitrite (IC₅₀ PON) and inhibit lipid peroxidation (IC₅₀ LPO) (Rezk et al. 2002)

3.1 μM and IC₅₀ LPO was 24 μM, while phloridzin's IC₅₀ PON was 55 μM and IC₅₀ LPO was 435 μM. The significance of these values can be understood when the authors also determined these values for a synthetic precursor of chalcones, 2-hydroxyacetophenone, whose IC₅₀ values were higher than 1000 μM. The authors proposed that the antioxidant pharmacophore in phloretin is the 2,6-dihydroxyacetophenone group due to its stabilization of radicals via tautomerization.



- 4 $R^2 = R^5 = H, R^3 = R^4 = OH, R^4 = R^6 = CH_3$ (2',3,4-trihydroxy-4',6'-dimethoxychalcone)
 5 $R^2 = R^5 = R^4 = R^6 = H, R^3 = R^4 = OH$ (2',3,4,4',6'-pentahydroxychalcone)
 6 $R^4 = R^5 = H, R^2 = R^3 = OH, R^4 = R^6 = CH_3$ (2,2',3-trihydroxy-4',6'-dimethoxychalcone)
 7 $R^4 = R^5 = R^4 = R^6 = H, R^2 = R^3 = OH$ (2,2',3,4',6'-pentahydroxychalcone)
 8 $R^1 = H, R^3 = R^4 = R^5 = OH, R^4 = R^6 = CH_3$ (2',3,4,5-tetrahydroxy-4',6'-dimethoxychalcone)
 9 $R^1 = R^4 = R^6 = H, R^3 = R^4 = R^5 = OH$ (2',3,4,4',5,6'-hexahydroxychalcone)

Fig. 4.11 Structures of hydroxychalcones 4–9 (Nishida and Kawabata 2006)

Nishida and Kawabata (2006) investigated the effect of number and arrangement of hydroxyl groups in six synthetic chalcones (4–9) for their DPPH radical-scavenging activity (Fig. 4.11).

Serifi et al. (2013) reported an antioxidant behavior of 2'-hydroxychalcones by means of cyclic voltammetry and chronoamperometry. From the 13 synthesized hydroxychalcones, compounds 10, 11, 12, and 13 showed the lowest IC_{50} for H_2O_2 scavenging (0.095, 0.228, 0.287, and 0.303 mM, respectively), which means they have higher ability to scavenge this oxidizing agent (Fig. 4.12). The IC_{50} in this

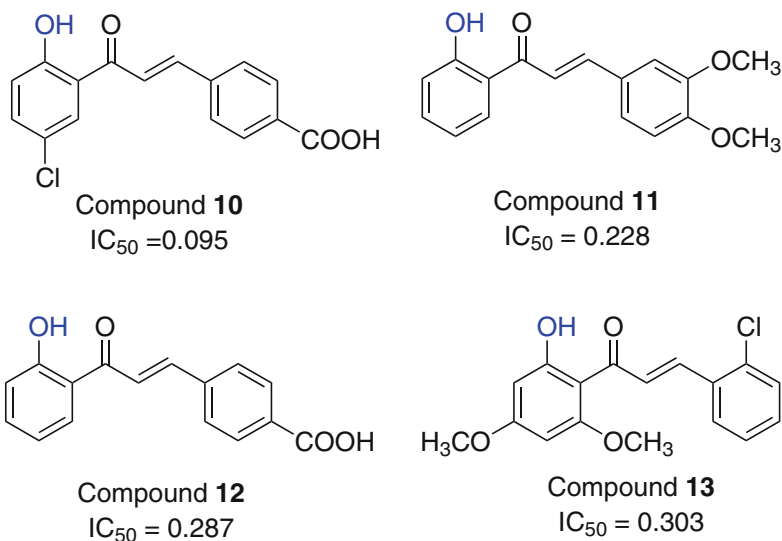


Fig. 4.12 Structures of 2'-hydroxychalcones (10–13) with reported antioxidant behavior; 2' hydroxyl groups are highlighted in blue; IC_{50} is expressed in mM as the concentration of the compound required to scavenge 50% of H_2O_2 radicals (Serifi et al. 2013)

case is the concentration of the chalcone required to scavenge 50% of radicals (Senevirathne et al. 2006).

Besides experimental studies, some theoretical studies have been made in order to better understand the structure–activity relationship (SAR) on the antioxidant activity of hydroxychalcones (Xue et al. 2012; Mathew et al. 2017; Niu et al. 2017). Two main mechanisms by which hydroxychalcones can act as antioxidants have been proposed, according to Scheme 4.2, though these mechanisms can occur in parallel.

Mechanism (i) is referred to as H-atom transfer, in which the ArOH itself becomes a radical; hydroxylated rings in chalcones can be easily converted to phenoxy radicals (ArO[•]). Mechanism (ii) is one electron transfer, in which the antioxidant agent gives an electron to the free radical and becomes a radical cation (ArO^{•+}).

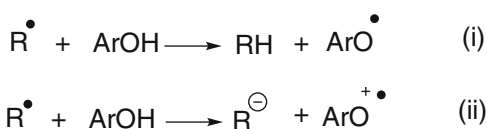
Experimental results have shown that chalcones have the potential for the scavenging of various radicals, and that the presence of hydroxyl groups may represent a higher efficiency of this activity (Xue et al. 2012). The antioxidant potential of molecules frequently calls attention for their possible therapeutic application in diseases or disorders associated with oxidative stress, such as inflammation and cancer (Padhye et al. 2010; Sikander et al. 2011). However, as described before, oxidative processes can also occur in biodiesel, representing a serious disadvantage for its usage.

In light of the scientific reports and factors associating hydroxychalcones with antioxidant activity, herein we report our study of the influence of two hydroxylated chalcones: (chalcones 16a and 16b; Fig. 4.13) in the oxidation of biodiesel.

4.2.1 Synthesis of Chalcones

Chalcones can be found as naturally in plants, fruits, and vegetables, which contain the key enzyme chalcone synthase (CHS). CHS is a member of the plant polyketide synthase superfamily and is structurally classified as a type III polyketide synthase enzyme. As outlined in Fig. 4.14, CHS catalyzes the sequential condensation of three acetate molecules derived from malonyl-CoA and *p*-coumaroyl-CoA (CoA = coenzyme A). CHS together with stilbene synthase (STS) generates the chalconaringenin (2',4', 6',4-tetrahydroxychalcone), which can be further cyclized to naringenin by action of chalcone isomerase (CHI) (step not shown, see Dao et al. 2011 for more details). The action of CHS with chalcone reductase (CHR) can

Scheme 4.2 Proposed mechanisms for antioxidant activity (Xue et al. 2012)



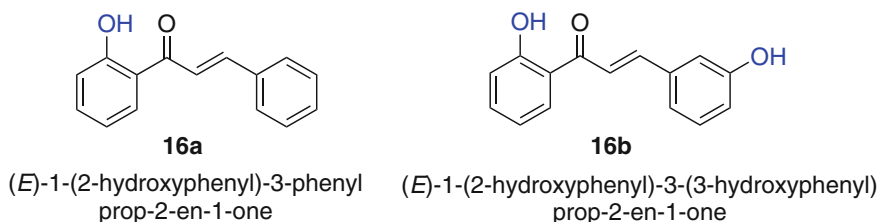


Fig. 4.13 Structures of chalcones 16a and 16b synthesized and assessed for antioxidant activity in biodiesel

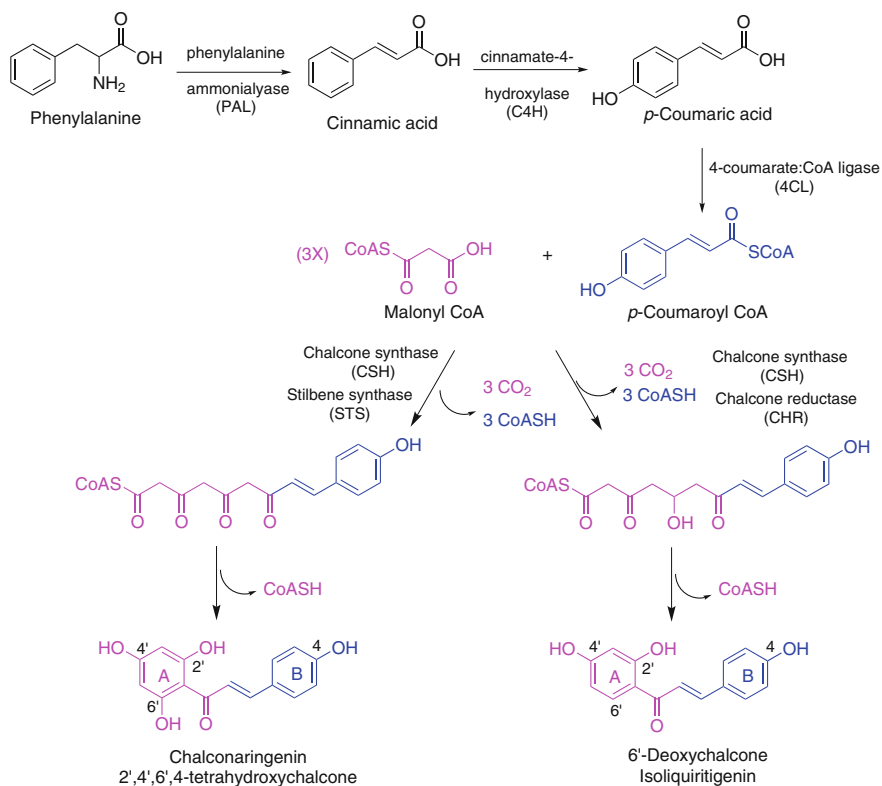


Fig. 4.14 Overview of the biosynthesis of chalcones: chalconaringenin (tetrahydroxychalcone) and isoliquiritigenin (6'-deoxychalcone). Modified from Veith and Grayer (2006) and Dao et al. (2011)

generate a 6'-deoxychalcone known as isoliquiritigenin (Veith and Grayer 2006; Dao et al. 2011; Orlikova et al. 2011).

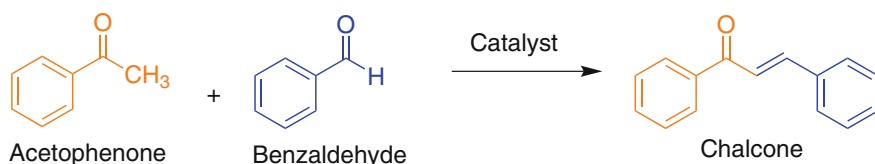
Other cyclization reactions are possible besides the one that yields chalcones, and CHS can accept other substrates as starting materials to generate corresponding

chalcones and lactone (cyclic esters) products, such as feruloyl-CoA, phenylacetyl-CoA, butyryl-CoA (Dao et al. 2011).

Nowadays, it is very practical to obtain series of chalcones by synthetic methods, and with high purities, which avoids many of the obstacles faced when these products are isolated from natural sources (Detsi et al. 2009; Aly et al. 2014; Mathew et al. 2017). The classical type of reaction carried to obtain chalcones is the Claisen–Schmidt condensation, in which a ketone reacts with an aromatic aldehyde, generally under basic catalysis (Dawane et al. 2009). This is possible because ketones, such as propanone or acetophenone, submitted to basic conditions form enolates, which react with the carbonyl group of aldehydes. It also involves dehydration to yield a product in which the double bond is conjugated both to a carbonyl group and to an aromatic ring as follows (Scheme 4.3).

An acceptable proposed mechanism for the formation of chalcones by Claisen–Schmidt condensation is shown in Fig. 4.15 (Zhuang et al. 2017).

The mechanism of Claisen–Schmidt condensation depicted in Fig. 4.15 shows that, initially, the oxygen of a base catalyst (such as NaOH or KOH) captures an acid hydrogen (α -hydrogen) from acetophenone, forming an enolate, which presents keto–enol tautomerism (shown in brackets). The enolate is a good nucleophile



Scheme 4.3 Claisen–Schmidt condensation reaction (Chavan et al. 2016)

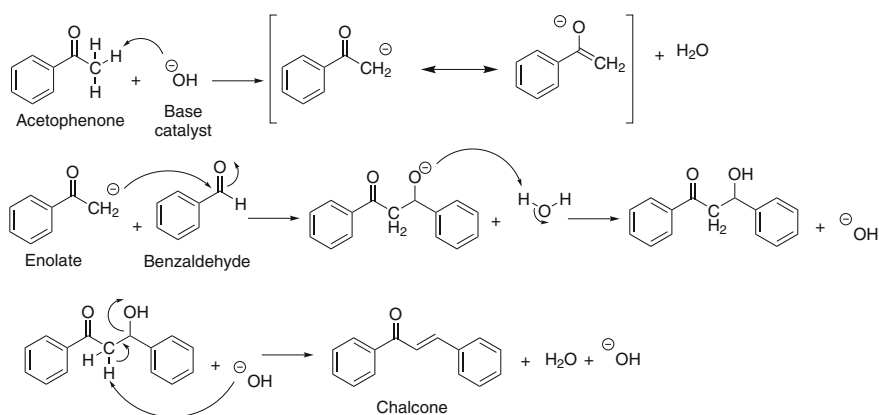


Fig. 4.15 Mechanism of Claisen–Schmidt condensation reaction to obtain chalcones

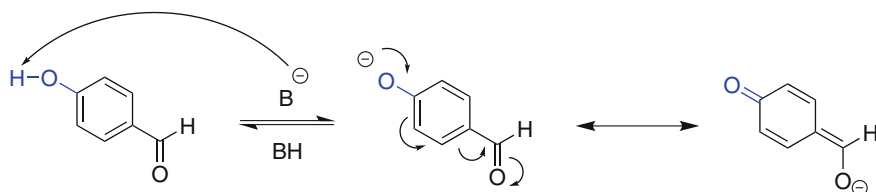
and attacks the partially positive carbonyl of the aromatic aldehyde, forming a tetrahedral intermediate (alkoxide). Under basic conditions, the alkoxide intermediate is protonated and the α -hydrogen (from the methylene group) is then removed, favoring the dehydration final step to yield the chalcone and to regenerate the basic media.

The synthesis of chalcones containing hydroxyl group (s) under basic conditions can be problematic because the electrophilicity of the aldehyde carbonyl carbon is reduced. The hydrogen from hydroxyl groups can be extracted by bases, forming a less reactive phenoxide anion (Scheme 4.4) or, in case of hydroxyl groups in the *ortho* position of the ring, intramolecular bonding with the carbonyl oxygen can be formed (Scheme 4.5) (Rozmer and Perjési 2016).

These factors result in decreased or no product formation so, in order to work around this problem, some authors use hydroxyl protective groups under basic conditions, such as methoxymethyl groups (MOM). The protective group is inserted prior to the Claisen–Schmidt condensation, which can be removed after the formation of the hydroxy-chalcone under acidic conditions (Scheme 4.6) (Nishida and Kawabata 2006; Detsi et al. 2009; Zhuang et al. 2017).

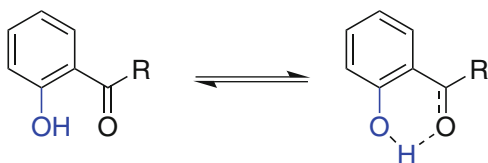
An alternative approach is the use of acids as catalysts for the synthesis of chalcones, which proceeds with the formation of an enol. Although both ketone and aldehyde need to be activated in acidic conditions, it can be directly applied for the synthesis of base-sensitive chalcones without prior use of protective groups (Petrov et al. 2008; Jayapal et al. 2010; Qian and Liu 2011).

In this way, the potential of chalcones, mainly hydroxychalcones, can be explored based on the relatively rapid, safe synthetic methods, and structure diversity of chalcone-based compounds in ways to apply them as antioxidant agents.

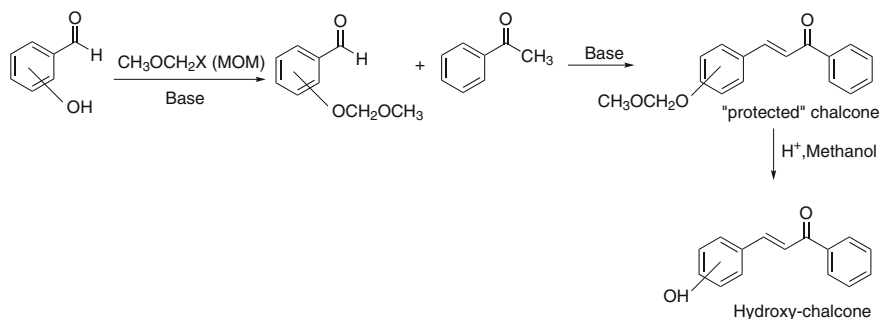


Scheme 4.4 Formation of phenoxide anion in hydroxy-aldehydes in basic media

Scheme 4.5 Formation of hydrogen intramolecular bond in *ortho*-hydroxy-substituted rings



R = H (benzaldehyde), CH₃ (acetophenone)



Scheme 4.6 Synthesis of hydroxyl chalcones using methoxymethyl (MOM) protective group

4.3 Methodology

4.3.1 Synthesis and Analysis of Biodiesel

Soybean frying oil was obtained from the restaurant of a University (Federal University of Pelotas, Brazil). Sequentially, the material was mixed with Celite in a proportion of 2 parts of oil to 4 parts of waste cooking oil. Afterward, the purified soybean oil was transesterified according to the methodology proposed by De Oliveira et al. (2013). The reaction was performed by the addition of 40 mL of a solution of sodium hydroxide 2.5% (m/v) in methanol in 100 mL of soybean oil, which was kept under reflux at 70 °C for one hour. The material was cooled to room temperature, then 60 mL of methanol and 1.5 mL of concentrated sulfuric acid were added to the system. The reaction was allowed to continue for another hour under reflux at 70 °C. Finally, the product was washed three times with distilled water, filtered through anhydrous sodium sulfate, and dried in a rotary evaporator.

Kinematic viscosity, acid value, iodine value, and flash point were determined according to ASTM standard methods. Saponification value was determined according to the AOAC method (AOAC 1997). The standard methods utilized to determine biodiesel parameters are shown in Table 4.2.

Fatty acid methyl esters (FAME) obtained from the transesterification reaction were identified in a gas chromatograph equipped with a flame ionization detector (GC-FID) (Shimadzu QP-2010). Quantitative analysis was carried out by area

Table 4.2 Standard methods for the characterization of biodiesel

Parameter	Standard method
Kinematic viscosity	ASTM D445 (ASTM 2006)
Acid value	ASTM D664-67 (ASTM 2011)
Iodine value	ASTM D459-97 (ASTM 1997)
Saponification value	AOCS CD3-25 (AOAC 1997)
Flash point	ASTM 93-94 (ASTM 1995)

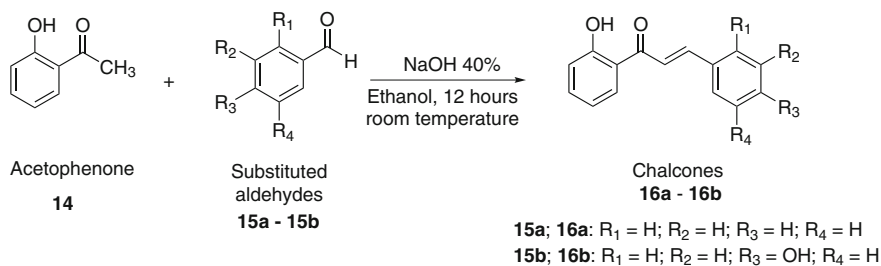
Table 4.3 Chromatographic analysis of fatty acid methyl esters from soybean frying oil

Parameter	Conditions
Column	(100 m × 0.25 m × 0.25 μm)
Carrier gas	Nitrogen
Injection volume	1 μL
Split ratio	1:25
Pressure	44.695 ψ
Flow	1.2 mL min ⁻¹
Injector temperature	250 °C
Heating ramp	100 °C (5 min)–7 °C min ⁻¹ to 200 °C (5 min)–5 °C min ⁻¹ to 230 °C (10 min)
Detector	Flame ionization detector
Detector temperature	250 °C

normalization using the GC Solution software in comparison to the FAME Mix C₄C₂₄ standard (Supelco). Details of chromatographic conditions can be seen in Table 4.3.

4.3.2 Synthesis and Characterization of HydroxyChalcones

Solvents and chemicals used in the synthesis were obtained from Sigma-Aldrich Co., St. Louis, MO, USA and used without further purification. Chalcones (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (16a) and (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl) prop-2-en-1-one (16b) were synthesized by Claisen–Schmidt condensation according to Wang et al. (2014) (Scheme 4.7). A solution of sodium hydroxide 40% in water (2.0 mL) was added to a round bottom flask containing 15 mL of ethanol and 1.0 mmol of 2-hydroxyacetophenone, in an ice bath. After 30 min, 1.2 mmol of the substituted aldehyde was added and the mixture was stirred for 12 h, at room temperature. The reaction was monitored by thin-layer chromatography in hexane/ethyl acetate 70/30%. The reaction mixture was neutralized with hydrochloric acid, and the filtered precipitate was purified by recrystallization with ethanol. Melting points were determined using capillary tubes on a Fisatom 430 apparatus. The synthetic chalcones had their structure and purity confirmed by gas chromatography coupled to mass spectrometry (GC-MS) equipped with an AOC-20i automatic injector and a Rtx-5MS 30 m × 0.25 mm × 0.25 μm column.



Scheme 4.7 Claisen–Schmidt condensation reaction to obtain chalcones 16a and 16b

4.3.3 Thermal Analysis

Oxidative stability of biodiesel without and with the addition of hydroxychalcones was assessed by differential scanning calorimetry (DSC), which was carried on a 200 F3 Maia equipment model (NETZSCH-GERÄTEBAU GMBH). Samples of biodiesel and chalcones were analyzed in closed aluminum pans, in triplicate, separately or mixed, in which the final concentration of chalcone in biodiesel was 1250 ppm. The experiments were conducted under nitrogen atmosphere at a flow rate of 50 mL min⁻¹ and under constant temperature heating of 10 °C min⁻¹ from 20 to 500 °C.

4.4 Results and Discussion

The crude waste cooking oil used in this work had a dark yellow and a viscous appearance due to its constant use, which is also linked to its high acidity and unpleasant odor. In order to be used as a fuel, it needed to surpass a pretreatment process with Celite so that most of its impurities could be eliminated. The clean up process was very efficient to remove most of the contaminants in the crude oil, such as solid particles and water, turning its color into a light yellow. These substances could interfere in the transesterification reaction leading to poor yields and a biodiesel with low quality (César et al. 2017).

The results of the physicochemical analysis of the biodiesel obtained from fried soybean oil (FSB) are shown in Table 4.4. These results can be compared to the biodiesel quality norms established in the USA (ASTM), European Union (EN), and Brazil (ANP).

According to Table 4.4, soybean frying biodiesel met all the regulated physicochemical parameters tested. Between tests was iodine value, which measures the degree of unsaturation of the fatty esters from the sample. The saponification value measures the amount of alkali needed to saponify a certain fraction of an oil or fat and usually increases as the length of the carbon chain decreases. Thus, it serves as a tool to indicate the formation of low molecular weight compounds throughout the

Table 4.4 Results and specifications soybean frying biodiesel

Specification	Result	ASTM	EN	ANP
Kinematic viscosity at 40 °C (mm ² s ⁻¹)	4.39	1.9–6	3.5–5	2,5–5,5
Acid value (mg KOH g ⁻¹)	0.5	Maximum of 0.5	Maximum of 0.5	Maximum of 0.8
Iodine value (g I ₂ g ⁻¹)	109.65	Not specified	Maximum of 120	Not specified
Saponification value (mg KOH g ⁻¹)	200.65	Not specified	Not specified	Not specified
Flash point (°C)	165	Minimum of 93	Minimum of 101	Minimum of 101

oxidation of biodiesel. Since this property is only a relative measure to determine the length of the carbon chains in oil, US and European standards do not regulate it (Knothe and Razon 2017).

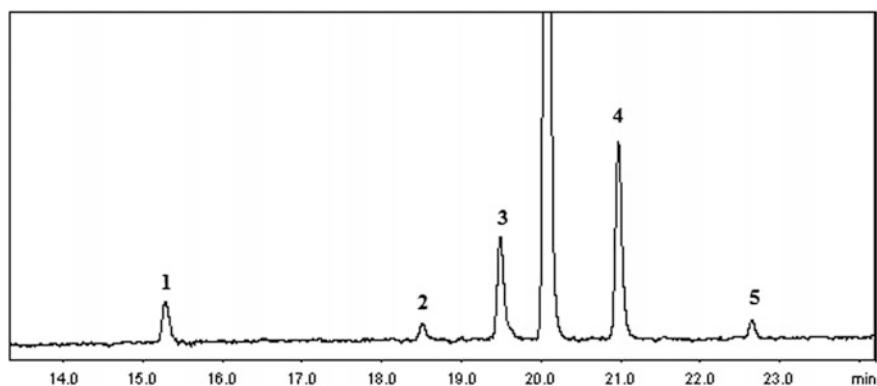
In turn, the acid value is the amount of alkali needed to neutralize a certain fraction of an oil or fat can be used to determine the presence of free fatty acids in the biodiesel sample. Therefore, it is an important parameter to regulate the quality of biodiesel since high acid values mean parts of the engine can be corroded (Georgogianni et al. 2007). Consecutively, kinematic viscosity is the amount of time needed for a liquid at 40 °C to flow through a viscometer. Generally, the viscosity of a liquid decreases as its level of unsaturation increases. Parallel to density, it also increases as the chain length of fatty acids becomes bigger or in the presence of contaminants such as soaps. This property is related to the amount of combustible injected in the engine and, thus, is of extreme relevance to the quality of biodiesel (Encinar et al. 2007).

Chromatographic analysis (Table 4.5; Fig. 4.16) revealed that soybean biodiesel is composed of several fatty acids. Nonetheless, linoleic acid (C18:2*n*6c) played a dominant role in the lipidic profile representing more than half (51.79%) of the composition of the biodiesel. Oleic acid (C18:1*n*9c) and palmitic acid (C16:0) were also important constituents representing 28.75 and 11.13%. Other biomolecules such as linolenic (C18:3*n*3) and stearic acid (C18:0) were found in small quantities. Since more than 85% of the fatty acids in the biodiesel were unsaturated, it can be said that the oxidative stability was relatively lower because these acids are more reactive toward oxidation reactions (Sajjadi et al. 2016).

In general, the transesterification reaction presented yields close to 96%. In a similar work, Georgogianni et al. (2007) isolated methyl esters of soybean frying oil in yields varying from 42 to 97% under mechanical stirring. Encinar et al. (2007) performed ethanolysis in waste frying oil under alkaline catalysis with yields ranging from 46.8 to 74.2% of oil conversion into esters.

Table 4.5 Fatty acid composition of soybean biodiesel

Fatty acid	Percentage (per area)	Peak number in Fig. 4.16
C16:0	11.13	1
C18:0	3.85	2
C18:1 <i>n</i> 9 <i>c</i>	28.75	3
C18:2 <i>n</i> 6 <i>c</i>	51.79	4
C18:3 <i>n</i> 3	4.46	5
Σ saturated	14.98	
Σ unsaturated	85.02	

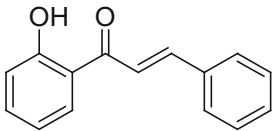
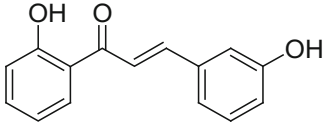
**Fig. 4.16** Chromatogram of fatty acids in waste soybean cooking oil

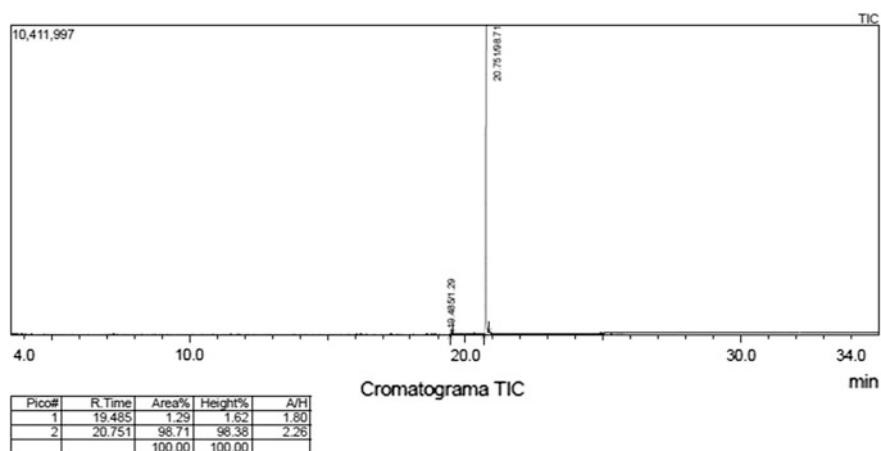
In relation to the synthetic chalcones, two products were successfully obtained in moderate yields of 46% for chalcone 16a and 32% for chalcone 16b. Wang et al. (2014) obtained the exact structure of chalcone 16a in a 55.7% yield. Kamboj et al. (2010) obtained the exact structure of chalcone 16b in a 52% yield under microwave irradiation, and Ohkatsu and Satoh (2008) obtained this product in a 13.9% yield under conventional conditions (without use of microwaves). Data of chalcones are shown in Table 4.6.

Results from spectrometric analysis are shown below. The chromatogram and mass spectrum of chalcone 16a are shown in Figs. 4.17 and 4.18, and chromatogram and mass spectrum for chalcone 16b are shown in Figs. 4.19 and 4.20.

It can be seen from the physical properties determined experimentally that the synthesized chalcones match the expected parameters, so their structures could be confirmed. Chalcone 16a presented an experimental melting point of 90–91 °C, which was very proximate to the result found in the literature for the same synthetic compound (89 °C) (Jhala et al. 2006). The same could be observed for chalcone 16b, which had an experimental melting point of 163–165 °C, proximate to the one found by Kamboj et al. (2010) (165–167 °C). It is common to observe some variations in experimental melting points to the ones found by other researchers,

Table 4.6 Data of synthesized hydroxychalcones

Code	Structure	Melting point (°C)	Exact mass
Chalcone 16a (<i>E</i>)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one		Exp.: 90–91 Lit.: 89 (Jhala et al. 2006)	224.08
Chalcone 16b (<i>E</i>)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one		Exp.: 163–165 Lit.: 165–167 (Kamboj et al. 2010)	240.08

**Fig. 4.17** Chromatogram of chalcone 16a (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one

mainly due to the different manipulations or solvents used in the recrystallization procedures. Chromatograms of both synthetic products show peaks at retention times of around 20 min, and the integration of the peaks generated the mass spectrum, which were in accordance to the exact masses of the chalcones. Figure 4.19 presents a molecular ion of m/z 224 in accordance to the exact mass of chalcone 16a (224.08), and Fig. 4.21 presented a molecular ion of 240 respective to the mass of 240.08 of chalcone 16b.

In the last few years, thermal analysis by DSC has been commonly used for the purpose of antioxidant activity evaluation (Dunn 2008). Two major quality control tests are assessed by DSC in industry: tests conducted under isothermal conditions indicate oxidation induction time (OIT), and the ones conducted at different temperature scans indicate the oxidation onset temperature (OOT). The difference

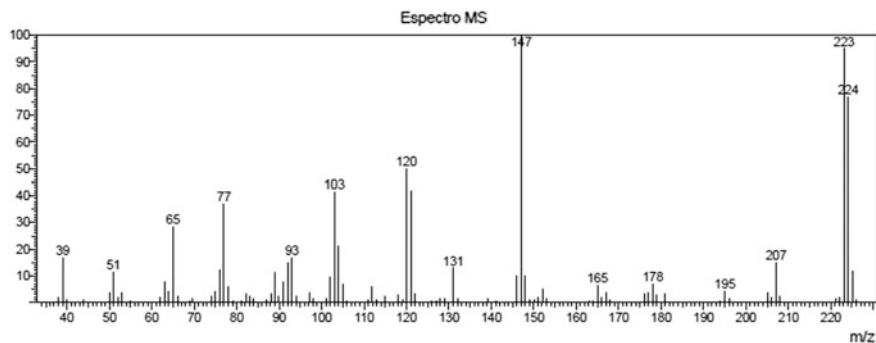


Fig. 4.18 Mass spectrum of chalcone 16a (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one

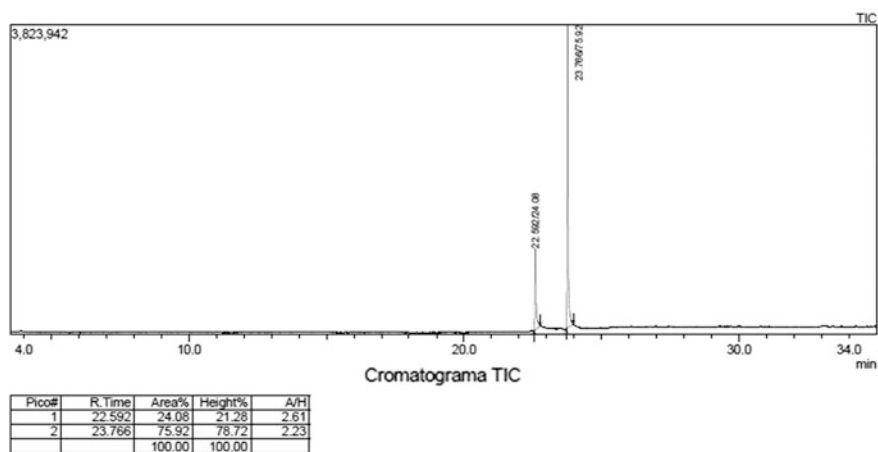


Fig. 4.19 Chromatogram of chalcone 16b (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one

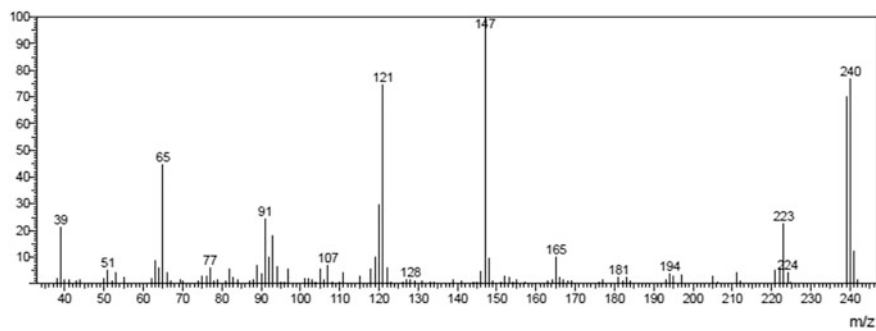


Fig. 4.20 Mass spectrum of chalcone 16b (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one

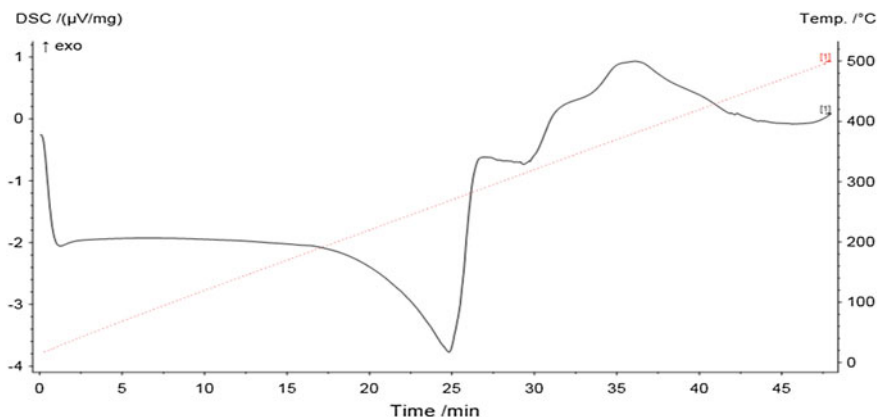


Fig. 4.21 DSC thermogram of fried soybean oil biodiesel only

between these calorimetric tests is that, under isothermal conditions, oxidation is registered as the time the sample exhibits a sudden exothermic event, whereas under temperature scanning experiments, oxidation is registered as the temperature the exothermic event occurs (Focke and Van Der Westhuizen 2010).

Recently, our laboratory showed the green synthesis of a series of chalcones with biological activity (Ritter et al. 2015) and deposited a patent (Brazilian patent BR 1020130300497) of hydroxy-functionalized chalcones applied as oxidative stabilizers in biodiesel (Oliveira et al. 2013). Results from DSC analysis are shown below in Figs. 4.21 (biodiesel only), 4.22 (chalcone 16a only), 4.23 (chalcone 16b only), 4.24 (biodiesel with chalcone 16a at 1250 ppm), and 4.25 (biodiesel with chalcone 16b at 1250 ppm).

It can be seen from Fig. 4.21 (thermogram of biodiesel only) an endothermic (downwards) event at a temperature range of 200–270 °C (~25 min) associated with the vaporization of methyl esters present in biodiesel (shown in Table 4.5). The variation of temperature will depend on the fatty acid composition due to their differences in structure (Misutsu et al. 2015). The other exothermic events (peaks facing upwards) starting at around 30 min (~300 °C) indicate oxidation processes affecting biodiesel and combustion of unsaturated fatty acids. Thermogram of chalcone 16a (Fig. 4.22) shows two endothermic events right before 100 °C temperature, which can be associated with the loss of water (dehydration) and with the melting point (89–91 °C; Table 4.6) of the synthetic molecule, respectively. The exothermic event happening at around 325 °C is associated with the oxidation of chalcone 16a. The same pattern was observed for chalcone 16b, as it can be seen from its thermogram in Fig. 4.23: two sharp endothermic events associated with dehydration and melting point (165 °C) and a clear exothermic event of oxidation at around 300 °C. Chalcone 16a blended with biodiesel at a 1250 ppm concentration does not show an antioxidant activity (Fig. 4.24), but rather caused an increase of the temperature in which biodiesel started to suffer oxidation, or an

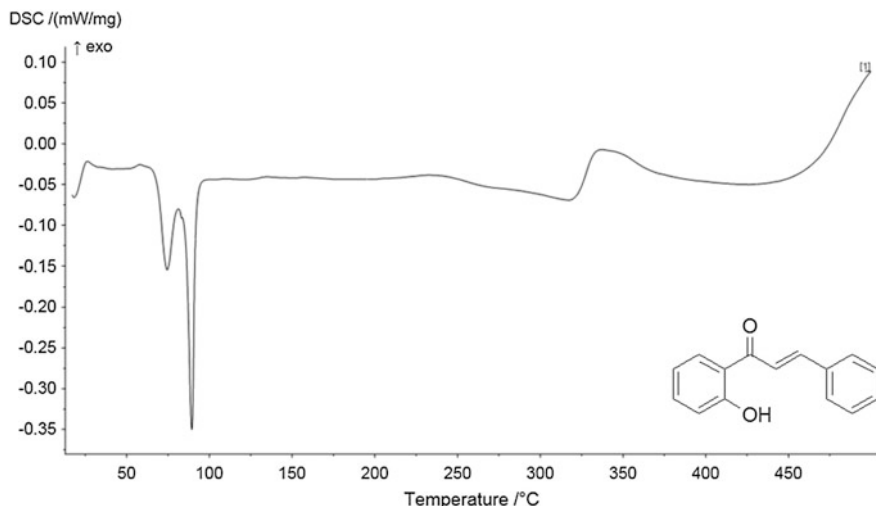


Fig. 4.22 DSC thermogram of chalcone 16a only

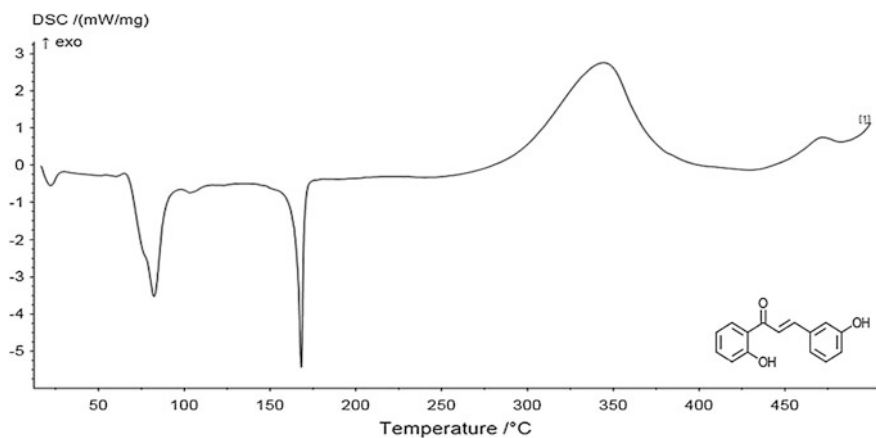


Fig. 4.23 DSC thermogram of chalcone 16b only

increase in OOT. Chalcone 16b showed a potent antioxidant effect as the thermogram depicted in Fig. 4.25 lacks any exothermic events of oxidation. It seems that events happening with the chalcone overlap the one observed in biodiesel (~25 min), and that the chalcone could be suffering or neutralizing oxidation events itself instead of biodiesel. The other slightly distorted endothermic event (27–35 min in Fig. 4.25) could be due to the formation of other stable products resulting from the oxidation of chalcone 16b. Their results showed that the chalcone 16b containing two hydroxyl groups was more effective than chalcone 16a, which has only one, but that both synthetic molecules influenced in the oxidative stability

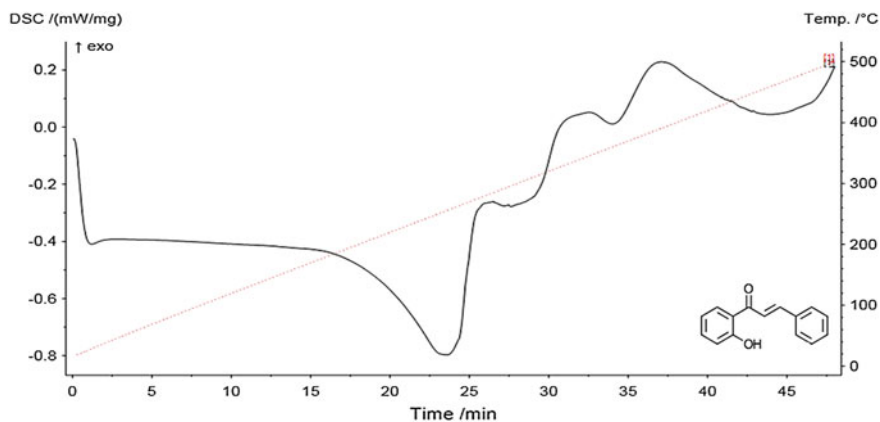


Fig. 4.24 DSC thermogram of biodiesel blended with chalcone 16a at 1250 ppm

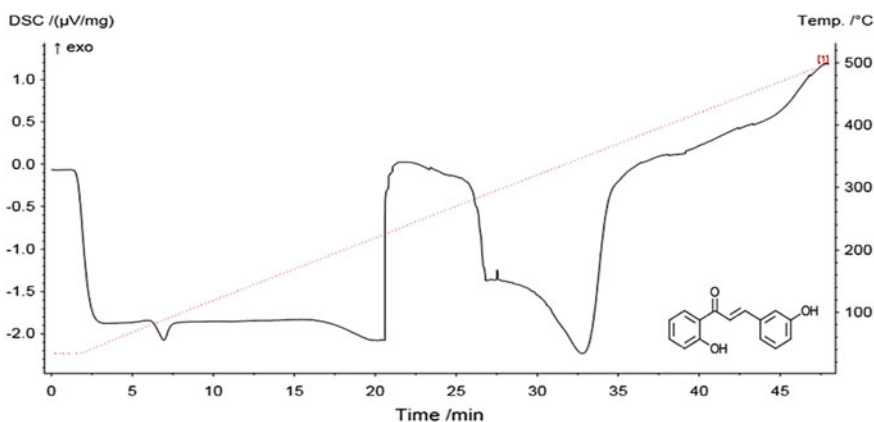


Fig. 4.25 DSC thermogram of biodiesel blended with chalcone 16b at 1250 ppm

of biodiesel. It is important to note the sample of biodiesel in this study was obtained from used frying oil, which itself has already been exposed to oxidative and hydrolysis processes. Also, the 1250 ppm concentration of chalcones blended with biodiesel is within the range of 500–2000 ppm normally used in similar studies (Dunn 2008). It is likely that a higher concentration of these compounds mixed with biodiesel would induct a more notable antioxidant effect. Thus, synthetic molecules based on the structural features discussed here are still promising agents to enhance biodiesel oxidative stability.

4.5 Conclusion

Biodiesel has many advantages compared to the ephemeral petrodiesel, but its potential has not yet been fully explored due to some drawbacks. In this chapter, it was demonstrated that oxidative stability is one of the major limitations associated with biodiesel. In this way, it is very important to search for agents that can avoid oxidative processes or enhance biodiesel stability. Phenolic compounds, specially natural or synthetic chalcones containing hydroxyl groups, have shown interesting antioxidant effects and, therefore, should be further explored as antioxidant agents or stabilizers to be applied along with biodiesel production. Finally, calorimetric analysis performed by DSC proved to be a very helpful tool to assess potential antioxidant effects resulting from the addition of isolated or synthetic compounds in biodiesel.

References

- Aly MRES, Fodah HHAER, Saleh SY (2014) Antiobesity, antioxidant and cytotoxicity activities of newly synthesized chalcone derivatives and their metal complexes. *Eur J Med Chem* 76:517–530
- Association of Official Analytical Chemists (AOAC) (1997) Section C: Commercial Fats and Oils, American Oil Chemists Society (AOCS) official method Cd 3–25 for saponification value. Association of Official Analytical Chemists, Washington
- ASTM D (1997) Standard test method for iodine value of drying oils and fatty acids. *Int Annual book of ASTM standards*
- ASTM Standard (1995) D93: standard test methods for flash point by Pensky-Martens closed cup tester. American Society for Testing and Materials, Philadelphia, PA (USA)
- ASTM Standard (2006) D445: standard test method for kinematic viscosity of transparent and opaque liquids (and calculation of dynamic viscosity). American Society for Testing and Materials, West Conshohocken, PA (USA)
- ASTM Standard (2011) Standard test method for acid number of petroleum products by potentiometric titration-ASTM D 664-11A. ASTM International, West Conshohocken, PA
- Atabani AE, Silitonga AS, Ong HC et al (2013) Non-edible vegetable oils: a critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. *Renew Sustain Energy Rev* 18:211–245
- Cao G, Sofic E, Prior RL (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic Biol Med* 22:749–760
- Chavan BB, Gadekar AS, Mehta PP, et al (2016) Synthesis and medicinal significance of chalcones—a review. *Asian J Biomed Pharm Sci* 56:1–7
- da César AS, Werderits DE, de Oliveira Saraiva GL, da Guabiroba RCS (2017) The potential of waste cooking oil as supply for the Brazilian biodiesel chain. *Renew. Sustain. Energy Rev* 72:246–253
- Dantas MB, Albuquerque AR, Barros AK et al (2011) Evaluation of the oxidative stability of corn biodiesel. *Fuel* 90:773–778
- Dao TTH, Linthorst HJM, Verpoorte R (2011) Chalcone synthase and its functions in plant resistance. *Phytochem Rev* 10:397–412

- Dawane BS, Konda SG, Shaikh BM, Bhosale RB (2009) An improved procedure for synthesis of some new 1,3-diaryl-2-propen-1-ones using PEG-400 as a recyclable solvent and their antimicrobial evaluation. *Acta Pharm* 59:473–482
- De Oliveira DM, Ongaratto DP, Fontoura LAM et al (2013) Obtenção de biodiesel por transesterificação em dois estágios e sua caracterização por cromatografia gasosa óleos e gorduras em laboratório de química orgânica. *Quim Nova* 36:734–737
- Detsi A, Majdalani M, Kontogiorgis CA et al (2009) Natural and synthetic 2'-hydroxy-chalcones and aurones: synthesis, characterization and evaluation of the antioxidant and soybean lipoygenase inhibitory activity. *Bioorganic Med Chem* 17:8073–8085
- Días-Tielas C, Graña E, Reigosa MJ, Sánchez-Moreiras AM (2016) Biological activities and novel applications of chalcones. *Planta Daninha* 34:607–616
- Dunn RO (2006) Oxidative stability of biodiesel by dynamic mode pressurized-differential Scanning Calorimetry (P-Dsc). *Am Soc Agric Biol Eng* 49:1633–1641
- Dunn RO (2008) Antioxidants for improving storage stability of biodiesel. *Biofuels, Bioprod Biorefining* 2:304–318
- Encinar JM, González JF, Rodríguez-Reinares A (2007) Ethanolysis of used frying oil. Biodiesel preparation and characterization. *Fuel Process Technol* 88:513–522
- Focke WW, Van Der Westhuizen I (2010) Oxidation induction time and oxidation onset temperature of polyethylene in air: testing Gimzewski's postulate. *J Therm Anal Calorim* 99:285–293
- Franceschelli S, Pesce M, Vinciguerra I et al (2011) Licocalchone-C extracted from glycyrrhiza glabra inhibits lipopolysaccharide-interferon-gamma inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. *Molecules* 16:5720–5734
- Fu J, Hue BTB, Turn SQ (2017) Oxidation stability of biodiesel derived from waste catfish oil. *Fuel* 202:455–463
- Georgogianni KG, Kontominas MG, Tegou E, et al (2007) Biodiesel production: reaction and process parameters of alkali-catalyzed transesterification of waste frying oils biodiesel production: reaction and process parameters of alkali-catalyzed transesterification of waste frying oils. *Energy* 3023–3027. <https://doi.org/10.1021/ef070102b>
- Gui MM, Lee KT, Bhatia S (2008) Feasibility of edible oil versus non-edible oil versus waste edible oil as biodiesel feedstock. *Energy* 33:1646–1653
- Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 13:572–584
- Herbinet O, Pitz WJ, Westbrook CK (2010) Detailed chemical kinetic mechanism for the oxidation of biodiesel fuels blend surrogate. *Combust Flame* 157:893–908
- Hoekman SK, Broch A, Robbins C et al (2012) Review of biodiesel composition, properties, and specifications. *Renew Sustain Energy Rev* 16:143–169
- Jayapal MR, Sreenivasa Prasad K, Sreedhar NY (2010) Synthesis and characterization of 2,4-dihydroxy substituted chalcones using aldol condensation by $\text{SOCl}_2/\text{EtOH}$. *J Chem Pharm Res* 2:127–132
- Jhala YS, Dulawat SS, Verma BL (2006) Solvent-free improved syntheses of some substituted 1, 3-diaryl-propenones and 3, 5-diaryl-6-carbethoxycyclohexenones under microwave irradiation and their antibacterial activity. *Indian J Chem* 45:466–469
- Kamboj RC, Arora R, Sharma G et al (2010) Eco-friendly synthesis and antimicrobial activity of chalcones. *Der Pharma Chem* 2:157–170
- Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technol* 86:1059–1070
- Knothe G (2006) Analyzing biodiesel: standards and other methods. *JAACS J Am Oil Chem Soc* 83:823–833
- Knothe G (2007) Some aspects of biodiesel oxidative stability. *Fuel Process Technol* 88:669–677
- Knothe G, Razon LF (2017) Biodiesel fuels. *Prog Energy Combust Sci* 58:36–59
- Kulkarni MG, Dalai AK (2006) Waste cooking oil - An economical source for biodiesel: a review. *Ind Eng Chem Res* 45:2901–2913
- Kumar N (2017) Oxidative stability of biodiesel: causes, effects and prevention. *Fuel* 190:328–350

- Lapuerta M, Herreros JM, Lyons LL et al (2008) Effect of the alcohol type used in the production of waste cooking oil biodiesel on diesel performance and emissions. *Fuel* 87:3161–3169
- Lee CK, Son SH, Park KK et al (2008) Licochalcone a inhibits the growth of colon carcinoma and attenuates cisplatin-induced toxicity without a loss of chemotherapeutic efficacy in mice. *Basic Clin Pharmacol Toxicol* 103:48–54
- Marcon NS, Colet R, Balen DS et al (2017) Enzymatic biodiesel production from microalgae biomass using propane as pressurized fluid. *Can J Chem Eng* 95:1340–1344
- Mathew B, Adeniyi AA, Joy M et al (2017) Anti-oxidant behavior of functionalized chalcone-a combined quantum chemical and crystallographic structural investigation. *J Mol Struct* 1146:301–308
- Mathiesena L, Malterud KE, Sund RB (1996) Hydrogen bond formation as basis for radical scavenging activity: a structure-activity study of C-methylated dihydrochalcones from *Myrica gale* and structurally related acetophenones. *Free Radic Biol Med* 22:307–311
- Mekhilef S, Siga S, Saidur R (2011) A review on palm oil biodiesel as a source of renewable fuel. *Renew Sustain Energy Rev* 15:1937–1949
- Misutsu MY, Cavalheiro LF, Ricci TG et al (2015) Thermoanalytical methods in verifying the quality of biodiesel. In: Biernat K (ed) *Biofuels—status and perspective*. InTech, Croatia, pp 251–269
- Mittelbach M, Schober S (2003) The influence of antioxidants on the oxidation stability of biodiesel. *JAOCS, J Am Oil Chem Soc* 80:817–823
- Nishida J, Kawabata J (2006) DPPH radical scavenging reaction of hydroxy- and methoxychalcones. *Biosci Biotechnol Biochem* 70:193–202
- Niu H, Wang W, Li J et al (2017) A novel structural class of coumarin-chalcone fibrates as PPARalpha/gamma agonists with potent antioxidant activities: Design, synthesis, biological evaluation and molecular docking studies. *Eur J Med Chem* 138:212–220
- Ohkatsu Y, Satoh T (2008) Antioxidant and photo-antioxidant activities of chalcone derivatives. *J Japan Pet Inst* 51:298–308. <https://doi.org/10.1627/jpi.51.298>
- Oliveira M, Rockembach CT, Dias D, et al (2013). Aplicação de Chalconas como Reagente Antioxidante em Biodiesel. Brazilian Patent BR 10 2013 030049 7 A2, 22 Nov 2013
- Orlikova B, Tasdemir D, Golais F et al (2011) Dietary chalcones with chemopreventive and chemotherapeutic potential. *Genes Nutr* 6:125–147
- Padhye S, Ahmad A, Oswal N et al (2010) Fluorinated 2'-hydroxychalcones as garcinol analogs with enhanced antioxidant and anticancer activities. *Bioorganic Med Chem Lett* 20:5818–5821
- Pereira CMP, Hobuss CB, Maciel JV et al (2012) Biodiesel renovável derivado de microalgas: avanços e perspectivas tecnológicas. *Quim Nova* 35:2013–2018
- Petrov O, Ivanova Y, Gerova M (2008) SOCl₂/EtOH: catalytic system for synthesis of chalcones. *Catal Commun* 9:315–316
- Pinto FV (2017) Boletim Mensal do biodiesel. In: Agência Nac. do Petróleo Gás Nat. e Biocombustíveis—ANP. Web Page. <http://www.anp.gov.br/wwwanp/publicacoes/boletins-anp/2386-boletim-mensal-do-biodiesel>. Accessed 28 Aug 2017
- Pinzi S, Garcia IL, Lopez-Gimenez FJ et al (2009) The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. *Energy Fuels* 23:2325–2341
- Pullen J, Saeed K (2012) An overview of biodiesel oxidation stability. *Renew Sustain Energy Rev* 16:5924–5950
- Qian H, Liu D (2011) Synthesis of chalcones via claisen-schmidt reaction catalyzed by sulfonic acid-functional ionic liquids. *Ind Eng Chem Res* 50:1146–1149
- Rezk BM, Haenen GRMM, Van der Vijgh WJF, Bast A (2002) The antioxidant activity of phloretin: The disclosure of a new antioxidant pharmacophore in flavonoids. *Biochem Biophys Res Commun* 295:9–13
- Righi AA, Alves TR, Negri G et al (2011) Brazilian red propolis: unreported substances, antioxidant and antimicrobial activities. *J Sci Food Agric* 91:2363–2370
- Ritter M, Martins RM, Rosa SA et al (2015) Green synthesis of chalcones and microbiological evaluation. *J Braz Chem Soc* 26:1201–1210

- Rizwanul Fattah IM, Hassan MH, Kalam MA et al (2014a) Synthetic phenolic antioxidants to biodiesel: path toward NO_x reduction of an unmodified indirect injection diesel engine. *J Clean Prod* 79:82–90
- Rizwanul Fattah IM, Masjuki HH, Kalam MA et al (2014b) Effect of antioxidants on oxidation stability of biodiesel derived from vegetable and animal based feedstocks. *Renew Sustain Energy Rev* 30:356–370. <https://doi.org/10.1016/j.rser.2013.10.026>
- Rockembach CT, Dias D, Vieira BM et al (2014) Synthesis of biodiesel from grape seed oil using ultrasound irradiation. *Rev Virtual Química* 6:884–897
- Rozmer Z, Perjési P (2016) Naturally occurring chalcones and their biological activities. *Phytochem Rev* 15:87–120
- Sajjadi B, Raman AAA, Arandiyani H (2016) A comprehensive review on properties of edible and non-edible vegetable oil-based biodiesel: composition, specifications and prediction models. *Renew Sustain Energy Rev* 63:62–92
- Saluja RK, Kumar V, Sham R (2016) Stability of biodiesel—a review. *Renew Sustain Energy Rev* 62:166–181
- Sarin R, Sharma M, Sinharay S, Malhotra RK (2007) Jatropha-Palm biodiesel blends: an optimum mix for Asia. *Fuel* 86:1365–1371
- Senevirathne M, Kim S-H, Siriwardhana N et al (2006) Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci Technol Int* 12:27–38
- Serifi O, Tsopelas F, Kypreou AM et al (2013) Antioxidant behaviour of 2'-hydroxy-chalcones: a study of their electrochemical properties. *J Phys Org Chem* 26:226–231
- Serqueira DS, Fernandes DM, Cunha RR et al (2014) Influence of blending soybean, sunflower, colza, corn, cottonseed, and residual cooking oil methyl biodiesels on the oxidation stability. *Fuel* 118:16–20
- Shahidi F, Wanasundara PK (1992) Phenolic antioxidants. *Crit Rev Food Sci Nutr* 32:67–103
- Sikander M, Malik S, Yadav D et al (2011) Cytoprotective activity of a trans-chalcone against hydrogen peroxide induced toxicity in hepatocellular carcinoma (HepG2) cells. *Asian Pacific J Cancer Prev* 12:2513–2516
- Singh A, Nigam PS, Murphy JD (2011) Renewable fuels from algae: An answer to debatable land based fuels. *Bioresour Technol* 102:10–16
- Singh SP, Singh D (2010) Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review. *Renew Sustain Energy Rev* 14:200–216
- Stuart BH (2012) *Forensic analytical techniques*. Wiley, New Jersey, 234 p
- Tomás-Barberán FA, Clifford MN (2000) Flavanones, chalcones and dihydrochalcones—nature, occurrence and dietary burden. *J Sci Food Agric* 80:1073–1080
- Veith NC, Grayer RJ (2006) Chalcones, Dihydrochalcones, and Aurones. In: Andersen OM, Markham KR (eds) *Flavonoids: chemistry, biochemistry, and applications*, pp 1003–1071. CRC Press Taylor & Francis Group, Florida
- Vieira BM, Elicker C, Nunes CFP et al (2016) The synthesis and characterization of *Butia capitata* seed oil as a FAME feedstock. *Fuel* 184:533–535
- Wang F-W, Wang S-Q, Zhao B-X, Miao J-Y (2014) Discovery of 2'-hydroxychalcones as autophagy inducer in A549 lung cancer cells. *Org Biomol Chem* 12:3062–3070
- Wang Y, Pengzhan Liu SO, Zhang Z (2007) Preparation of biodiesel from waste cooking oil via two-step catalyzed process. *Energy Convers Manag* 48:184–188
- Xin J, Imahara H, Saka S (2009) Kinetics on the oxidation of biodiesel stabilized with antioxidant. *Fuel* 88:282–286
- Xue Y, Zheng Y, An L et al (2012) A theoretical study of the structure-radical scavenging activity of hydroxychalcones. *Comput Theor Chem* 982:74–83
- Yaakob Z, Narayanan BN, Padikkaparambil S et al (2014) A review on the oxidation stability of biodiesel. *Renew Sustain Energy Rev* 35:136–153
- Zhuang C, Zhang W, Sheng C et al (2017) Chalcone: a privileged structure in medicinal chemistry. *Chem Rev* 117:7762–7810